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Control of swine pseudorabies in China: Opportunities and limitations

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ABSTRACT

Pseudorabies (PR), also known as Aujeszky's disease (AD), is caused by pseudorabies virus (PRV) or called suid herpesvirus 1 (SuHV-1). It is an economically significant viral disease of pigs and other animals. Although the disease has been eradicated in commercial swine populations of some countries using gE-deleted vaccines and differentiating infected from vaccinated animals (DIVA) strategy, PR continues to be one of the most important diseases of pigs in many countries, particularly in regions with dense pig populations, including China. This article reviews the current situation of PR in China, including epidemiology, diagnostic assays, control strategies and challenges of the disease. PR has been endemic in most provinces of China largely due to the lack of appropriate compulsory vaccination campaigns of pigs, sufficient awareness and biosecurity measures, although gE-deleted vaccines based on the Bartha-K61 strain and regional DIVA-based eradication programs have been widely used in the past decades. Notably, since 2011, an emerging variant PRV with enhanced pathogenicity has become prevalent in vaccinated swine herds in many regions of China and the disease situation is worsening. Control and eventual eradication of PR remain a big challenge in China, and strengthened control measures based on updated DIVA strategy are urgently needed toward national eradication of PR.

Contents

1. Introduction

Pseudorabies (PR), also known as Aujeszky's disease, is an economically important viral disease of pigs and other animals in many countries. The disease is caused by suid herpesvirus 1 (SuHV-1), or called pseudorabies virus (PRV) or Aujeszky's disease virus (ADV). SuHV-1 is a member of the genus *Varicellovirus* of the subfamily *Alphaherpesvirinae* within the family *Herpesviridae*. Pigs

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are the natural host for PRV and the only animals to become latent carriers, although the virus can infect numerous other species of mammals, including ruminants, carnivores and rodents (Mettenleiter, 2000; Pomeranz et al., 2005).

In pigs infected with PRV, the clinical manifestations vary from subclinical signs to death. In newborn piglets as well as other susceptible species, PRV infections are often fatal, and animals die from central nervous system disorders. In contrast, older pigs develop primarily respiratory symptoms. Like other alphaherpesviruses, PRV usually establishes a life-long latent infection in the host peripheral nervous system. Latently infected pigs can be a source of reinfections when the latent viral genome reactivates spontaneously or is reactivated by stress and triggers virus shedding. In pregnant sows, PRV infections may result in reproductive failure including abortions and/or stillbirths (Rziha et al., 1986; Tong and Chen, 1999; Klupp et al., 2004).

Due to control efforts and strict implementation of national eradication programs, including large-scale compulsory vaccination with gE-deleted vaccines and the DIVA (differentiating infected from vaccinated animals) strategy, PR has been eradicated from domesticated pigs in North America and a number of European countries (Müller et al., 2011; OIE, 2012). However, PR remains one of the most important diseases of swine in many countries, particularly occurring in regions with dense pig populations, including China, the biggest pork producer in the world (Tong and Chen, 1999; Pomeranz et al., 2005; Yang, 2015).

2. Epidemiology

In China, PRV infection was first identified in cats by Liu in 1947, and subsequently documented in cattle and swine (Zhou and Sun, 1957; Guo, 1963). It was reported that PR had been endemic in China before 1960s (Zhou and Sun, 1957; Ou and Huang, 1958; Cao, 1959), but it did not cause significant economic losses (Yuan et al., 1986; Tong and Chen, 1999). By early 1980s, PRV infections had been identified in 18 regions (14 provinces, 1 municipality and 3 autonomous regions of China) in diverse species, mainly in pigs but also in cattle, sheep, goats, cats, dogs and minks (Fig. 1A and Table 1; Yuan and Wu, 1986). These animals are infected with PRV mainly through direct/indirect contact with infected pigs or feeding of infected pork due to poor biosecurity measures. Since 1980s, PR outbreaks have risen and spread widely around the country, which was coincident with the increase in the intensity of swine production in China (Yuan and Wu, 1986; Zhang and Chen, 2008). In addition, a high proportion of pigs infected with PRV become latently infected in the field (Sabó, 1985), which was considered to be the most possible source of infection (Yuan and Wu, 1986). During that time, the morbidity and mortality in newborn piglets of infected pig herds were 70–100%. To control PRV infections of pigs in China, the Bartha-K61 vaccine strain was imported from Hungary in 1979 and evaluated for its safety and efficacy (Yuan et al., 1983, 1985, 1986). Since late 1980s, Bartha-K61 vaccines have been widely applied in China, resulting in

Table 1
Geographic distribution and recorded year and species of first pseudorabies occurrence in China.

Region	Year	Species
Heilongjiang	1957, 1981	Pig, dog, cat
Liaoning	1982	Pig, cattle
Jilin	1975, 1985	Pig, cattle
Inner Mongolia	1975	Mink
Guangdong	1958, 1980	Pig, cattle
Fujian	1962, 1974	Pig, cattle
Shanghai	1947, 1978	Cat, pig
Jiangsu	1959	Cattle, pig
Jiangxi	1977	Pig
Sichuan	1959, 1982	Pig
Guizhou	1979	Pig
Yunnan	1984	Cattle
Shaanxi	1978–1979	Pig, cattle
Shandong	1980	Goat, sheep
Henan	1979	Cattle, sheep, cat, dog
Xinjiang	Before 1980	Pig
Tibet	1980	Cattle, sheep
Taiwan	1971	Pig
Hunan	1991	Pig
Beijing	1992	Pig
Hong Kong	1990s	Pig
Hubei	1990	Pig
Tianjin	1997	Pig
Chongqing	1990s	Pig
Guangxi	1990s	Pig
Hainan	Early 2000s	Pig
Gansu	Late 1990s	Pig
Qinghai	Late 1990s	Pig
Ningxia	Late 1990s	Pig
Hebei	1997	Pig
Zhejiang	1994	Pig
Anhui	Early 2000s	Pig
Shanxi	2001	Pig

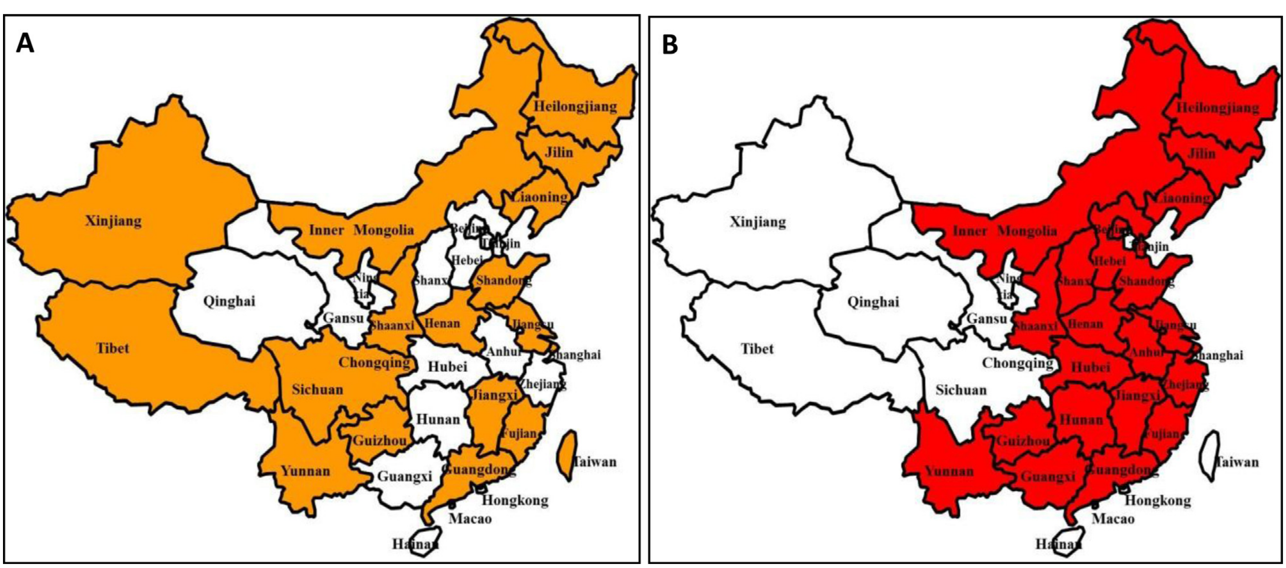

Fig. 1. Geographic distribution of PRV infections in China. (A) Classical PRV strains infections before 1980; (B) PRV variants infections since 2011.

relatively favorable control of PR and the morbidity and mortality in newborn piglets of infected swine herds were less than 10% in Bartha-K61-vaccinated swine herds (Tong and Chen, 1999; Kong, 2000). However, PR has been endemic or sporadic in all the regions of China by the mid-2000s, largely due to the lack of compulsory vaccination campaigns of pigs, DIVA strategy, insufficient regulations and poor biosecurity measures (Lou and Du, 1999; Tong and Chen, 1999; Han et al., 2001; Zhang and Chen, 2008).

More seriously, since late 2011, PRV variants have emerged in a large number of Bartha-K61-vaccinated swine herds in many regions of China. According to a recent report, 80.1% of the investigated farms (213 out of 266) from 23 regions of China suffered from PRV variant infections (Fig. 1B). Furthermore, overall PRV infection rates or gE-antibody positive rates increased up to over 50% from below 20% in 58.2% (124/213) of the PRV variants-infected farms (Yang, 2015), indicating the high prevalence of the PRV variants in China. The PRV variant infections have caused significant economic impact to the swine production. In one swine herd alone, about 2600 newborn piglets and 200 sows died from the PRV variant infections in Henan Province, resulting in direct economic losses of at least one million Chinese yuan ($156,000) (Ye, 2013).

Vaccination/challenge experiments in sheep and pigs showed that the traditional Bartha-K61 vaccine could not provide complete protection against the current prevalent PRV variants in China (Wu et al., 2013; An et al., 2013; Yu et al., 2014; Wang et al., 2014; Luo et al., 2014). The analysis of genome-wide variations of the current isolates revealed extensive variations, including substitutions, insertions and/or deletions that occurred in most viral proteins compared with previous isolates worldwide, including Bartha-K61 strain (An et al., 2013; Yu et al., 2014; Luo et al., 2014; Ye et al., 2015), emphasizing that the current isolates epidemic in China are quite different from previous ones, which could also explain the low efficacy of the traditional Bartha-K61 vaccine.

To date no survey has been conducted to illustrate PR infections in wild boar in China and well-designed surveillance is needed to elucidate their possible impact on PR prevalence in domestic pigs.

3. Diagnosis

Specific and sensitive diagnostic methods are an indispensable part of the PR control and eradication programs.

Before 1980, diagnostic methods for PR included virus isolation, virus neutralization test (VNT) and experimental infection of animals. Since 1990s, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and real-time PCR have been widely used in rapid diagnosis of PR. At present, there is no reference diagnostic laboratory for PR and various commercial and non-profit institutions are involved in diagnostic services in China. Several commercially available ELISA kits, imported or homemade, have been licensed and widely used in China. Currently, there is no standardization for the diverse assays, resulting in inconsistent diagnostic results.

PCR and gB/gE-based ELISAs are routinely performed in most diagnostic laboratories in China. gB-ELISA is used for assessing the level of immunity induced by vaccination and gE-ELISA for the differentiation between infected and vaccinated pigs. Real-time PCR, VNT and virus isolation are feasible only in some professional laboratories to make a definite diagnosis.

Different assays, such as PCR, real-time PCR and loop-mediated isothermal amplification (LAMP), have been developed in China for detection of PRV or/and differentiation of wild-type PRV and gene-deleted vaccine strain (Fan et al., 2003; Zhang et al., 2008; Tian et al., 2009; Yang et al., 2011; Zheng et al., 2013), some of which have been routinely used in laboratories and/or in the field.

The currently available serological tests employed for DIVA strategies are also applicable for the PRV variant infections circulating in Chinese pig populations. Most commonly used PCR protocols are also capable of detecting these new variants. However, the current PCR methods cannot distinguish the variant strains from the vaccine or classical strains. The novel PCR and real-time PCR methods are being developed for the differential detection of PRV strains (Meng et al., unpublished data).

4. Control and prevention strategies

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. Twenty-seven animal diseases were listed in the program, including PR. 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), but a compulsory vaccination policy has not been carried out by far.

Table 2
Different types of genetic engineering vaccines against pseudorabies developed in China.

Types of vaccines	Examples	Features	References
Gene-deleted vaccines	- Single gene-deleted vaccine: TK gene-deleted - Double gene-deleted vaccine: TK/gG gene-deleted (HB-98 strain); gE/gI gene-deleted (PRV gE−/gI−/GFP+ strain) - Triple gene-deleted vaccine: gE/gI/TK gene-deleted (SA215 strain)	Attenuated virulence; excellent immunogenicity; allowing differentiation of infected from vaccinated animals (DIVA); most gene-deleted vaccines are attenuated in central nervous system and unlikely to lead to latent infections	(Wang et al., 1996; Zhu et al., 2004; Xu et al., 2004; He et al., 2005, 2006)
DNA vaccines	Plasmids pcDB, pcDC and pcDD expressing the gB, gC and gD genes of PRV; plasmid sgC-M284 expressing the gC gene of PRV	Stable expression of antigenic proteins of PRV; inducing PRV-specific neutralizing antibodies; allowing DIVA; high costs	(Hong et al., 2002; Xiao et al., 2004; Tong et al., 2006; Fan et al., 2009)
Subunit vaccines	A novel PRV subunit vaccine (PRV ISCOM)	High safety and stability; easy to store and transport; allowing DIVA; poor immunogenicity	(Ye et al., 2002)
Live virus-vectored vaccines	Recombination vaccinia viruses V-50A, V-50B and V-50C expressing the gp50 gene of PRV; PRV-vectored vaccines	Live-virus vectored vaccines providing protection against PR and other diseases; allowing DIVA	(Lou et al., 1996)

Wide-scale vaccination against PR with gene-deleted live vaccines (Bartha-K61 strain or its derivatives) has been carried out in China since early 1990s, and routine vaccination has been conducted in most farms except some backyard and remote villages (Tong and Chen, 1999). Bartha-K61 strain was attenuated after continued passages on porcine kidney cells, chicken embryos or embryo fibroblast cells (McFerran and Dow, 1970). This vaccine has been proved to be safe and efficacious (Yuan et al., 1983, 1985, 1986), and has played a critical role in the control of PR. Although vaccination has resulted in decreased PR outbreaks and reduced economic losses in China (Tong and Chen, 1999; Kong, 2000), PR has been endemic or sporadic in most regions of China in the past decades (Lou and Du, 1999; Zhang and Chen, 2008).

To completely control PR, some large-scale farms have implemented PRV eradication programs with the guidance and support of scientists and the government. Since 2000, PRV has been eradicated in some farms and regions by using DIVA strategy based on gE-deleted vaccines (mostly Bartha-K61 strain) and intense awareness and effective biosecurity measures. For most farmers, more attention is paid to vaccination rather than biosecurity measures. Due to the voluntary vaccination policy, China suffers from incomplete vaccination coverage, especially in remote villages and backyard farms.

Apart from the Bartha-K61 strain, various genetic engineering vaccines against PR have been developed, including gene-deleted vaccines (Wang et al., 1996; Zhu et al., 2004; Xu et al., 2004; He et al., 2005, 2006), DNA vaccines (Hong et al., 2002; Xiao et al., 2004; Tong et al., 2006; Fan et al., 2009), subunit vaccines (Ye et al., 2002) and live virus-vectored vaccines (Lou et al., 1996) (Table 2). The gE/gI/TK-gene-deleted PR vaccine SA215 strain was licensed in 2003, which represents the first genetic engineering animal vaccine in China (Zhu et al., 2004; Xu et al., 2004). The TK/gG-gene-deleted PR vaccine HB-98 strain was licensed in 2006, which was considered to be suitable for emergency vaccination in case of PR outbreaks (He et al., 2005, 2006). Both of the licensed vaccines have been used in the field.

Recent studies have shown that the traditional Bartha-K61 vaccine could not provide complete protection against the current prevalent PRV variants in Chinese swine population (Wu et al., 2013; An et al., 2013; Yu et al., 2014; Wang et al., 2014; Luo et al., 2014). Whether other currently available double- (HB-98 strain) or triple-gene-deleted vaccines (SA215 strain) could provide effective protection against the new PRV variants are unclear. To effectively control the infections by the recently emerged PRV variant, several novel vaccines based on current PRV variants have been developed, including the gE/gI-deleted (Wang et al., 2014) and gE/gI/TK-deleted vaccines (Cong et al., 2016) based on the PRV TJ strain, the gE/gI/TK-deleted vaccine based on the PRV HN1201 strain (Zhang et al., 2015), and the killed gE/gI-deleted vaccine based on the PRV ZJ01 strain (Gu et al., 2015). These vaccines have been reported to provide effective protection against the PRV variant infections, but the safety and efficacy of these candidate vaccines are still under evaluation.

5. Problems and challenges

PR is endemic in most regions of China, although gene-deleted live vaccines, such as Bartha-K61 strain, have been widely used for decades. PR is not under effective control due to the following problems.

5.1. Co-infections with other pathogens

With intensive pig production, it is common for swine to be concurrently infected with more than one pathogen. It was reported that PRV can co-infect with a variety of pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV), porcine circovirus type 2 (PCV2), porcine parvovirus, *Mycoplasma suis*,*Toxoplasma gondii*, *Actinobacillus pleuropneumoniae*, *Streptococcus spp*., etc., making the disease more complicated (Zhang and Chen, 2008; Wang et al., 2013).

5.2. Vaccination failure

In the field, vaccination failure or incomplete protection often occurred due to poor quality vaccines (insufficient antigens, or virus being inactivated due to cold-chain problems), improper immunization schedules (incorrect immunization timing, interval, dose and route), or interference by anti-PRV maternal antibodies, co-administration of other vaccines (such as PRRSV vaccine), diseases, drugs, etc. (Pomorska-Mól et al., 2010). Interference of PR vaccine by PRRS or CSF modified live vaccines has been demonstrated (Suradhat et al., 2001; Wang et al., 2013). In most backyard and small farms, almost no vaccination is carried out against any disease including PR, resulting in insufficient vaccination coverage.

5.3. Poor biosecurity

In China, pig farms especially small and medium-sized farms pay little attention to biosecurity, which makes PR control and eradication more difficult (Tong and Chen, 1999; Yang, 2015). Many farmers rely on vaccination rather than anti-epidemic and sanitary measures and they never perform regular surveillance to remove PRV carriers. Some farmers would mix the pigs imported from other farms with their own pigs immediately without any quarantine and surveillance, which greatly increases the chances of PRV introduction. Quite a few small pig farms are “open” to the outside without access control and daily disinfection of premises, personnel, visitors and vehicles, etc. In some farms, infected or dead pigs are not burned or buried and instead moved to the market, resulting in wide spread of the disease in China.

5.4. Persistent infections within a swine herd

Currently, almost no regional or national PR eradication program has been put into practice in China. Generally, pigs that survive from PRV infection become latently infected. If the infected pigs are not screened and removed from a swine herd, they will shed virus, resulting in infections of susceptible pigs and persistence or perpetuation of PRV in the herd. In fact, due to the limitation of costs and technologies, many Chinese farmers rarely or never do serological or virological surveillance, and gE-positive pigs go unnoticed in the herds.

5.5. Limited information on PRV epidemiology

No comprehensive epidemiological study of PR has been performed for domestic pigs in the last decades (Tong and Chen, 1999). Therefore, it is necessary to strengthen epidemiological survey to obtain comprehensive data of PR situation in China. On the other hand, there is little epidemiological information regarding PRV infections in wild boar populations in China. Considering the potential reservoir role of wild boar and their wide distribution and the high mobility in China, the regional and national surveillance of PRV in wild boar populations is urgently needed.

6. Prospects

For control and eradication of PR, safe and effective vaccines along with differential diagnostic methods are very important.

Considering the worsening situation of PRV infections in swine populations of China due to the emergence of the novel PRV variants, a task of top priority will be to develop a safe and efficacious vaccine against the currently prevalent PRV variants. Moreover, PRV can establish a persistent infection and conceal itself in the trigeminal ganglion following acute infection. Accordingly, there is an urgent need to develop more sensitive and convenient assays for identification and removal of the latent carriers.

A national compulsory vaccination has to be carried out together with comprehensive measures, including virological and serological surveillance and strict biosecurity procedures. Meanwhile, national-wide intensive surveillance of PR is needed. Little is known about the epidemiology of PRV in animals other than pigs and efforts should be made to determine the real status of PRV infections in these animals, especially in wild boars. We should learn more from the countries where PRV has been eradicated in commercial herds. In China, some scientists urged that the national eradication programs should be exercised (Chen, 2000), and the Chinese government has listed PR in the priority diseases to be controlled and demanded the eradication of the disease in breeding pigs in the National Middle-to-Long Term Plan for Animal Disease Control (The State Council of the People's Republic of China, 2012). It is possible to eradicate the disease in China, in view of gE-deletion vaccines and accompanying gE-ELISA kits being currently available.

7. Conclusion

This review summarized the epidemiology, diagnosis and vaccines of PR and discussed the problems for the prevention and control of PR in China. The emergent PRV variants pose a new challenge to the control of PR and eradication programs based on updated DIVA strategy are urgent to achieve the complete control of the disease in China.

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