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Full Length Research Paper

Investigation on clinical healthy swine carrier status of *Streptococcus suis* in Hebei Province of China

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A total of 600 samples of nose swabs collected from Hebei Province, China, were examined by polymerase chain reaction (PCR) for the presence of *Streptococcus suis* in healthy swine and the serotype were identified. Results showed that 148 strains (24.67%) of 600 tested were positive with *S. suis,* including 27 strains (18.24%) were identified to be type 7, 24 strains (16.22%) were type 2 and 20 strains (13.51%) were type 9. But serotypes of other 75 strains (50.68%) were undetermined. To our knowledge, this is the first epidemiological investigation of *S. suis* in healthy swine from Hebei Province of China.

Key words: *Streptococcus suis,* polymerase chain reaction (PCR), serotype, epidemiological.

INTRODUCTION

Streptococcus suis is an important pathogenic bacteria hazard in modern swine industry. It can be divided into 35 serotypes (type 1 to 34 and 1/2) according to the differences in antigenic properties of polysaccharide capsular. *S. suis* serotype 2 is considered to be the most widely popular and highest pathogenicity isolated in both swine and humans, which is also an important zoonotic pathogen. It reported that slaughterhouse employees can be infected with *S. suis* and to be considered as an occupational disease (He et al., 2000; Hu et al., 2000). In recent years, *S. suis* outbreaks in many countries from Europe, Americas and Asian. It can not only cause huge losses to the world's swine industry, but also endangers with public health and safety (Staats et al., 1997; Touil et al., 1998; Torremorell et al., 1998). *S. suis serotype 2* (SS2) was discovered firstly in Guangdong Province, China, in 1990. There were two large outbreaks of SS2, people had been found infected with SS2 died in Jiang su, Si chuan Province, in 1998 (Shen et al., 2000; Liu et al., 2005). In addition to SS2, SS1, SS7, SS9, etc are also important serotypes in pigs. In order to investigate the presence of *S. suis* in normal swine herds in Hebei Province, 600 nasal swabs were collected from Shijiazhuang, Xingtai, Zhangjiakou, Cangzhou, Tangshan, Qinhuangdao and other areas of Hebei Province in May 2009 to October 2009, and then detected by polymerase chain reaction (PCR). This study can provide information for epidemiological investigation of *S. suis*, and has great significance for further monitoring and effective prevention to *S. suis*.

MATERIALS AND METHODS

Strains and antisera

S. suis standard strain SS2 was provided by Professor Lu Chengping, Nanjing Agricultural University, China. Standard strains SS1, SS7, SS9 was donated by researcher Cai Xuehui, Harbin Veterinary Research Institute China. Standard antiserum of *S. suis* type 1, type 2, type 7, type 9, 1/2 and 14 types were taken from college of Veterinary Medicine, Nanjing Agricultural University.

Medium and reagents

Medium was purchased from Qingdao Haibo biotech companies. Brain heart infusion broth was purchased from Oxoid company,

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Table 1. Primers of *S. suis.*

Target genes	Primers'sequences(5′ to 3′)	Annealing temperature (°C)	Length (bp)	References
gdh-1	GCAGCGTATTCTGTCAAACG	55	689	Okwumabua et al. (2003)
gdh-2	CCATGGACAGATAAAGATGG			
cps1I-1	GGCGGTCTAGCAGATGCTCG	55	441	Smith et al. (1999)
cps1I-2	GCGAACTGTTAGCAATGAC			
Cps2J-1	ATGTTTGGAATACGCAGAGCAAAGAT	55	351	Wang et al. (2009)
Cps2J-2	CAACAAGGGCTATTAAAGATACCGC			
cps7H-1	AGCTCTAACACGAAATAAGGC	55	251	Wang et al. (2009)
cps7H-2	GTCAAACACCCTGGATAGCCG			
cps9H-1	GGCTACATATAATGGAAGCCC	55	388	Smith et al. (1999)
cps9H-2	CCGAAGTATCTGGGCTACTG			

ExTaq polymerase (5U/L), dNTPs (2.5 mmol/L each), 10 × PCR buffer (containing $MgCL_2$), DNA Marker DL2000 were purchased from TaKaRa Company, bacterial genomic DNA extraction kit purchased from Tiangen Biotech(Beijing) CO.,LTD.

Primers

Five pairs of primers were designed according to the reference (Okwumabua et al., 2003; Smith et al., 1999; Wang et al., 2009) respectively and synthesized in Sangon Biotech (Shanghai) Co., LTD. The details of the primers were listed in Table 1.

Sample collection

600 nasal swabs were collected from healthy pigs (different growth stage) on farms of Hebei Province in China, such as Shijiazhuang, Xingtai, Zhangjiakou, Cangzhou, Tangshan and Qinhuangdao. Samples were collected and processed to refrigerated storage.

Bacteria culture and identification

100 µl samples of nasal swab were inoculated into 2 ml of Streptococcus liquid selection medium (containing 15 µg/ml polymyxin B, 30 µg/ml nalidixic acid and 0.2 g/ml crystal purple) for 18 to 24 h at 37°C, *S. suis* was observed in Gram's method by a microscope.

Preparation of the templates

1000 µl Gram-positive *Streptococcus* culture liquid were centrifuged at 10000r / min for 1 min, supernatant was discarded, resuspended with 200 µl ddH_2O and then boiled for 10 min, after cooling, centrifuged at 7000r / min for 5 min, supernatant were stored at -20°C. DNA was extracted using Bacteria genomic DNA extraction kit.

PCR identification of *S. suis*

Firstly, GDH sequence of *S. suis* was applied to identify the strain. Samples were also identified by PCR based on the *S. suis* serotype 1,2,7,9. The final PCR volume was 25 µl, the reaction components are as follows: 10×PCR bufferr (Mg^{2+} Plus) 2.5 µl, dNTPs Mixture (2.5 mM) 2.0 µl, upstream and downstream primer 1.0 µl, Ex Taq DNA polymerase 0.2 µl (5 U), DNA template 2.5 µl, added ddH_2O to 25 µl. PCRs consisted of 30 cycles of denaturation for 5 min at 95°C, then 95°C denaturation 15S (gdh and cps1I) or 94°C for 30 s (Cps2J, Cps7H, Cps9H), annealing at 55°C for 45 s, and extension for 30 s at 72°C. A final extension was performed for 10 min at 72°C. PCR reaction condition of the rest genes are the same as except for annealing temperature. Simultaneously, negative control was designed. The PCR products were detected by electrophoresis in 1.2% agarose gel.

Isolation and identification of *S. suis*

The strain was identified as *S. suis* in morphology. *S. suis* is Gram-positive cocci in pairs or short chains of broth cultures by microscopy. Bacterial liquid rules on blood agar plates, at 37°C for 18 ~ 24 h, 3～6 colonies each plate with α hemolytic, smooth, moist, white translucent, diameter 1 ~2 mm were picked selectively, and inoculated into 2 ml 5% bovine serum of THB, suspected of *S. suis* were stored at 4°C. Finally, PCR methods established above were used to identified the isolated bacteria.

Serum agglutination test

Each drop of diagnose serum antibodies known and bacilli were mixed in the slide, a few minutes after, it was identified as positive when there was emergence of visible agglutination. Also set up normal saline as control. PCR identification as *S. suis* serotype 2 were to have further identification with 1/2 Standard antiserum, PCR identification as *S. suis* serotype 1, and *S. suis* serotype 14 and 1/2 standard anti-serum were used for further identification, respectively.

RESULTS

S. suis carrying in different regions

PCR analysis showed that 148 samples in 600 nasal

Table 2. Results of *S. suis* positive rates.

Regions	Samples number	SS positive number	Positive rate (%)
Qinhuangdao	100	30	30
Tangsan	100	32	32
Xingtai	100	25	25
Shijiazhuang	100	34	34
Zhangjiakou	100	11	11
Cangzhou	100	17	17
Total	600	148	24.67

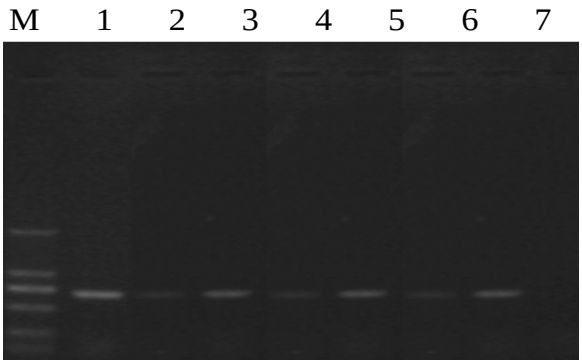

Figure 1. PCR result of *gdh* gene from *S. suis.* M: DS2000 Maker; 1-7, *gdh* positive strain; 8, negative control.

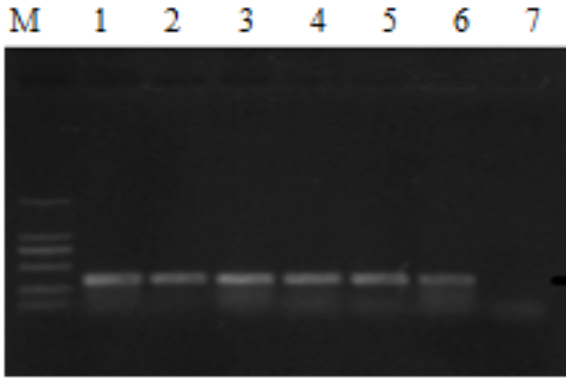

Figure 2. PCR result of *cps*2J gene from *S. suis* M: DS2000 Maker; 1-6, *cps*2J positive strain; 7, negative control.

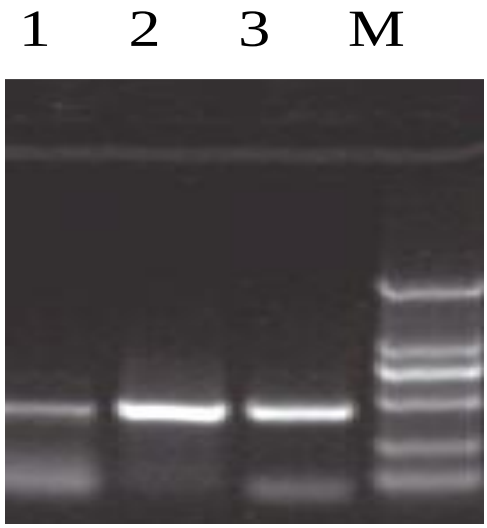

Figure 3. PCR result of cps1I gene from *S.suis* M: DS2000 Maker; 1-2, cps1I positive strain; 3, positive control.

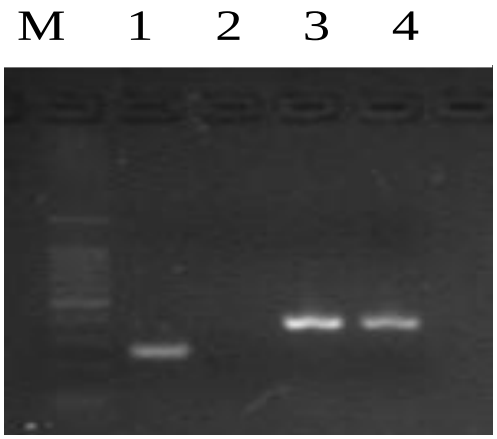

Figure 4. PCR result of cps7H and cps9H gene from *S. suis* M: 100 bp DNA ladder;1, cps7H positive strain; 3-4, cps9H positive strain.

swabs were positive for *S. suis*, the positive rate was 24.67%, results were shown in Table 2. Zhangjiakou, Cangzhou positive rates were 11 and 17%, significantly lower than other regions, the difference was significant ($p<0.01$), Qinhuangdao, Tangshan, Shijiazhuang positive samples were high, were 30, 32 and 34%, significantly higher than other regions, which showed that the *S. suis* infection had some regional differences (Table 2). The electrophoresis graphs of gdh, cps2J, cps1I, cps7H and cps9H genes of some strains respectively are shown as Figures 1, 2, 3 and 4.

Table 3. Major pathogenic *S. suis* serotype carrying cases in different regions.

Region	Total sample number	*S.suis* positive number				
		SS1 n (%)	SS2 n (%)	SS7 n (%)	SS9 n (%)	others n (%)
Qinhuangdao	100	1(1.0)	6(6)	4(4)	1 (1)	18(18)
Tangshan	100	0	5(5)	5(5)	10(10)	12(12)
Xingtai	100	0	4(4)	1(1)	6(6)	14(14)
Shijiazhuang	100	0	4(4)	12(12)	4(4)	14(14)
Zhangjiakou	100	0	1(1)	2(2)	0	8(8)
Cangzhou	100	1(1)	4(4)	3(3)	0	9(9)
Total	600	2(0.33)	24(4)	27(4.5)	20(3.3)	75(12.5))

Table 4. Different *S. suis* serotypes mixed infection.

Total sample number	SS positive sample number	SS positive sample number				
		SS2+SS7 n (%)	SS2+SS9 n (%)	SS7+SS9 n (%)	SS2+SS7+SS9 n (%)	SS1+ SS2 n (%)
600	148	6(1)	4(0.67)	10(1.67)	3(0.5)	1(0.17)

Major pathogenic *S. suis* serotype carrying cases in different regions

PCR test results showed that each serotype carrying case as follows: SS7 were the highest (4.5%), followed by SS2 up to 4%; SS9 of 3.3%; SS1 was the least, only 0.33%. The positive rate of SS7 in Shijiazhuang or Qinhuangdao was significantly ($p<0.01$) higher than other areas; SS1 positive rate was lower, in addition to one was detected in Qinhuangdao, Cangzhou, other regions were not detected positive. The positive rate of SS9 in Qinhuangdao was significantly higher than other regions ($p <0.01$) (Table 3).

S. suis serotype mixed infections in different parts

Statistics of nasal swab test results showed that the same one sample could be infected with two or more different serotypes of *Streptococcus suis*. But the samples were limited，the proportion in the sample was less than 5%. The samples of infected with both SS7 and SS9 sample were the most, up to 1.67% of the total sample, both SS2 and SS7 were the second, accounting for 1%, both SS2 and SS9 infection accounted for 0.67%. There were also infected with SS2, SS7 and SS9 or more serotypes, but the proportion was very small, only 0.5%. (Table 4).

Bacterial isolatation and PCR results

Gram-positive cocci in pairs or short chains of 528 broth cultures by microscopic examination were isolated, 61 *S. suis* strains were obtained, isolated rate was 11.55%. SS2 and SS7 were the most, each 11 stains was (2.08%). SS9 were 8 (1.52%), SS1 was not isolated. No-finalized SS were 31 stains (5.87%).

Serum agglutination test

Results for 30 isolates with a slide serum agglutination test showed that 11 *S. suis* serotype 2 bacilli only had antiserum agglutination with *S. suis* type 2, not with *S. suis* 1/2 type, so it was judged as *S. suis* type 2. *S. suis* serotype 7 (11) and type 9 (8) bacilli had specific agglutination with *S. suis* serotype 7, type 9 antiserum respectively. All results were consistent with the PCR analysis results.

DISCUSSION

The *S. suis* detection rate is different in the normal swine herds in different regions of China. Yang et al. (2009) reported 14 strains of *S. suis* in 248 pig tonsils were detected collected from 20 different regions of China, *S. suis* isolated from the tonsils were obtained from southern region of China, which may be related to *S. suis* outbreak and its hot and humid climate in south. Lu et al. (2008) isolated a highly pathogenic *S. suis* type 2 containing eight major virulence factor from 40 tonsil collected from a slaughterhouse, Jiangsu Province. Luo et al. (2009) investigated the carrying *S. suis* of healthy pigs from nasal swabs, throat swabs and tonsil in Ziyang, *S. suis* type 2 was only detected, *S. suis* carrier rate was 14.93%, concentrated in July, has a more obvious

seasonal characteristics. However, there were no related reports about carrying *S. suis* cases in clinical healthy pig herds in Hebei Province. In this study, 600 healthy pigs nasal swabs were first detected by PCR and isolate collected from 6 different regions of Hebei Province, 148 samples were found to be SS-positive (24.67%), mainly prevalent serotypes of *S. suis* type 1 , type 2, type 7, type 9, total 73, other types were 75, was lower than *S. suis* detected in Heilongjiang (29%), Jilin (27%), Liaoning (34%) Province, report by Shu-Jie Wang et al. (2009). It confirmed that *S. suis* is also widespread in Hebei Health pig herds, and the coexistence of multiple serotypes, indicating that pigs carrying *S. suis* serotype is complex and diverse in normal pigs farms.

Study found that SS7 had the highest detection rate, 4.5%, followed by SS2, SS9, and the detection rates were 4 and 3.3% respectively, these results suggested SS2, SS7 and SS9 were the most important popular serotypes in the Hebei region of China, and SS2 was zoonotic disease. More attention should be paid to this disease. SS7 was the highest detection strain. The authors found the pig cases infected with SS7 in Hebei, therefore, it needs to conduct deeper research on SS7 and strengthen prevention and control. SS1 carrier rate was relatively low (0.33%). There were multiple *S. suis* serotypes in the same nasal swabs by PCR, but it was small rate related to the carrier rate of each serotypes, its epidemiological significance remains to be studied. The study also found that, samples collected from Zhangjiakou, Cangzhou was significantly lower than other places of the province, can be inferred there are some regional differences in SS infection or the incidence of different farms will be different.

PCR is the most commonly method for the detection of *S. suis*. But PCR cannot distinguish between *S. suis* type 1 and type 14, *S. suis* 1/2 and type 1, type 2. To make test results more accurate, standard antiserum of *S. suis* type 2, type 7, type 9, 14 and 1/2 prepared by our laboratory were used to have a re-examination for this epidemiological survey. *S. suis* type 1 and 1/2 were not isolated in this study, it may be relative to the two serotypes little in Hebei Province.

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