Recent Proceedings on Prevalence and Pathogenesis of *Streptococcus suis*

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Abstract

Streptococcus suis (S. suis) is an important zoonotic pathogen that causes huge economic losses in the pig industry, as well as severe illness and even death in humans. The outbreak of human infection of *S. suis* in China in 2005 led to significant human morbidity and death, prompting an increase in global studies of *S. suis*. In recent years, important advances have been made regarding the etiology, genomics, excavation of virulence genes, and vaccine research in *S. suis*. A number of countries and regions have identified their predominantly serotypes. The development of genome sequencing technology has laid an important foundation for the study of pathogenic mechanisms. For example, 89K PAI was found in representative virulence strains in China, and several studies have been carried out to confirm multiple genes which carries are closely related to virulence. Also, the functions of some regulatory genes represented by the two-component signal transduction system have been analyzed. The development of inactivated vaccines, natural avirulent vaccines, gene-deletion attenuated vaccines, subunit vaccines, and glycoconjugate vaccines have greatly contributed to the prevention and control of the disease in the future. This article aims to summarize the research progress to provide directions for future research and the prevention of *S. suis*.

Introduction

Streptococcus suis is an important zoonotic pathogen that causes enormous economic losses in the global swine industry. It is also considered as an emerging zoonotic pathogen with the potential to cause a wide variety of diseases including septicemia, meningitis, pneumonia, endocarditis, and arthritis in both pigs and humans (Segura *et al*., 2014). A total of 29 serotypes of *S. suis* have been identified on the basis of various capsular antigens (Nomoto *et al*., 2015; Okura *et al*., 2016; Oh *et al*., 2017; Tohya *et al*., 2017), and some serotypes such as serotype 1, 2, 5, 9, 14, 16, 28, and 31 can infect humans (Lun *et al*., 2007; Gottschalk *et al*., 2010; Nutravong *et al*., 2014; Taniyama *et al*., 2016). Since the first human case of *S. suis* was reported in Denmark in 1968, over 1,000 cases have been reported in over 30 countries with intensive pig production. Most cases were European, Asian, and North American countries and regions. From the 29 serotypes of *S. suis*, serotype 2 is the most dominant serotype infecting humans, causing serious illness and streptococcal toxic shock syndrome (STSS), which was the main cause of death in the 2005 outbreak in Sichuan province of China (Feng *et al*., 2010).

Epidemiology

Numerous *S. suis* serotypes are pathogenic, but there are differences in the major serotypes prevalent in different countries (Oh *et al*., 2017). For example, in China, serotype 2 was the prevailing serotype, followed by serotype 1 and serotype 3 (Wei *et al*., 2009; Li *et al*., 2012). In Canada, serotype 3 was the most common serotype until 2009, but serotype 2 was the most common in 2011 (Gottschalk *et al*., 2013). In South Korea, serotypes 7 and 21 were most

commonly isolated from pigs during the 2010-2013 period (Gurung *et al*., 2015). In contrast, in Thailand, serotype 23 was the most common, followed by serotypes 9, 7, and 2 (Thongkamkoon *et al*., 2017).

In the last 20 years, *S. suis* has caused huge losses to the swine industry in many Asian countries, and is the most frequently isolated pathogenic bacterial pathogen in most pig farms in China. Two large outbreaks of *S. suis* in China have been identified since the 1990s. The first outbreak occurred in 1998 in the Jiangsu Province, in which 25 humans were infected, 14 died, and approximately 80,000 pigs were infected (Tang *et al*., 2006). Sick pigs showed a high fever, shortness of breath, head, neck, and abdominal bleeding. All 25 patients were adult males who had contact with dead or sick pigs, 16 of whom presented with STSS and nine presented with meningitis (Zhu *et al*., 2001). The second outbreak took place in 2005 in Sichuan Province, resulting in 215 human infections and 39 deaths (Yu *et al*., 2006). All infected people were mainly adult male farmers with recent exposure to carcasses of pigs which died of unknown causes or sick pigs. Three distinct clinical symptom settings have been observed, as 61 had STSS and the others were sepsis, meningitis, or both (Yu *et al*., 2006). Both outbreaks were caused by the sequence type 7 (ST7), an emerging highly virulent *S. suis* clone (Ye *et al*., 2008).

In the northern and northeastern regions of Thailand, *S. suis* is a major pathogen causing a public health concern (Kerdsin *et al*., 2011). The transmission of this pathogen in humans in these areas is due to specific ethnic practices, such as the consumption of raw pork and meat products (Fongcom *et al*., 2001). There

was a serious *S. suis* outbreak in northern Thailand in 2010, involving 171 human cases (Thongkamkoon *et al.*, 2017).

In Vietnam, *S. suis* type 2 is the most commonly detected organism causing acute bacterial meningitis in adults. Mai et al. studied 450 patients with suspected bacterial meningitis and showed *S. suis* was the etiologic pathogen in 151 of the patients (Mai *et al*., 2008). In 2007, Wertheim et al. identified *S. suis* from 43 meningitis patients from a national hospital in Hanoi (Wertheim *et al*., 2009). The risk factors of *S. suis* infection in humans were investigated by a different research group. Nghia et al. conducted a case–control study and determined that eating "high-risk" dishes popular in parts of Asia, occupational exposure to pigs and pig products, and preparation of pork in the presence of skin lesions are the most important risk factors associated with *S. suis* bacterial meningitis (Nghia *et al*., 2011).

Healthy pigs also carry *S. suis* on their tonsils which significantly impacts swine productivity, animal welfare, and human health. In collaboration with the University of Cambridge, we have investigated the prevalence and population biology of the *S. suis* isolates from the clinically healthy pig herds of China and the UK. This *S. suis* population showed a higher diversity than the disease-associated isolates on serotypes and sequence types. A significant effect of temperature is identified on carriage of *S. suis* on the tonsils of healthy pigs (Zou *et al*., 2018).

Genomics

Genome sequencing is extremely important for understanding the characteristics

of *S. suis*. To date, more than 1000 genome sequences of *S. suis* strains are available in public databases. For example, whole genomes of the two virulent serotype 2 strains 98HAH12 and 05ZYH33 were isolated from the outbreaks in China in 1998 and 2005, respectively, and were sequenced in 2007. By comparing these genomes, a novel 89-Kbpathogenicity island (PAI) was present in both strains, but not in P1/7 (Chen *et al*., 2007). Subsequent studies demonstrated that some genes in this 89K PAI are strongly associated with virulence and virulence regulation, such as the two two-component signal transduction systems (TCSTS) and six stand-alone transcriptional regulators (Li *et al*., 2008; Xu *et al*., 2018). Also, SalK/SalR and NisK/NisR of TCSTS have been confirmed to be related to virulence, resistance to PMN-mediated killing, and phagocytosis by macrophages (Li *et al*., 2008; Xu *et al*., 2014). Among the six stand-alone transcriptional regulators, TstS was obviously upregulated *in vivo* which promotes STSLS (Xu *et al*., 2018). In addition, a Type-IVC secretion system consisting of four genes has been identified, which can transfer secretion of plasmid type 89K to other bacteria. It is also related to the virulence, and knockout studies of the genes impaired its ability to trigger host immune response (Zhang *et al*., 2012b; Li *et al*., 2011c; Zhao *et al*., 2011; Yin *et al*., 2016).

In 2009, Holden et al. analyzed the whole-genome sequences of three *S. suis* strains, European P1/7 from pigs, and two strains from human cases: SC84 from China and BM407 from Vietnam (Holden *et al*., 2009). The P1/7 and SC84 chromosomes have a conserved structure, while the BM407 chromosome has a large inversion. Three 90-Kb regions that carry some drug resistance genes

were found in the two isolates from humans. Upon comparing the P1/7 chromosome with those of other *Streptococcus* species, *S. suis* was phylogenetically distinct, although many housekeeping genes, such as fatty acid metabolism genes, nucleotide biosynthesis genes, and macromolecule biosynthesis genes display the highest levels of conservation. In contrast, some mobile genetic elements in the P1/7 genome have the lowest conservation compared with those in other *Streptococcus* species.

In addition to serotype 2, the genomes of several other serotype strains were also resolved, which indicated that there is significant genomic diversity among the different strains of *S. suis* (Zhang *et al*., 2011b). What important information can we glean from the genomic differences among various types of *S. suis*? Weinert et al.studied the genetic differences among the systemic, respiratory, and non-clinical *S. suis* isolates from pigs and humans using high-quality clinical data and genome sequences, and found that the clinical *S. suis* isolates have a smaller genome size than the non-clinical ones, but are more likely to encode virulence factors. Human disease isolates are limited to a single-virulent population, originating in the 1920s when pig production was intensified. No consistent genomic differences are observed between pig and human isolates. High rates of recombination occur in the genomes of this bacterium, suggesting that virulence of *S. suis* may increase anywhere in the world (Weinert *et al*., 2015).

Virulence factors and pathogenesis

Virulence-associated factors have been identified, and they provide a likely

explanation as to why the strain is so highly virulent since the outbreak of *S. suis* serotype 2 in China in 2005 (Chen *et al*., 2007). Although some comprehensive studies have been performed, some results were contradictory due to differences in strain backgrounds and different animal models of infectious disease in the evaluation of virulence (Auger *et al*., 2017; Segura *et al*., 2017).

The polysaccharide capsule (CPS) is considered to be essential for bacterial virulence by inhibiting the signaling pathways involved in phagocytosis (Smith *et al*., 1999; Segura *et al*., 2004; Chabot-Roy *et al*., 2006; Lecours *et al*., 2011) and by the evasion of neutrophil extracellular traps (NETs) (Zhao *et al*., 2015). However, a few avirulent *S. suis* strains also contain CPS *(Berthelot-Herault et al*., 2001; Berthelot-Herault *et al*., 2005), indicating that the virulence is multifactorial and does not exclusively rely on CPS structure. Furthermore, CPS structure may interfere with some important virulence properties, such as the inhibition of bacterial adherence, invasion of host cells (Benga *et al*., 2004; Tenenbaum *et al*., 2009; Segura *et al*., 2016), and biofilm formation (Tanabe *et al*., 2010). Interestingly, the CPS structure could be retrieved after *in vivo* passage of a non-encapsulated *S. suis* (Auger *et al*., 2016), indicating that CPS structure could be regulated *in vivo,* and that its contribution to virulence is complicated. In fact, CPS structure could be regulated by several factors, such as the availability of glucose or other carbohydrates, pH, and temperature (Smith *et al*., 2001; Wu *et al*., 2011). In particular, glucose availability could also regulate CPS gene expression through catabolite control protein A (CcpA) *(Willenborg et al*., 2011), which is also why virulence-associated factor HP0197 contributed to the virulence through the regulation of CCPA activity (Zhang *et al*., 2012a; Yuan

et al., 2013). Together, these studies indicate that CPS is an important virulence factor in the behavior of *S. suis*.

Suilysin (SLY) is a member of the pore-forming cholesterol-dependent cytolysin family of toxins (Palmer, 2001; Xu *et al*., 2010). Examination of the crystal structure further confirmed the cytotoxic properties of SLY (Xu *et al*., 2010). For a long time, SLY was thought to contribute to meningitis (Takeuchi *et al*., 2014), supported by the fact that SLY can remodel the cytoskeleton of human brain microvascular endothelial cells by activating RhoA and Rac1 GTPase (Lv *et al*., 2014) and can increase vascular permeability through the blood-brain barrier (Chen *et al*., 2016; Liu *et al*., 2017). The toxic effect of SLY benefits the adherence of the pathogen to airway cells, which subsequently causes loss and apoptosis of ciliated cells for invasion (Meng *et al*., 2016). Therefore, SLY plays an important role in the invasion of host cells and induction of cell death (Allen *et al*., 2001; Lun *et al*., 2003; Vanier *et al*., 2004; Ferrando *et al*., 2015; Meng *et al*., 2016). In addition, SLY is involved in inflammatory responses through TLR4 (Lecours *et al*., 2011; Bi *et al*., 2015; Zhang *et al*., 2016a; Zhang *et al*., 2016b), and SLY induced platelet aggregation and also platelet-neutrophil complexes formation through the pore-dependent Ca2+ influx (Zhang *et al*., 2016a; Zhang *et al*., 2016b). It has been reported that SLY is partially involved in cytokine release and also contributes to bacterial escape of opsonophagocytosis (Lecours *et al*., 2011). Therefore, SLY is also an important virulence factor (Allen *et al*., 2001), which has been the target of drugs in previous studies (Li *et al*., 2017a; Zhang *et al*., 2018b). Notably, the expression level of SLY seems to be associated with the virulence of *S. suis* strains (He *et al*., 2014; Takeuchi *et al*.,

2014). Nonetheless, many virulent strains, especially those from North America, do not produce SLY, which suggests that SLY is not a requirement for the virulence of *S. suis* (Berthelot-Herault *et al*., 2000; Fittipaldi *et al*., 2011).

Muramidase-released protein (MRP) and extracellular factor protein were originally identified to be associated with the virulence of *S. suis* (Vecht *et al*., 1991). Previous work has shown that MRP binds to fibrinogen and facilitates attachment to and traversal of human brain microvascular endothelial cells by increasing transendothelial cell permeability, thereby promoting the development of *Streptococcus suis* meningitis (Wang *et al*., 2015). However, a subsequent study indicated that the virulence of MRP-deficient mutant was not decreased in comparison with that of the parental strain (Baums and Valentin-Weigand, 2009), suggesting that MRP is required for the virulence of some strains.

Factor H (FH) is an important negative regulator of the alternative complement pathway. *S. suis* can secrete FH-binding protein (FHBP) to bind the host complement component C3 and FH that reduces opsonophagocytosis. FHBP can also enhance the adherence to and invasion of host cells (Li *et al*., 2016), thereby contributing to the virulence (Pian *et al*., 2012). In addition, globotriaosylceramide (Gb3) has been identified as the receptor of FHBP, which contributes to *S. suis* infection-induced activation of myosin light chain 2 through Rho/ROCK signaling in hCMEC/D3 cells. This also contributes to the traversal of *S. suis* across the human blood-brain barrier. However, more comprehensive studies are required to determine the role of FHBP in unlocking the blood-brain barrier (Kong *et al*., 2017) and accessing the central nervous system (Auger and

Gottschalk, 2017). Nonetheless, a double mutant-lacking FHBP and FHBP was similarly phagocytosed by human macrophages and killed by pig blood when compared to the wild-type strain. This suggests that the recruitment of factor H to the *S. suis* cell surface is multifactorial and redundant (Roy *et al*., 2016). In fact, more factor H-binding proteins were identified to be required for the virulence of the bacteria (Li *et al*., 2017c).

Oxidative stress is an ubiquitous challenge faced by pathogens, and stress response systems can play an important role in the virulence of pathogenic bacteria (Requena, 2012). *S. suis* has evolved certain strategies to tolerate oxidative stress, which in turn contributes to its virulence. For example, *S. suis* can express NADH oxidase (Zheng *et al*., 2017) and superoxide dismutase (Fang *et al*., 2015) to scavenge reactive oxygen species. The improved resistance to reactive oxygen species is also a factor through which hsdS *(Xu et al*., 2017a), a serine/threonine phosphatase 1 (Fang *et al*., 2017), heme-binding protein SntA (Wan *et al*., 2017), FNR-like protein (Willenborg *et al*., 2016), and PnuC (Li *et al*., 2018a) contribute to *S. suis* virulence.

In addition to receptor-mediated capture and phagocytosis, neutrophils can also attack pathogens by an antimicrobial mechanism called NET-mediated bacterial killing, which plays an important role in the clearance of *S. suis in vivo (Zhao et al*., 2016). *S. suis* can induce the formation of NETs both *in vitro* and *in vivo* (de Buhr *et al*., 2014; de Buhr *et al*., 2017). Interestingly, the bacterium has evolved certain strategies to evade this killing, and specifically can inhibit the formation of NETs through biofilms (Ma *et al*., 2017), resist the NET-mediated trapping

through CPS structure (Zhao *et al*., 2015), and degrade the NET structure to resist killing through the secretion of DNase SsnA (de Buhr *et al*., 2014; de Buhr *et al*., 2015).

Interestingly, the *S. suis* ST7 strain contains an 89-Kb genomic island, which includes genes encoding the components of a type IV secretion system involved in the pathogenesis of *S. suis* (Li *et al*., 2011c; Zhao *et al*., 2011). Subtilisin-like protease-1 secreted through the type IV secretion system contributes to the high virulence of *Streptococcus suis* 2 (Yin *et al*., 2016), and a novel PPIase molecule, SP1, has been shown to interact with a component of innate immunity, peptidoglycan recognition protein (PGLYRP-1), and to perturb the PGLYRP-1-mediated bacteriostatic effect by interacting with the protein PGLYRP-1 (Wang *et al*., 2017b).

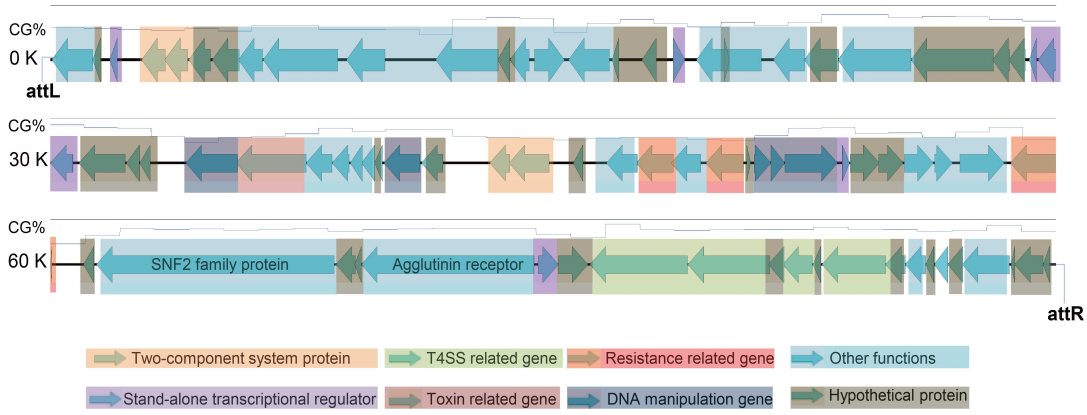

Figure 1. The structure schematic model of 89K PAI . Important elements are presented in different colors.

In addition to the identification of these novel virulence-associated factors, characterization of the pathogenic roles of virulence factor proteins of other pathogenic bacteria homologous to those in *S. suis* was also performed. Immunoglobulin A protease (IgAP) was identified and confirmed to be involved in the virulence of *S. suis* (Zhang *et al*., 2010; Zhang *et al*., 2011a), although the identified IgA protease is part of the zinc metalloprotease family and may have other functions besides this (Bek-Thomsen *et al*., 2012). Interestingly, the findings also showed that Mac is an important marker of virulence for other *Streptococcus* strains but is not essential for *S. suis* virulence in strain P1/7 in natural, healthy hosts without specific IgM. Moreover, the immunogenicity of Mac does not appear to correlate with its significance for virulence (Xiao *et al*., 2017a). The IgM degrading enzyme could reduce the amount of IgM bound to the bacterial surface, which is a novel complement evasion mechanism (Rungelrath *et al*., 2018).

A few regulators were confirmed to be involved in the virulence of *S. suis* in addition to these virulence-associated factors. Fifteen groups of two-component systems including one orphan response regulator were predicted in two virulent *S. suis* 2 strains (98HAH12 and 05ZYH33), and nine of them have been confirmed to regulate the virulence (Li *et al*., 2008; Li *et al*., 2011a; Xu *et al*., 2014; Yuan *et al*., 2017; Chang *et al*., 2018; Velikova, 2018; Zhong *et al*., 2018; Zheng *et al*., 2018a). In addition to the two-component system, there are other Stand-Alone Transcriptional Regulator that regulate the virulence of *S. suis*. For example, the Rgg regulator regulates genes associated with non-glucose carbohydrate metabolism, DNA recombination, protein biosynthesis, and others

affecting *S. suis* 2 metabolism and virulence (Zheng *et al.*, 2011). Two Spx ortholog regulators, SpxA1 and SpxA2, have been associated with multiple stress tolerance and virulence of *S. suis* 2 (Zheng *et al.*, 2014). Also, TstS regulator located at 89K PAI has been identified to regulate the virulence of *S. suis* 2 and to stimulate the release of cytokines (Xu *et al.*, 2018).

In addition to the regulation of CPS synthesis by CcpA, *S. suis* can also control manganese homeostasis by MntE, which explains why MntE is involved in the virulence of *S. suis* (Xu *et a*l., 2017b). Also, (p)ppGpp synthetases can regulate the expression of virulence-related genes involved in morphology and virulence (Zhu *et al.*, 2016). In addition, MsmK is an ATPase that contributes to the utilization of multiple carbohydrates and host colonization of *S. suis*. Additionally, STK and CodY have been confirmed to be central regulators of its virulence (Tan *et al.,* 2015; Tan *et al.*, 2017), and GidA, a tRNA modification enzyme, was confirmed to contribute to its growth and virulence (Gao *et al.*, 2016). In addition to these regulators, small RNA rss04 was also reported to regulate virulence by regulating capsule synthesis and inducing biofilm formation, as determined in a mouse infection model (Xiao *et al.*, 2017b).

It has been recognized that the identification and characterization of novel virulence-associated factors are effective ways of understanding the pathogenesis of *S. suis*. Additional studies have also identified several other virulence-associated factors, such as Formate-tetrahydrofolate ligase (Zheng *et al.*, 2016), HP1330 (Zhang *et al.*, 2017b), SadP (Ferrando *et al.*, 2017), SBP2 (Yu *et al.*, 2016), vapE (Ji *et al.*, 2016), oligopeptide-binding protein (OppA)

(Zheng *et al*., 2018b), SssP1 (Zhang *et al*., 2018a), lysM (Wu *et al*., 2016), and Dnase (Haas *et al*., 2014). However, in line with the concern of Segura et al. (YEAR) that the identified factors might be strain-specific (Segura *et al*., 2017), the identification of critical virulent factors requires more comprehensive studies.

These identified novel factors strengthen our understanding about pathogenesis, but the studies that mainly focused on these virulent factors could not fully elucidate the primary pathogenic mechanism of *S. suis.* As any infectious disease is the result of the interaction of pathogen and host, more concern should be focused on the host response to infections in further studies.

Vaccine development

The development of vaccines against *S. suis* is an optimal approach to controlling its infection. The first vaccine developed for this purpose was based on all of its bacterins. An early experimental study reported that formalin-killed pathogenic SS2 could stimulate a complete protective response against homologous challenge in piglets (Holt *et al*., 1990). However, a field study reported that the immunization of pigs with a commercial bacterin vaccine failed to protect against nursery mortality (Torremorell *et al*., 1997). Aside from the use of whole *S. suis* inactivated vaccine, the inactive method (Holt *et al*., 1990; Pallares *et al*., 2004), very high doses of bacterin (Holt *et al*., 1990), and the adjuvant used in the formulation (Pallares *et al*., 2004) are important for the protective efficacy of the vaccine. In fact, Chinese swine farms began to use SS2 whole bacterins vaccine, which was prepared using high doses of bacterin in oil adjuvant and conferred a detectable level of CPS2 antibodies, as determined

using ELISA (Jin *et al*., 2006). Subsequently, the prevalence of serotype 2 has decreased significantly, although it is still quite prevalent in China. However, the protection conferred by all bacterins is either serotype or strain-dependent (Pallares *et al*., 2004). There are several serotypes that are prevalent in swine farms in different countries, including China (Wei *et al*., 2009), for which the development of novel vaccines is required.

The development of a live avirulent vaccine may provide better protective efficacy, as strong humoral immunity is induced following challenges with live bacteria (Buttner *et al*., 2012). Temperature-sensitive mutants of *S. suis* were tested as vaccines and conferred protection only against homologous challenge in mice (Kebede *et al*., 1990). Developed non-encapsulated isogenic mutants would be avirulent and may provide better cross-protection because CPS is serotype-specific. A live vaccine was developed based on a non-encapsulated serotype 2 mutant; it induced partial protection only against mortality and failed to prevent the development of clinical signs in pigs challenged with the wild-type strain (Wisselink *et al*., 2002). However, another study indicated that pigs vaccinated with a non-encapsulated mutant exhibited a survival rate of 100% and presented only minor clinical signs after challenge in the wild-type strain (Fittipaldi *et al*., 2007). The protection against different serotypes should be confirmed by further studies. Besides disruption of the CPS gene, disruption of other virulence-associated genes would also be promising for the development of an attenuated vaccine. For example, a double-deletion mutant (SsPep/SsPspC–/–) (Hu *et al*., 2015), a five-deletion mutant (sly, scpA, ssnA, FHBP, and ssads) (Li *et al*., 2018b), and a mutant with ssnA deletion (Li *et al*.,

2017b) have offered good protection. In contrast, the isogenic OFS mutant failed to induce opsonizing antibodies and protection (Kock *et al*., 2009). In addition to the constructed isogenic mutants, isolated native avirulent strains could also be used as strains for vaccines (Quessy *et al*., 1994; Quessy *et al*., 1995; Busque *et al*., 1997). For example, Yao et al used an avirulent strain from healthy pigs for a vaccine, which induced complete protection against SS2 infection (Yao *et al*., 2015; Wang *et al*., 2017a).

Another strategy to develop a universal vaccine is based on immunogenic proteins, which could confer cross-protection. For example, subunit vaccines using suilysin (Jacobs *et al*., 1996; Du *et al*., 2013) or MRP and extracellular protein factors (Wisselink *et al*., 2001) have been shown to protect pigs against homologous and heterologous strains. However, in some geographical regions, their application is hindered by the presence of a substantial number of virulent strains that do not express these proteins.

SAO (Li *et al*., 2006; Li *et al*., 2007; Hsueh *et al*., 2017) and the 38-kDa protein (Okwumabua and Chinnapapakkagari, 2005) were identified as vaccine candidate antigens. However, it is necessary to identify more immunogenic proteins based on the strategy of developing a universal vaccine. To date, several technologies have been applied to identify novel immunogenic proteins (Zhang and Lu, 2007a; b; Jing *et al*., 2008; Zhang *et al*., 2008; Gu *et al*., 2009; Liu *et al*., 2009; Mandanici *et al*., 2010; Gomez-Gascon *et al*., 2012) such as enolase (Esgleas *et al*., 2008; Esgleas *et al*., 2009; Feng *et al*., 2009; Zhang *et al*., 2009b), 6-phosphogluconatedehydrogenase (Tan *et al*., 2008; Tan *et al*.,

2009), SsuiDRAFT 0103 (Aranda *et al*., 2008), cation-regulated proteins (Aranda *et al*., 2009), HP0197 (Zhang *et al*., 2009a), RTX family exoprotein A, epidermal surface antigen, immunoglobulin G-binding protein (Liu *et al*., 2009), SAT/HP0272 (Chen *et al*., 2010; Mandanici *et al*., 2010), pilus subunit (Garibaldi *et al*., 2010), SsPepO (Li *et al*., 2011b), HP0245 (Li *et al*., 2011d), SsnA (Gomez-Gascon *et al*., 2014; Gomez-Gascon *et al*., 2016), Lmb (Zhang *et al*., 2014), AbpB (Huang *et al*., 2015), IdeSsuis (Seele *et al*., 2015), Sbp (Zhou *et al*., 2015), the type II histidine triad protein HtpsC (Li *et al*., 2015), and IgA protease (Fu et *al*., 2016). However, it should be noted that there are contradictory results about the protective efficacy of some antigens. For example, enolase was confirmed to be both protective (Feng *et al*., 2009; Zhang *et al*., 2009b) and nonprotective (Esgleas *et al*., 2009). This may have been due to different adjuvants used in the formation of the vaccine. Therefore, the development of technology for evaluating the protection conferred by a subunit vaccine is important, and an assay based on porcine dendritic cells has been developed to assess the immunological behavior of vaccines and the polarizing effect of adjuvants (Martelet *et al*., 2017).

CPS is the most external bacterial layer in contact with the host, and the antibodies are highly opsonizing and protective *(Charland et al., 1997; Calzas et al*., 2017). However, free CPS is nonimmunogenic, and carbohydrate-based vaccines (glycoconjugate vaccines) were successfully discovered for many encapsulated pathogens, such as *Haemophilus influenzae* (Hiberix), *Neisseria meningitidis* (MenACWY), and *Streptococcus pneumoniae* (PCV13) (Roy and Shiao, 2011; Bottomley *et al*., 2012). The structure and method of biosynthesis

of capsular polysaccharides of serotypes 2 and 1/2 have now been characterized (Van Calsteren *et al*., 2016), and a serotype 2 capsular polysaccharide glycoconjugate vaccine was confirmed to induce potent IgM and isotype-switched IgGs in mice and pigs, yielding functional activity in vitro and protection against lethal challenge in vivo. These are all features of a T-dependent response (Goyette-Desjardins *et al*., 2016), which suggests the possibility of developing polyvalent glycoconjugate vaccines against *S. suis* infection.

Conclusions and future perspectives

With the rapid development of genome sequencing technology and the progress of molecular biology technology, important progress has been made in the understanding of the pathogenesis of *S. suis*. Despite these advances, there are still many limitations. Future studies on *S. suis* should include the following aspects: continue etiology work to elucidate changes in prevalent serotypes, focus on pathogen-environment-host interactions, and develop novel multivalent vaccines and diagnostic reagents.

Acknowledgements

This work was supported by grants from the National Key R&D Program of China (2017YFD0500200), the National Natural Science Foundation of China (31772714, 31572501), the Hubei Province Natural Science Foundation for Innovative Research Groups (2016CFA015), and the earmarked fund for China Agriculture Research System(CARS-35).

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