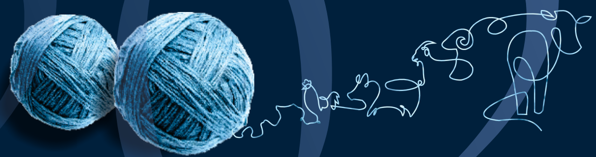
14th INTERNATIONAL SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

2023



PROCEEDINGS

4 - 6 October 2023, Belgrade, Serbia

Institute for Animal Husbandry

Belgrade - Zemun, SERBIA

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CONTENT

POSTER SECTION

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STREPTOCOCCUS SUIS, TWO-FACED GAME CHANGER

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Invited paper

Abstract: *Streptococcus suis* infection is one of the major health problems in the swine industry worldwide. During the last decade, the number of reported human cases due to *S. suis* has dramatically increased, and while most sporadic human cases of infection appear to be due to close occupational contact with pigs/pork products. *S. suis* infection is considered to be multifactorial, with transition from subclinical to clinical that depends on many factors. These factors can be divided in two groups, host-based and external factors. Pathogenesis of *S. suis* infection can be divided into 4 phases: adherence to and colonisation of mucosal and epithelial surfaces, invasion into deeper tissues and entering the bloodstream, crossing blood-brain barrier and inflammation. *S. suis* virulence-associated factors are divided into the following 4 groups: surface/secreted elements, enzymes (such as including proteases), transcription factors and regulatory systems and other factors (such as transporting and secreting systems). Therefore significant research support is needed to obtain a vaccine as a valuable and universal protection against disease caused by *S. suis* strains and thus national and international support will be crucial for the aim many researchers hope for.

Key words: *Streptococcus suis*, pigs, commensal, pathogen, virulence factors

Introduction

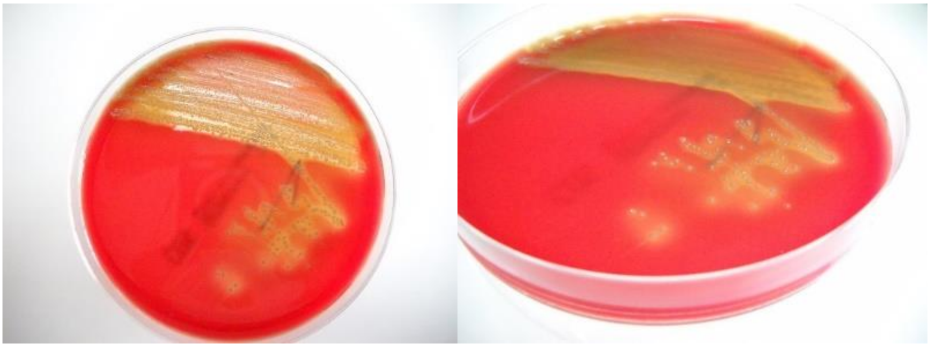
Streptococcus (*S. suis*) is a commensal of the swine respiratory system, in particular of the tonsils and nasal cavities, but it can also cause serious infections with high mortality rate.

Streptococcus suis infection is one of the major health problems in the swine industry worldwide. This pathogen is the most prominent cause of meningitis and septicemia in the porcine industry, however, other pathological conditions have also been described, such as arthritis, endocarditis, pneumonia, and septicemia with sudden death.

S. suis is primarily considered a major swine pathogen, but it has been increasingly isolated from a wide range of mammalian species, birds and even fish species. These findings suggest the existence of complex epidemiological patterns of the infection, since other animal species might also be a source of swine infection (*Gottschalk et al., 2010b).* Human *S. suis* infections have usually been considered sporadic (*Arends and Zanen, 1988*). During the last decade, the number of reported human cases due to *S. suis* has dramatically increased, and while most sporadic human cases of infection appear to be due to close occupational contact with pigs/pork products, particularly in Western countries (farmers, veterinarians, butchers, food processing workers, etc.), two epidemics were recorded in China in 1998 and 2005 (*Goyette-Desjardins et al., 2014*). However, the important outbreak in China that occurred in 2005 and that affected more than 200 people with a mortality rate of nearly 20% changed the perspective on the threat of *S. suis* to human health.

Microorganism characteristics, number and distribution of serotypes

Streptococcus suis is a facultative anaerobic, Gram-positive coccoid bacterium that has the ability of capsule synthesis and secretes heamolysin. The cell wall antigenic components of *S. suis* are similar to those displayed by group D streptococci (*Stanojkovic et al., 2012*). However, *S. suis* is not genetically associated with group D streptococci *(Kilpper-Balz and Schleifer, 1987).* The organism grows well on media usually used for isolation of streptococci, most frequently sheep blood agar, and forms glistening, round, slightly grey alpha haemolytic colonies (Picture 1). *S. suis* has vary variable biochemical properties (*Stanojković et al., 2014*) and thus must be confirmed by serotyping.



Picture 1. Alpha haemolytic colonies of *Streptococcus suis* on CNA 5 % sheep blood agar (*Stanojković et al., 2012*)

Previously, *S. suis* had been classified into 35 serotypes (serotype 1/2, and 1–34) (*Higgins and Gottschalk, 1995*) and then reduced to 33 serotypes because serotypes 32 and 34 were determined to be *Streptococcus orisratti*, streptococci that can be often isolated from rats (*Hill et al., 2005*). More recently, it was proposed to remove serotypes 20, 22, 26 and 33 from the *Streptococcus suis* taxon (*Tien et al., 2013*). Hence, it is currently considered that there are 29 true *Streptococcus suis* serotypes.

During the last 12 years, more than 4500 serologically confirmed strains recovered from diseased pigs have been reported. Globally, the most dominant serotypes isolates from clinical cases in pigs are serotypes 2, 9, 3, 1/2 and 7, while 15.5% were so called non-typable strains. However, there is clear geographical distribution of serotypes.

Goyette-Desjardins et al. (2014) summarize strain prevalence in Europe and America. In Canada the most prevalent serotype is serotype 2, while in United States serotype 3 is the most prevalent. In these countries there is only a slight difference in percentages of prevalent strains, demonstrating similar distribution of serotypes when data from Canada and the USA are combined. Both, serotypes 2 and 3 are the most prevalent from diseased pigs with 24.3% and 21.0% prevalence respectively, followed by serotypes 1/2, 8 and 7 (*Goyette-Desjardins et al., 2014*). This can be explained by easy and free movement of animals from United states to Canada and vice versa. In South America, all results came from Brasil, stating that serotype 2 is the most prevalent with 57.6% reported cases followed by serotypes 1/2, 14, 7 and 9. In Asia, the majority of results regarding serotype affiliation came from China and South Korea. In China the most prevalent serotypes detected in infected pigs are, in decreasing order of prevalence, serotypes 2, 3 4, 7, and 8. On the contrary, in South Korea serotype 2 had a prevalence of only 8.3%, the same as serotypes 8 and 33 while the most dominant were serotypes 3 and 4 with 29.2|% and 20.8% respectively, while serotypes 16 and 22 had distribution of 4.1%. Other Asian countries reported many human cases of disease but strains isolated from pigs only refer to slaughterhouses and healthy pigs. Similarly, in Japan there have been 10 human *S. suis* cases reported but studies on the distribution of isolates from ill pigs have not been published ately and all of the research dates before 1987. In Cambodia, Philippines, Laos and Singapore, human cases were diagnosed recently but there are no data avilable on the epidemiology of *S. suis* infections in pigs.

In Europe, the largest number of *S. suis* serotypes isolated from clinically ill pigs belongs to serotypes 1 to 8 *(Reams et al., 1996; Higins and Gottschalk, 2005)*. Most of the *S. suis* serotype distribution reports date before year 2000. *S. suis* serotype 2 was the most common in clinical cases in Italy, France and Spain, whreas serotype 9 was more frequent in the Netherlands, Germany and Belgium. Recent conducted reaserch on serotype distribution in Spain suggest that serotype 2 is no longer the most prevalent serotype, and that serotype 9 is the one most frequently isolated from diseased pigs. Behind serotype 9 is serotype 2, followed by serotypes 7, 8 and 3 (*Luque et al., 2010*). In Netherlands, serotype 9 was the most prevalent in data collected between 2002-2007 followed by serotypes 2, 7, 1 and 4. Contrary to the fact that serotype 9 becomes most prevalent in some countries, there were no human cases reported that were associated with this serotype. In Belgium and United Kingdom, serotype 1 was the predominant in ill pigs while in Denmark serotype 7 was the most frequent one. In Southern Europe, serotype distribution was done in Serbia where serotype 2 was the only serotype found in piglets that had clinical symptoms of meningitis (*Stanojkovic et al., 2015*). Beside that various *S. suis* serotypes were found in healthy animals (*Stanojković, 2012*).

S. suis infection in pigs

The natural habitat of *S. suis* is the upper respiratory tract of pigs, more particularly the tonsils and nasal cavities, but also the genital and digestive tracts (*Goyette-Desjardins et al., 2014*). Almost 100% of pig farms worldwide have carrier animals, and that puts *S. suis* as one of the most important bacterial pig pathogens with quite established infection patterns.

Transmission of *S. suis* among animals is considered to be mainly through the respiratory route. Indeed, investigation of presence of alpha haemolytic streptococci, enterococci and streptococci-like bacteria in tonsil and nose swabs of clinically healthy pigs in one research (*Stanojković et al., 2012*) showed that most species belonged to *S. suis (*64%*).*

The sow is also a source of infection. Gilts and sows may harbor *S. suis* in the uterus or vagina, but no male reproductive organs have been shown to be infected. Piglets born to sows with uterine or vaginal *S. suis* infections become infected at birth, before birth, or soon after birth (*Robertson and Blackmore, 1989*), but mostly when passing through the birth canal.

Transmission of virulent strains between herds usually occurs by the movement of healthy carrier animals. The introduction of carrier pigs harboring virulent strains (breeding gilts, boars, weaners) into a noninfected recipient herd may result in the subsequent onset of disease in weaners and/or growing pigs (*Higgins and Gottschalk, 2005*). Horizontal transmission is important especially during outbreaks when diseased animals shed higher numbers of bacteria, increasing transmission by direct contact or aerosol (*Cloutier et al., 2003*).

S. suis type is an important contaminant of feces, dust and water. In water, the organism survives for 10 min at 60°C and for 2 h at 50°C. At 48°C, *S. suis* can survive in carcasses for 6 weeks (*Clifton-Hadley et al., 1986).* At 0°C, the organism can survive for 1 month in dust and for over 3 months in feces, whereas at 25°C, it can survive for 24 h in dust and for 8 days in feces. *Dee and Corey (1993)* have also been shown that to transmission of *S. suis* strains can be through fomites, such as manure-covered work boots and needles. *S. suis* can be inactivated using many disinfectants, such as diluted bleach. Organic matter reduces effectiveness of chemical disinfectants and should be completely removed with thorough washing prior to application. Even though *S.suis* survives in water up to 2 hours at 50°C but only 10 minutes at 60°C, use of heated pressure washers compared with non-heated is of limited value since water cools rapidly on surfaces negating potential benefit (*Clifton-Hadley and Enright 1984*).

Vectors of *S. suis* can play a role in disease transmission. Houseflies can carry *S. suis* strains for 2 for 5 days, and have been shown to easily transmit the disease migrating between farms (*Enright et al., 1987*). Mice can be experimentally infected orally or intranasally with *S. suis* type 2, and the transfer of organisms from orally infected mice to uninoculated mice has been established (*Williams et al., 1988; Robertson and Blackmore, 1990*). Transmission of disease between mice and pigs is believed to occur (*Williams et al., 1988*).

All categories of pigs can be affected by the disease caused by *S. suis*, including suckling piglets, older piglets and fatteners. *S. suis* carriage rates may vary between herds and can range from 0% to up to 80-100% *(Amass et al., 1997)*. More than one serotype of *S. suis* often colonizes individual pigs. In one study, 31% of pigs had only one serotype of *S.suis* in their nasal cavities, 38% had two or three serotypes, and 6% had more than four serotypes *(Monter Flores et al.,* *1993)*.According to *Silvonen et al. (1988)* even if all the pigs in the herd are infected with some strains of *S. suis* clinically apparent disease varies and is usually below 5%. The prevalence of and the morbidity and mortality from *S. suis* vary among herds.

Even when the pig carrier rate is near 100%, the incidence of the disease varies from period to period and is usually less than 5% *(Clifton-Hadley et al., 1986)*. Clinical signs can vary between herds, depending on the pathogenesis of the disease. Pigs with per acute *S. suis* infection may be found dead with no previously noticed signs of disease or die within hours of the onset of clinical signs. In the acute form of the disease, clinical signs may include fever (up to 42°C), depression, anorexia and lassitude, followed by one or more of the following: ataxia, incoordination, tremors, opisthotonus, blindness, loss of hearing, paddling, paralysis, dyspnea, convulsions, nystagmus, arthritis, lameness, erythema, and/or abortion *(Staats et al., 1997)*. So, we can conclude that meningitis is the major feature of *S. suis* infection in pigs but other organs (joints, heart, lungs, reproductive organs etc.) can also be affected.

S. suis infection in humans

Different from pigs infection, the main route of entry of *S. suis* in humans is thought to be through contact of cutaneous lesions, most usually on the hands and arms, with contaminated animals, carcasses or meat, although in some cases, no wound was detected; bacteria may colonize the nasopharynx, as observed in swine; and the gastrointestinal tract, as suggested by diarrhea as a prodromal symptom *(Fongcom et al., 2001; Wertheim et al., 2009)*. The outbreak in China in 2005 caused by *S. suis* affected more than 200 people, with almost 20% mortality rate. This epidemic has completely changed the perception of the danger which this pathogen presents to human health *(Stanojkovic et al., 2014)*. Period of incubation ranges from just a few hours to few days *(Fongcom et al., 2001)*. Just like in pigs *S. suis* produces meningitis as the main feature of disease but cases of endocarditis, pneumonia, peritonitis, arthritis and other less common clinical signs can be seen as the part of generalized septicemia *(Arends and Zanen, 1988)*. Also, there have been described per acute infections related to this pathogen which were usually in the form of streptococcal toxic shock-like syndrome (STSLS) with almost 20% death cases. In Western countries, *S. suis* disease has been considered a rare event in humans. Most cases of human infection are related to close contact with meat or live animals: pig farmers, abattoir workers, persons transporting pork, meat inspectors, butchers, and veterinarian practitioners *(Tang et al., 2006)*.

According to *Hoa et al. (2011)* slaughterhouse pigs are a major reservoir of *Streptococcus suis* serotype 2 capable of causing human infection. *Cheung et al.*

(2008) examined 78 samples of raw pork lean meat from retail markets and wet markets and determined that *S. suis* can be found in every sample although in different levels (MPN/g). Similar meat prevalence in Serbia *(Stanojković et al., 2016)* showed serotype 2 was the most isolated serotype from fresh pork with 46,1 % of isolated *S. suis* serotypes followed by serotype 9, 7, 3, 1 and 4. Slaughtered pigs had similar prevalence of *S. suis* strains just like those data reported for clinically ill pigs.

Stanojkovic et al. (2016) found that there was a significant difference in the presence of *S. suis* strains on the basis of sample collected. In above mentioned authors research hog head was highly contaminated with *S. suis* serotype 2 strains (prevalence of 25%). This result is maybe expected since *S. suis* is normal inhabitant of respiratory system such as tonsils, and also slaughtered pigs are held in that kind of position that allows water to spread bacteria from hind part of the body to the head. Same authors found that presence of *Streptococcus suis* serotype 2 in liver, kidneys, shoulder, ham, loin and belly was 20%, 12%, 5%, 5%, 5%, and 10% respectively. Have found overall prevalence of *S. suis* serotype 2 in pork of 12.8%. Same authors mentioned that prevalence of *S. suis* serotype 2 in fresh meat was 10.8% but it was not clear referring to the part of the body that fresh meat was taken from. These authors detected 15.4% prevalence of *S. suis* serotype 2 in liver and other offal and demonstrated that *S. suis* accumulates in the kidney during *S. suis* infection.

It can be concluded that processing and consuming of uncooked or partially cooked pork meat in Asian countries is major risk factor for infection.

Different from Asian countries infected persons in Western countries are usually adult males and this can be readily explained, since many acquire the disease following occupational exposure to pigs or pork products. Affected humans had usually close contact with pigs or meat and very often small cuts on their hands *(Stanojkovic, 2012)*. *Stanojkovic et al. (2012)* found that *S. suis* can readily isolated from butchers knives. Also, there are reports that confirm carrier state in humans, especially abattoir workers *(Sala et al., 1989; Rohas et al., 2001)*. *Strangmann et al. (2002)* determined nasopharyngeal carriage rate of *S. suis* serotype 2 in the high-risk group (butchers, abattoir workers, and meat processing employees) was 5.3%, while those without contact with pigs or pork consistently tested negative. This kind of nasopharyngeal carriage rate has also been shown in pigs *(Higgins and Gottschalk, 2005)*.

In humans, *S. suis* usually produces a purulent meningitis but also endocarditis, cellulitis, peritonitis, rhabdomyolysis, arthritis, spondylodiscitis, pneumonia, uveitis, and endopthalmitis have also been reported *(Gottschalk et al., 2010b; Wertheim et al., 2009)*. Also, there have been described per acute infections related to this pathogen which were usually in the form of streptococcal toxic shock-like syndrome (STSLS) that has been associated with most of the death cases in China 2005 epidemics *(Lun et al., 2007)*. The most important often mentioned sequela of *S. suis* infection are vestibular dysfunction or unilateral or bilateral hearing loss.

S. suis as commensal

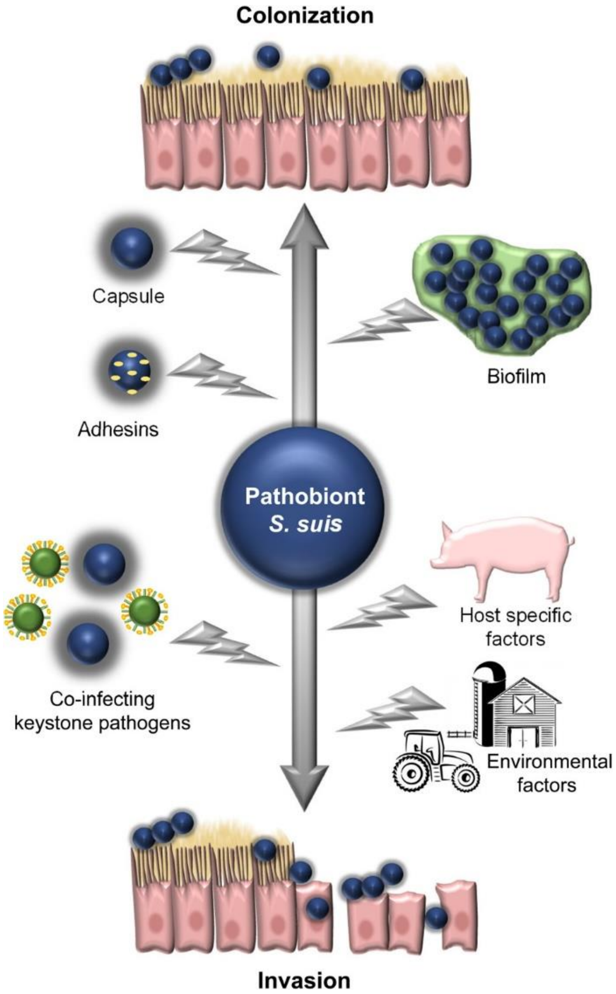
S. suis is a commensal bacterium with a natural habitat being usually the tonsils and nose cavities of healthy pigs. *S. suis* two million (mega) base pairs (2 Mbp) genome contains sequences that encode variety of factors such as adhesins and enzymes which enable it to colonize pigs tonsils in cohabitation with other bacteria Bacterial adherence is the first step and maybe crucial for development of a carrier state.

In recent years there have been many researches regarding mechanisms of adherence and tissue tropism of *S. suis*, but at the moment exact adhesin responsible for infection can not be precisely explained. Study by *Chuzeville et al. (2017)* reports that *S. suis* serotype 2 and 9 strains express genes coding for multimodal adhesion proteins known as antigen I/II (AgI/II) which in the presence of salivary glycoproteins AgI/II leads to the aggregation of *S. suis*, adherence, and colonization of the upper respiratory tract of pigs. In serotype 9, the AgI/II is responsible for aggregation and biofilm formation and these aggregated group of bacteria are partially resistant to and protected from high acid content (low pH) in the stomach which leads to colonization of the intestine (*Chuzeville et al., 2017*).

Adhesin FhB (factor H-binding protein) of *S. suis* is responsible for binding of the blood complement H factor which results in enhanced adherence of the bacteria to epithelial and endothelial cells and protecting bacteria from complement mediated phagocytosis. (*Roy et al., 2016*).

Polysaccharide capsule is the major factor involved in the pathogenesis of *S. suis* infection. Capsule is down regulated by *S. suis* genes and its thickness depends on the environment in which the bacterial cell resides. According to *Gottschalk and Segura (2000)* capsule thickness increases during epithelial invasion and adhesion and is involved in enabling *S. suis* to escape phagocytosis. Capsule synthesis is regulated is regulated by transcriptional regulator catabolite control protein A (CcpA) (*Willenborg et al., 2011*). Non-encapsulated phenotype strains lack the ccpA gene.

Biofilm is one of the components that enable the bacteria to colonize tissues, resist host defense and antibacterials, but is also involved in pathogen-comensal relationship. Biofilm formation is regulated by luxS gene (coding for the enzyme S-ribosylhomocysteinase, LuxS). It has been reported that virulent strains of *S. suis* have a higher ability to produce biofilms than avirulent strains (*Wang et* *al., 2011a*). Biofilm formation results in lower expression of virulence-associated genes with less damage to the host tissue, thus can explain the colonization of host with virulent strains while being harmless with no signs of disease.



Picture 2. *S. suis* infection (*Vötsch et al., 2018*)

Commensal to pathogen transition

S. suis infection is considered to be multifactorial, with transition from subclinical to clinical that depends on many factors. These factors can be divided in two groups, host- based and external factors. Genetics, age, pre-existing diseases and infections, immunosuppresssion are one of the most prominent host-based factors. Climatic changes in the breeding objects, poor hygiene, bad ventilation, weaning, overcrowding, and other external stressors are usually the key starters of clinical infection (*Vötsch et al., 2018*). *S. suis* strain virulence and the presence of virulence factors is also an important feature for pathogenesis. Human infection usually depends on the route of infection and immunocompetence of the human host.

Pathogenesis of *S. suis* infection can be divided into 4 phases: adherence to and colonisation of mucosal and epithelial surfaces, invasion into deeper tissues and entering the bloodstream, crossing the blood-brain barrier and inflammation. All of these phases are mediated by specific virulence factors and none of the phases will be explained here regarding their complicity and in limited knowledge for the some parts of pathogenesis.

Feng et al. (2014) classified *S. suis* virulence-associated factors into the following 4 groups: surface/secreted elements, enzymes (such as including proteases), transcription factors and regulatory systems and other factors (such as transporting and secreting systems). First group of the surface/secreted elements enzymes includes:

- capsular polysaccharides (*cps*);
- extracellular protein factor *(epf)*;
- fibronectin binding adhesin (*fbps*);
- muramidase released protein (*mrp*);
- protein of 38 kDa localized on bacterial surface (*38 kDa*);
- secreted thio-activated heamolysin (suilysin)
- surface-associated subtilisin-like serine protease (*SspA*)
- histidine triade immunogenic cell surface protein (*htpS*);
- Sat surface protein (*sat*);
- serum opacity factor (*ofs*);
- surface antigen protein (*sao*);
- sortase A (*SrtA*), catalyzing cell wall sorting reaction;
- pili

The second enzyme group of virulence factors is represented by more than 20 bacterial enzymes such as:

- GlnA, glutamine synthetase (*glnA*);
- Gdh, glutamate dehydrogenase (*gdh*);
- enolase (*eno*) catalyzing dehydration of 2-phosphoglycerate to phospho-enolpyruvate enzyme catalyzing lipoteichoic acid (LTA)-d-alanylation (*dltA*);
- peptidoglycan N-acetylglucosamine deacetylase (*pgdA*);
- inosine 5-monophosphate dehydrogebase [Impdh] (*impdh*);
- N-acetylneuraminic acid (sialic acid) synthetase (*neuB*);
- UDP *N*-Acetylglucosamine 2-Epimerase (*neuC*)
- glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)
- DNase (112 kDa)
- IgA1 (*IgA1*) protease cleaving immunoglobulin A;
- superoxide dismutase (*sod*)
- adenosine synthase (*Ssads*)
- LuxS or S-ribosyl homocysteinase

Transcription factors include more than 15 elements which include:

- AdcR (*adcR*)
- catabolite control protein A (*ccpA*)
- ArgR (*argR*)
- Rgg (*rgg*)
- Fur (*fur*)
- PerR (*perR*)

The fourth group of transporters/secretion systems comprises following factors:

- VirA (*virA*)
- Trigger factor (*Tig*)
- FeoBA *(feoBA)*
- Type IV like-secretion system (T4SS-like system)

Conclusion

Streptococcus suis is a swine pathogen that causes important economic losses in the swine industry worldwide. This bacterium has great ability to adapt to the present host, being commensal, but also to be the cause of wide range of pathological findings, including meningitis, septicemia and endocarditis, but also the cause of sudden death. The task of battling the disease is not easy because of the great diversity among virulent *S. suis* strains with many virulence-associated factors. It is very difficult to implement effective preventive measures in pigs but also for the persons that come into close occupational contact with pigs and pig products, especially employees of the meat industry. The goal of providing a functional vaccine against *S. suis* infection is being undertaken with promising results.

Therefore significant research support is needed to obtain vaccine as a valuable and universal protection against disease caused by *S. suis* strains and thus national and international support will be crucial for the aim many researchers hope for.

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