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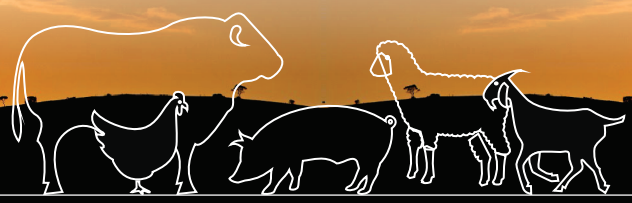

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STREPTOCOCCUS SUIS, MOST COMMON SEROTYPES ISOLATED FROM DISEASED PIGLETS IN SOME FARMS IN SERBIA

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Invited paper

Abstract: The aim of this study was to establish serotype affiliation of *S. suis* strains in piglets that died after septicemia and meningitis clinical symptoms similar to those caused by this pathogen. The material analyzed in this study included 104 brain, kidney, joint, lung and blood samples, both part of the organs and swabs from the organs from deceased piglets that had clinical feature of meningitis. Bacterial strains were selected on the basis of colony morphology, catalase negativity, hemolytic characteristics (α haemolysis) they produce on blood agar, and their microscopic appearance. For identification of bacteria, classical and commercial tests API 20 Strep and Rapid ID32 STREP (bioMérieux, France) were used. Serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens (Quellung reaction) was used in order to determine the serotypes of the isolated strains. *S. suis* serotype 2 was the most common among the 78 isolated strains (47,5%), and the only one isolated from all collected samples, serotype 9 was the second most frequent serotype isolated from diseased piglets with the prevalence of 28,2%, and it was not isolated from piglets blood, while other serotypes (1, 3, 4 and 8) were isolated in far less percentage. The results of this study showed that *S. suis* serotypes isolated from clinicaly ill piglets had prevalence similar to the prevalence reported in major European pig producing countries. Prevalence of serotype 2 *Streptococcus suis* was the highest, although it is noted that prevalence of serotype 9 in disesed pigs was remarkably increased since some earlier research in Serbia.

Key words: *Streptococcus suis*, serotype prevalence, diseased piglets

Introduction

Streptococcus suis is a normal inhabitant of the respiratory system of pigs (*Gottschalk et al., 2007*). Contrary to the fact that it is normal inhabitant, *S. suis* is the major pathogen causing significant losses on the pig farms. *S. suis* can often be isolated from tonsils, nasal cavities as well as from genital and gastrointestinal system of healthy animals. Since it is a very good colonizer of the mucosal surfaces, clinically healthy pigs are the main reservoir of infection, and the most important link in the epidemiology of human infections caused by *S. suis*. With almost 100% of pig farms worldwide having carrier animals, *S. suis* is one of the most important bacterial pig pathogen. Transmission of S. suis among pigs is mainly by respiratory route, but also some other routes have important role. From the moment it is first described as the important pathogen, *Streptococcus suis* has been identified in all countries with developed pig industries (*Stanojković et. al., 2011*). *S. suis* can cause diseases in pigs of all age categories, including suckling piglets, older piglets and pigs. *Streptococcus suis* is major pathogen causing meningitis in pigs, but also can cause septicemia, endocarditis, pneumonia and arthritis. Sometimes in per acute cases of infection pigs are found dead with no previously noticed signs of disease. In addition, it is an emerging zoonotic agent responsible for septicemia with or without septic shock, meningitis and other less common infections in humans.

Since first described in Denmark in 1968, over 1600 human cases of *S. suis* infection have been reported with many more probably never diagnosed or misdiagnosed. 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. As of 2006, the number of human cases reported in Asia has increased. In fact, in some Asian countries, the general population is at risk. *S. suis* is the most common cause of adult meningitis in Vietnam, the second most common in Thailand and the third most frequent cause of community-acquired bacterial meningitis in Hong Kong.

S. suis is encapsulated, gram positive species , that occur single, in pairs or occasionaly in short chains. It has components of cell wall antigens similar to those displayed by group D streptococci. However, *S. suis* is not genetically associated with group D streptococci. *S. suis* is a very heterogeneous species. So far, 35 *S. suis* serotypes have been described on the basis of the composition of the capsular polysaccharide. The organism grows well on media usually used for isolation of streptococci, most frequently sheep blood agar, and forms glistening, round, slightly grey alpha haemolitic colonies. It can growth well in aerobic conditions but the growth is enchanced by microaerophilic atmosphere. Isolation and

identification of strains is relatively easy, especially in the cases of diseased animals. *S. suis* has vary variable biochemical properties (*Stanojković et al., 2014*) and thus must be confirmed by serotyping. Although S. suis can be easily identified by veterinary laboratories that are aware of pathogen, many human veterinary laboratories misidentify it as enterococci, Streptococcus pneumoniae, viridans streptococci or even Listeria monocytogenes and mainly by use of rapid multitest biochemical kits (*Gottschalk et al., 2010*). This confusion may have led to the misdiagnosis of S. suis infections in the past. Serotyping is the only accurate method for definitive diagnosis of S. suis infection. This can be done by two methods. First, by serological method that is performed by either co-agglutination, capillary precipitation test or Neufeld's capsular reaction using reference antisera. Second method is serotyping using PCR method in which CPS (capsular) genes are amplified by either simplex or multiplex PCR. Polymerase chain reaction (PCR) tests have been used to directly detect S. suis DNA from samples with a high sensitivity. Some S. suis isolates do not agglutinate with any of the antisera directed against 35 serotypes and these are identified as non-typable isolates.

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.

In Canada the most prevalent serotype is serotype 2 while in United State serotype 3 is the most prevalent. In these countries there is only 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 freely movement of animals from United states to Canada and vice versa.

In South America , all results came from Brasil, stating that serotype 2 being the most prevalent with 57,6% reported cases followed by serotypes 1/2, 14, 7 and 9. In Asia majority of results regarding serotype affiliation came from China and South Korea. In China the most prevalent serotypes of infected pigs are in decreasing order of prevalnce, serotype 2, 3, 4, 7 and 8. On the contrary in South Korea serotype 2 had a prevalence of only 8,3% , the same ase 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. Similary, in Japan there have been reported 10 human S. suis cases reported but studies on the distribution of isolates

from ill pigs have not been reported lately and all of the research dates before 1987 year. 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, 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 (*Schultsz et al., 2012*). 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 only done in Serbia where serotype 2 was the only serotype found in piglets that had clinical symptoms of meningitis (*Stanojkovic et al., 2012*). Beside that various *S. suis* serotypes were found in healthy animals (*Stanojković, 2012*).

Multilocus sequence typing (MLST) distinguishes a large number of genotypes while using genetic variations that accumulate very slowly, in house keeping genes, and has allowed global and longterm epidemiology for many important meningitis-causing bacteria by determining the ST's present within a population. In 2002 , King et al. established a model of MLST for S. suis using seven house-keeping genes (*cpn*60 , *dpr*, *rec*A , *aro*A , *thr*A, *gki* and *mut*S).Many laboratories through the world use MLST model to determine sequence types (ST) of isolated S. suis strains. MLST allows gathering genetic diversity information of S. suis strains within the different serotypes. More recently, studies have begun combining data obtained from MLST with the presence or absence of different S. suis virulence-associated markers at the gene and protein levels including the suilysin (SLY, encoded by the *sly* gene), muramidase-released protein (MRP, encoded by the *mrp* gene), extracellular factor (EF, encoded by the *epf* gene) and different pili in order to compare ST's data with phenotypic characteristics.

The aim of this study was to establish serotype affiliation of *S. suis* strains in piglets that died after septicemia and meningitis clinical symptoms similar to those caused by this pathogen.

Materials and methods

The material analyzed in this study included 104 brain, kidney, joint, lung and blood samples, both part of the organs and swabs from the organs from deceased piglets that had clinical feature of meningitis. Samples were transported in trypton soy broth (Oxoid, England) within 2 h of sampling and incubated for 4 hours at 37 °C, and by using standard microbiological methods, were inoculated on Columbia CNA agar with 5% sheep blood (bioMérieux, France), and incubated for 24 h in conditions at 37 °C. Bacterial strains were selected on the basis of colony morphology, catalase negativity, hemolytic characteristics (α haemolysis) produced on blood agar, and their microscopic appearance. For identification of bacteria, classical and commercial tests API 20 Strep and Rapid ID32 STREP (bioMérieux, France) were used. Serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens (Quellung reaction) was used in order to determine the serotypes of the isolated strains.

Results and Discussion

From the 104 tested samples, 78 strains of *S. suis* were isolated. Table 1 shows the number and percentage of determined serotypes of *S. suis.*

Table 1. The number and percentage of isolated *S. suis* serotypes.

Serotypes described	Number of isolated serotypes	Isolated serotypes (%)
S. suis serotype 1	1	1,3
S. suis serotype 2	37	47,5
S. suis serotype 3	3	3,8
S. suis serotype 4	4	5,1
S. suis serotype 7	9	11,5
S. suis serotype 8	2	2,6
S. suis serotype 9	22	28,2

S. suis serotype 2 was the most common among the 78 isolated strains (47,5%), and the only one isolated from all collected samples samples. This result is similar to results that were obtained in most European countries before year 2000, China, Canada, Brasil and results all taken globally. On the contrary serotype 2 had prevalence in South Korea of only 8,3% and it was second frequent isolated serotype in the United states, Netherlands, Germany and Belgium. Also, serotype 2 is no longer dominant in Spain as it was in our research. Serotype 9 was the second most frequent serotype isolated from diseased piglets with the prevalence of 28,2%, and it was not isolated from

piglets blood. We ackonowledge that this serotype had higher distribution than expected because in other research in Serbia prevalence was not in this high percentage. This is in correlation to serotype distribution in Spain where serotype 9 prevalence became most isolated *S. suis* serotype. Also, these results follow pattern in most European countries in increased isolation frequency of *S. suis* serotype 9 from diseased pigs. Other serotypes were isolated in far less number. Serotypes 1, 3, 4 and 8 were isolated in one, three, four and two piglets respectively. Serotype 1 was isolated only from joint sample, serotype 3 from joint and kidney samples, serotype 4 from all samples except lung, and serotype 8 was isolated from brain and kidney samples. Results referring to serotypes 1, 3, 4 and 8 are similar to those in Europe except these serotypes in some countries have different order in distribution frequency. For example, in Spain the second most common serotype is serotype 2 followed by serotypes 7, 8 and 3, while in the Netherlands second common serotype 2 is followed by serotypes 7, 1 and 4. In China serotypes following most common serotype 2 are serotypes 3, 4, 7 and 8. Significant difference is noted in South Korea where serotypes 3 and 4 are the most common ones, in in United States where serotype 3 is the most frequently isolated, in Belgium and United kingdom where serotype 1 had highest distribution and in Denmark where serotype 7 was the most prevalent serotype.

Regarding results in this research we acknowledge that these results are similar to those of other authors from Europe. Although, differences can be found in prevalence of serotypes 2 and 9 which change their places as the first and the second most common serotypes in most important pig producing countries in Europe.

Conclusion

The results of this study showed that *S. suis* serotypes isolated from clinicaly ill piglets had prevalence similar to the prevalence reported in major European pig producing countries. Prevalence of serotype 2 *Streptococcus suis* was the highest, although it is noted that prevalence of serotype 9 in diseased pigs is increasing. One thing that has not been done yet in Serbia is *S. suis* multilocus sequence typing (MLST) in order to distinguish different genotypes isolated from pigs as well as from human infections and in some future reaserch it is essential to obtaine these kind of results.

Streptococcus suis, najčešće izolovani serotipovi kod obolele prasadi na nekim farmama u Srbiji

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Rezime

Cilj ovog istraživanja je bio da se utvrdi serotipska pripadnost sojeva S. suis kod prasadi koja su uginula posle kliničkih znakova septikemije i meningitisa sličnih onima koje izaziva ovaj patogen. Materijal korišćen u analizama se sastojao od uzoraka krvi, mozga, bubrega i pluća, kako delova tih organa ,tako i briseva istih. Bakterijski sojevi izolovanih bakterija su selekcionisani na osnovu morfologije kolonija, negativnosti u testu katalaze, hemolize na krvnom agaru (alfa hemoliza) i mikroskopskog pregleda. Za identifikaciju bakterija klasični i komercijalni testovi API 20 Strep i Rapid ID32 STREP (bioMérieux, France) su korišćeni a seološka tipizacija sojeva S. suis je serumima specifičnim za kapsularne antigene ove bakterije (Statens Serum Institute, Danska) takozvanom Quellung reakcijom. *S. suis* serotip 2 je u našem istraživanju među 78 izolovanih sojeva bio najfrekventniji izolovani serotip (47,5%), i jedini koji je bio izolovanih iz svih uzoraka, serotip 9 je bio sledeći drugi po prevalenciji (28,2%) i nije bio izolovan iz krvi obolelih svinja dok je prevalencija ostalih izolovanih serotipova (1, 3, 4 and 8) bila znatno niža. Rezultati ove studije su pokazali da je S. suis izolovan kod klinički obolelih prasadi imao prevalenciju sličnu onoj ustanovljenoj u Evropskim zemljam sa razvijenim svinjarstvom. Iako je prevalencija serotipa 2 *S. suis* bila najviša utvrđeno je da se distribucija serotipa 9 ove bakterije značajno povećava.

References

GOTTSCHALK M., SEGURA M., XU J. (2007): Streptococcus suis infections in humans: the Chinese experience and the situation in North America. Animal Health Research Reviews, 8, 29-45.

GOTTSCHALK M., XU J., CALZAS C., SEGURA M. (2010): Streptococcus suis: a new emerging or an old neglected zoonotic pathogen? Future Microbiology, 5, 371-391.

HIGGINS R., GOTTSCHALK M. (1990): An update on Streptococcus suis identification. Journal of Veterinary Diagnostic Investigation, 2, 249-252.
LUQUE I., BLUME V., BORGE C. ET AL (2010): Genetic analysis of Streptococcus suis isolates recovered from diseased and healthy carrier pigs at different stages of production on a pig farm. Veterinary Journal, 186, 396-398.
SCHULTSZ C., JANSEN E., KEIJZERS W. ET AL (2012): Differences in the population structure ofinvasive Streptococcus suis strains isolated from pigs and from humans in The Netherlands. PLoS One, 7, e33854.
STANOJKOVIĆ A. (2012): Investigation of presence and serotype affiliation of Streptococcus suis species in samples originating from pigs. Doctoral dissertation.
STANOJKOVIĆ A., AŠANIN R., AŠANIN J., PALIĆ K., STANOJKOVIĆ A., ŽUTIĆ J. (2011): Investigation of presence of α haemolytic streptococci, enterococci and streptococci-like bacteria in different materials originating from pigs. Veterinarski glasnik 65, 3-4, 203-213.
STANOJKOVIĆ A., AŠANIN R., MIŠIĆ D., AŠANIN J., STANOJKOVIĆ-SEBIĆ A. (2012): The presence and serological types of Streptococcus suis strains isolated from pigs originating from some farms in Serbia. Fresenius Environmental Bulletin, 21, 11a, 1-4.
STANOJKOVIĆ A., PETROVIĆ M. M., ŠKRBIĆ Z., MANDIĆ V., STANIŠIĆ N., GOGIĆ M., STANOJKOVIĆ-SEBIĆ A. (2014): Biochemical characteristics of *Streptococcus strains* isolated from healthy and deceased pigs. Biotechnology in animal husbandry, 30, 4, 699-704.