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PREVALENCE OF *STREPTOCOCUS SUIS* SEROTYPE 2 STRAINS ISOLATED FROM MAJOR PARTS OF FRESH PORK MEAT

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Abstract

The goal of this paper is to present the prevalence of major disease causing sertotype 2 of Streptococcus suis in meat ready for retail market shipment. Streptococcus suis is one of the most important pig pathogen causing septicemia, meningitis and other infections in affected animals. In addition this bacteria is an emerging zoonotic pathogen. Human infections are usually after close contact with pigs or their products. A total of 180 samples of raw pork meat (200 gr each) were taken at sloughterhouse. Samples were taken from different parts of pork, already prepared for market: liver, kidneys, shoulder, ham, loin, belly, and head area. Results have shown that 18 isolates were identified as Streptococcus suis serotype 2 from 180 samples examined. The rate of prevalence was 10% exactly. 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. Streptococcus suis serotype 2 had been isolated in all collected samples. Prevalence of serotype 2 in liver, kidneys, shoulder, ham, loin, belly and head was 20%, 12%, 5%, 5%, 5%, 10% and 25 % respectively. It is known that besides occupational exposure and meat processing in sloughterhouses, consuming of uncooked or partially cooked pork products is also a risk factor for infection. Therfore it should be advised that pork need to be thoroughly cooked/baked and not to be in contact with raw meat in any way.

Key words*: Streptococcus suis, serotype, pork meat, prevalence.*

INTRODUCTION

Streptococcus suis is one of the most important pig pathogen causing septicaemia, meningitis and other infections in affected animals. In addition this bacteria is an emerging zoonotic pathogen responsible for increased number of diseased humans, with illness that may be fatal. Especially during the last 15 years this increased number of human infections due this pathogen has been recorded, and while most of these cases are sporadic, in Asia two epidemics are documented.

Streptococcus suis is coccoid, facultative anaerobic, Gram positive bacterium that synthesize capsule and secrete haemolysin. Most of the strains are alpha haemolytic on sheep blood agar and seen as as single cells, in pairs or short chains on microscopic slides.

S. suis is very heterogeneous species, at the moment 33 serotypes have been recognized on the basis of the composition of capsule. During the last 25 years *S. suis* is considered to be one of the most important cause of severe economic loss in major pig breeding countries. *S. suis* is a normal inhabitant of the pigs respiratory system, mostly of the tonsils and nasal cavities, and can often be isolated from the genital and gastrointestinal systems in healthy animals. Transmission of *S. suis* among animals is considered to be mainly through the respiratory route *(*Higgins and Gottschalk, 2005*)*. 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 (*Gottschalk et al., 2010*)*. *S. suis* can be also easily isolated from noses and tonsils of live pigs, as well as from pig carcasses and butchers' knives (Stanojkovic et al., 2012).

All categories of pigs can be affected by the disease caused by *S. suis*, including suckling piglets, older piglets and fatteners. Colonization of pigs with *S. suis* occurs at early stage of life,

often through vertical transmission from carrying sows. *S. suis* carriage rates may vary between herds and can range from 0% to up to 80-100% (Amass et al., 1997).

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%. Meningitis is the major feature of *S. suis* infection in pigs but other organs (joints, heart, lungs, reproductive organs etc.) can also be affected.

The largest number of *S. suis* serotypes isolated from clinically ill pigs belongs to serotypes 1 to 8 (Reams et al., 1996; Higins and Gotschalk, 2001) . In the European and Asian countries, *S. suis* serotype 2 is usually the most present one (Wisselink et al., 2000), however, in some European countries with a developed pig production, such as the Netherlands, Spain and Germany, *S. suis* serotype 9 is the most common serotype causing disease in pigs.

Human infections are usually after close contact with pigs or their products. This includes farmers, farm workers, veterinarians and butchers. According to Arends and Zanen (1988) the annual risk of developing *S. suis* meningitis among abattoir workers and pig breeders has been estimated to be 3.0 cases per 100,000 population while the risk is lower for butchers, at 1.2 cases per 100,000 population in developed countries. 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 (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 i sar., 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 septicaemia (Arends and Zanen, 1988; Huang et al., 2005). 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. In China 2005 epidemics there have been 215 cases of infection while 38 of them died mainly as a results of STSLS.

According to Hoa et al. (2011) slaughterhouse pigs are a major reservoir of *Streptococcus suis* serotype 2 capable of causing human infection. But this results were obtained from tonsil samples at the slaughterhouse. 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). Authors concluded that sometimes standard culture methods can't efficiently recover *S. suis* from samples. *S*. suis was isolated from 6.1% of raw pork meat from 3 of the 6 wet markets in Hong Kong.

It can be assumed that processing and consuming of uncooked or partially cooked pork meat is also a risk factor for infection. Also, local cuisine specialities such as raw or half-cooked intestines, uterus, tonsils or fresh pig blood can be important sources of infection. In Thailand there is an increasing trend of the incidence of the disease, mainly because of consumption of half-cooked/baked meat.

Studies of *Streptococcus suis* regarding prevalence are mainly directed to clinical cases in pigs and humans and discharges, tonsils, blood, brain and spinal fluids as a specimens. There are little date of this pathogen prevalence in raw meat. In this context, the paper presents an analysis of *Streptococcus suis* presence in different parts of fresh pork meat.

MATERIALS AND METHODS

A total of 180 samples of raw pork meat (200 gr each) were taken at 4 different regional slaughterhouses in Serbia. Samples were taken randomly from different parts of pork, already prepared for market: liver, kidneys, shoulder, ham (leg), loin, belly, and head area. Mentioned parts were chosen since these are the most commonly consumed parts of pork.

All samples were homogenized, inoculated on Columbia CNA agar with 5% sheep blood and incubated aerobically for 24 h at 37 °C. Bacterial strains were selected on the basis of

colony morphology, haemolytic characteristics that they produce on blood agar (picture 1), absence of growth in 6.5% NaCl broth and their microscopic appearance. For primary identification of bacteria, classical and commercial tests API 20 Strep and Rapid ID32 STREP (bioMérieux, France) were used. In order to achieve definitive identification and determine the serotypes of the isolated strains, serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens was used.

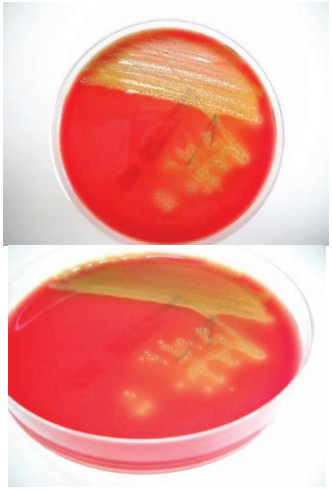

Figure 1. α Haemolysis on blood agar by *S. suis* strains

RESULTS AND DISCUSSIONS

Results have shown that 18 isolates were identified as *Streptococcus suis* serotype 2 from 180 samples examined. The rate of prevalence was 10% exactly. Except serotype 2 of *S. suis*, there have been found additional 21 strains of *S. suis* serotypes, such as serotypes 1, 3, 4, 7 and 9 with number of 3, 4, 2, 4 and 8 respectively (Figure 1). Prevalence of serotype 2 was major objective of this research as this serotype is almost always a cause of human *S. suis* infections.

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. Our data shows that serotype 2 is the most frequent serotype and in accordance to distribution of serotypes in Europe. Hoa et al (2011) found that *S. suis* serotype 2 was the most common serotype isolated from the sampled pigs, indicating that *S. suis* serotype 2 is highly prevalent in slaughterhouse pigs in southern Vietnam. In contrast to above mentioned and our research, in a study of slaughterhouse pigs in Korea, *S. suis* serotype 2 strains were absent, while serotype 9 was the most common serotype (Han et al., 2001)

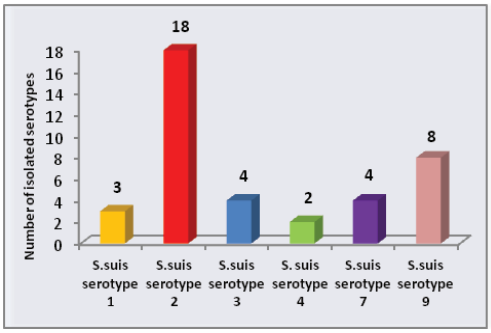

Figure 1. Number of isolated S. suis serotypes from fresh pork

Streptococcus suis serotype 2 had been isolated in all collected samples. Prevalence of serotype 2 in liver, kidneys, shoulder, ham, loin, belly and head was 20%, 12%, 5%, 5%, 5%, 10% and 25 % respectively (Table 1).

There was a significant difference in the presence of *S. suis* strains on the basis of sample collected. In this research hog head was highly contaminated with *S. suis* serotype 2 strains. This result was 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.

Noppon et al. (2014) have found overall prevalence of S. suis serotype 2 in pork of 12,8% which is similar to 10% prevalence in our research. In a research of previously mentioned authors results of prevalence of *S. suis* serotype 2 in fresh meat was 10,8% and it was not clear referring to the part of the body that fresh meat was taken from. In our study fresh meat samples from ham, loin and shoulder had lowest presence of bacteria of 5%.

Table 1. The number and percentage of isolated *Streptococcus suis* serotype 2 isolates

Meat type	Number of samples collected	Prevalence of S. suis N° (%)
Liver	25	5 (20)
Kidney	25	3 (12)
Shoulder	20	1 (5)
Ham	20	1 (5)
Loin	20	1 (5)
Belly	20	2 (10)
Head	20	5 (25)
Total	180	18 (10)

This figure is similar to results obtained by Nguyen et al. (2008) who found *S. suis* contamination of 6.1% of raw pork meat from 3 of the 6 wet markets in Hong Kong. Somehow lower isolation of *S. suis* in fresh meat from ham, loin and shoulder can be explained by fast removal of selected parts and absence of contact of these parts with other parts of pork that can be expected to be highly contaminated such as head, kidneys or sometimes skin.

Internal organs such as liver and kidneys had higher presence of *S. suis* serotype 2 strains of 20% and 12%. This is similar to 15,4% prevalence of *S. suis* serotype 2 in liver and other offal reported by Noppon et al. (2014). Although healthy at the moment of slaughter, higher kidney presence can maybe connected to findings presented by Nakayama et al. (2011) who demonstrated that *S. suis* accumulates in the kidney during *S. suis* infection.

CONCLUSIONS

Serotype 2 of *Streptococcus suis* is the most frequently isolated serotype of this bacteria in fresh pork meat. It can be readily isolated from almost every part of pork prepared to be sent to retail markets. The most contaminated parts of the pork are head, liver and kidney while ham (leg), loin and shoulder area had low prevalence of *S. suis* serotype 2 strains. It is known that besides occupational exposure and meat processing in slaughterhouses, consuming of uncooked or partially cooked pork products is also a risk factor for infection.

Therefore it should be advised that pork need to be thoroughly cooked/baked and not to be in contact with raw meat in any way.

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