NOTE *Public Health*

Serotype- and virulence-associated gene profile of *Streptococcus suis* isolates from pig carcasses in Chiang Mai Province, Northern Thailand

Kanruethai WONGSAWAN[1)]*, Marcelo GOTTSCHALK[2)] and Prasit THARAVICHITKUL[3)]

[1)]*Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand*
[2)]*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montréal, St-Hyacinthe, Québec, J2S 2M2, Canada*
[3)]*Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

(Received 23 July 2014/Accepted 30 September 2014/Published online in J-STAGE 3 November 2014)

ABSTRACT. In this present study, the serotype of 40 *Streptococcus suis* isolates from submaxillary glands of pig carcasses sold in wet markets in Chiang Mai Province, northern Thailand, was investigated. Eleven serotypes, including types 2, 3, 4, 5, 7, 8, 9, 17, 21, 22 and 31, were found in the isolates by a Multiplex PCR combined with serum agglutination. Of the eleven serotypes present, type 3 was the most prevalent, while types 2, 4, 5 and 21 were of primary interest due to their human isolate serotype. The *mrp*+/*epf* − /*sly* − genotype was found to be the most prevalent genotype. This study indicates the importance of effective control of human *S. suis* infection due to raw pork or pig carcass handling in northern Thailand.

KEY WORDS: pig carcass, raw pork, serotype, *Streptococcus suis*, virulence-associated gene profile

doi: 10.1292/jvms.14-0380; *J. Vet. Med. Sci.* 77(2): 233–236, 2015

Streptococcus suis is one of the most extensively emerging worldwide swine pathogens, in addition to being a zoonotic agent for people who come in close contact with pigs or pork-derived products [4]. Presently, 35 serotypes of *S. suis* have been described. Infection is still frequently found in people from the northern region of Thailand. Almost all cases have a background history of consumption of raw pork, raw pig blood or internal organs of pigs [11–13, 21, 25]. This traditional consumption behavior is considered to be a major risk factor in this region. *S. suis* is a genetically diverse agent, and the difference in virulence among the *S. suis* strains has been previously determined. Although the presence of three virulence-associated factors, suilysin (SLY, encoded by gene *sly*), a hemolysin which has a cytotoxic effect and two proteins of unknown function, the muramidase-released protein (MRP, encoded by gene *mrp*) and the secreted extracellular factor (EF, encoded by gene *epf*) [20], cannot categorically predict the virulence of an *S. suis* serotype 2 strain [3], genes encoding these virulence-associated factors have been widely used to genetically characterize *S. suis* strains. Due to limited access to costly *S. suis* antisera in Thailand, in the past, many *S. suis* isolates from pigs were considered untypable strains when tested with the few serotype-specific PCR tests available. This led to a loss of authentic data regarding the serotype of pig isolates. Thus, the objective of this study was to serotype untypable *S. suis* strains isolated from the submaxillary glands of pig carcasses sold in retail wet markets in Chiang Mai Province, Northern Thailand, by serotyping based on PCR detection of serotype-specific genes and using serotype-specific antibodies. Additionally, to genetically characterize *S. suis* strains, the presence of three virulence-associated genes encoding MRP, EF and SLY was also investigated.

A total of 40 *S. suis* isolates collected in the 2001–2002 period were included in this study. All of them were isolated from the submaxillary glands of pig carcasses purchased from six wet markets in Chiang Mai Province, northern Thailand, and kept in bacterial storage medium at −80°C. The bacterial isolates were recovered from bacterial stock cultures by subculture on Columbia blood agar (Oxoid, Hampshire, U.K.) containing 5% (v/v) defibrinated sheep blood and incubated at 37°C in 5% CO_2 for 24 hr. The colonies were confirmed as *S. suis* by both the API 20 Strep kit (BioMerieux, Marcy l'Etoile, France) and the *gdh*-specific PCR amplification product [18]. Serotyping was performed first using PCR detection of capsular (*cps*) biosynthesis genes for serotypes 1, 14, 2, 1/2, 9 and 16 [20, 23], which represent those that are mostly isolated from pigs and human cases (types 2, 14, 16) in Southeast Asia, Europe and Canada [6, 15, 19, 21, 24, 26, 27, 29]. Five pairs of *cps*-specific primers and *gdh*-specific primers were used, as illustrated in Table 1. The 50 μl PCR mixture contained 1 μg of DNA template, 25 μl of Red Dye PCR Master Mix (Merck, Mumbai, India) and 0.1 μM of each primer. *S. suis* strain P1/7 was used as the positive control of *gdh* and *cps* 2J genes, while streptococcal isolates (*Streptococcus pneumoniae*, *Streptococcus bovis* and *Streptococcus agalactiae*) were used as negative controls. PCR amplification was carried out in a thermal cycler under the

*CORRESPONDENCE TO: WONGSAWAN, K., Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand.
e-mail: KanreuthaiW@gmail.com

Table 1. Oligonucleotide primer sequences used in this study

Gene	Primer sequence (5′–3′)	PCR product size (bp)	References
gdh	AAGTTCCTCGGTTTTGAGCA, GCAGCGTATTCTGTCAAACG	566	18
*cps*1J	TGGCTCTGTAGATCATTCTGCT, TGATACGTCAAAATCCTCACCA	637	20
*cps*2J	TTTCTCGGGAGGGTTACTTC, TTTGGAAGCGATTCATCTCC	498	20
*cps*9H	GGGATGATTGCTCGACAGAT, CCGAAGTATCTGGGCTACTGA	303	20
*cps*16G	ATGATTTTTGTAACTGTAGG, CCAGCTTTTCTATTTCTTTC	487	23
mrp	CGGAGAGGAACTGATACGA, CAGCTGCAGCCAAGAGCTGACTTAGGA	515	[GenBank: A24025]
mrp variant (*mrp**)	GACAGATGGTGAGGAAAATGG, TGAGCTTTACCTGAAGCGGT	1148	20
epf variant (*epf**)	GCTACGACGGCCTCAGAAATC, TGGATCAACCACTGGTGTTAC	626 for *epf* and 1278–2993 for *epf**	28
sly	CGGATCCTCAAAGCTTGACTTACGGGCC, CAGCTGCAGCCACCATTCCCAAGCTAATCC	1282	17

Table 2. Serotypes and virulence-associated gene profiles of *Streptococcus suis* strain isolates from the submaxillary glands of pig carcasses in Chiang Mai, northern Thailand, collected in 2001–2002

Strains	Total of isolates (%)	Virulence-associated gene profile Total of isolates (%)				
		mrp+/*epf*–/*sly*–	*mrp*+/*epf*–/*sly*+	*mrp*+/*epf**/*sly*+	*mrp*–/*epf*–/*sly*–	*mrp*–/*epf*–/*sly*+
Serotype 2	5 (12.5)	0	0	0	0	5 (12.5)
Serotype 3	7 (17.5)	6 (15)	0	0	1 (2.5)	0
Serotype 4	4 (10)	0	2 (5)	1 (2.5)	1 (2.5)	0
Serotype 5	2 (5)	0	0	0	2 (5)	0
Serotype 7	1 (2.5)	0	0	0	0	1 (2.5)
Serotype 8	1 (2.5)	0	0	0	0	1 (2.5)
Serotype 9	1 (2.5)	0	0	0	1 (2.5)	0
Serotype 17	1 (2.5)	1 (2.5)	0	0	0	0
Serotype 21	1 (2.5)	1 (2.5)	0	0	0	0
Serotype 22	1 (2.5)	1 (2.5)	0	0	0	0
Serotype 31	1 (2.5)	0	0	0	1 (2.5)	0
Untypable[a)]	15 (37.5)	9 (22.5)	2 (5)	0	2 (5)	2 (5)
Total	40	18 (45)	4 (10)	1 (2.5)	8 (20)	9 (22.5)

a) Isolates did not agglutinate with any of the 34 serotype antisera and were negative by serotyping using PCR.

following conditions: initial denaturation for 2 min at 94°C, 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 50°C and extension for 1.30 min at 72°C, followed by final extension for 10 min at 72°C. Amplified PCR products of *cps*-positive strains were sequenced and compared with the GenBank database using the BLAST software maintained by the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST).

Only 6 of the 40 *S. suis* isolates were successfully serotyped by PCR; five were either *cps* type 2 or type 1/2, and one was *cps* type 9. The remaining 34 isolates were PCR negative and inferred to be serotypes other than these, and they were further identified by a coagglutination test with 34 serotype-specific antibodies (serotype 1/2 reacts with both serotypes 1 and 2 specific-antibodies) at the Reference Laboratory for *S. suis* serotyping, Faculty of Veterinary Medicine, University of Montreal, Canada. Since PCR cannot differentiate serotype 2 from serotype 1/2, the five *cps*2 or *cps*1/2 PCR-positive isolates were also tested with specific antibodies. Thus, a total of 39 isolates were further identified by a coagglutination test with serotype-specific antibodies. The 35 serotypes of *S. suis* reference strains and the reference strains of other bacterial species were used as positive and negative controls, respectively. The results confirmed that all five serotype 2 or 1/2 PCR-positive isolates were, in fact, serotype 2. Six serotypes were found from 34 PCR-negative isolates, namely, serotypes 3, 4, 5, 7, 8 and 31. Collectively, of the 40 isolates tested, eight serotypes of *S. suis* were found (Table 2). Eighteen isolates (45%) did not agglutinate with any of the 34 serotype antisera. The presence of non-typable

strains in our study is consistent with findings of Kerdsin *et al.* [10] and Maneerat *et al.* [14], who showed that half of the isolates recovered from asymptomatic pigs were untypable. In order to complete the serotyping, these untypable strains were tested with a recently described two-step multiplex PCR assay for the serotyping of all *S. suis* serotypes [16]. The serotypes of only three of the untypable strains by co-agglutination where positively serotyped by PCR, and they included serotypes 17, 21 and 22 (Table 2). These strains are probably not encapsulated. The remaining untypable strains may correspond to already described serotypes with some mutations in the *cps* genes that prevent the PCR test from correctly identifying them. They may also belong to either unencapsulated or true encapsulated strains that belong to novel, not-yet described serotypes [6].

Most typable strains were serotypes other than serotype 2, with serotype 3 being the most prevalent (7 of 25), suggesting a wide spread of *S. suis* in certain sites of pig carcasses, including the head/neck region, from which the submaxillary gland was taken. This agrees with findings from Hong Kong, where an *S. suis* serotype other than serotype 2 was found in the head/neck region, bones and tails of pig carcasses sold in wet markets [9]. However, *S. suis* serotype 2, the most common cause of human infection in Thailand, was also found among typable strains from the submaxillary glands of pig carcasses in our study (5 of 25). This finding strongly suggests that handling and consumption of raw pork represent major risks for transmission of *S. suis* serotype 2 to humans in this region. The population-based study of Takeuchi *et al.* (2012) [21] supported our suggestion; the results showed that 70% of *S. suis*-infected patients (mostly serotype 2) in Phayao Province in Northern Thailand had recently consumed raw pork dishes. Although serotype 2 is the most prevalent type recovered from humans, zoonotic cases involving serotypes 1, 4, 5, 14, 16, 21 and 24 have also been reported [1, 5, 8, 12, 13, 15], with serotypes 5 and 24 being the most recently reported cases in Thailand [12]. Serotype 5 strains have also been isolated from humans in U.S.A. and Europe [7, 8]. Serotypes 4 and 5 were identified in our study, and their numbers were similar to those found for serotype 2, suggesting their long-term existence in this area and their potential to cause disease in both swine and humans. Serotypes 3, 7, 8 and 31 were also present in pig carcasses, indicating that they are probably virulent for pigs. Although they have so far not been isolated from human patients, an eventual role in zoonosis cannot be completely ruled out.

To genotype *S. suis* isolates, three virulence-associated genes, *mrp, epf* and *sly*, were amplified by multiplex PCR. The variants of *mrp* (*mrp**) and *epf* (*epf**) genes were also detected to characterize *S. suis*. The primer sequences of virulence-associated genes [17, 20, 28] are illustrated in Table 1. The *S. suis* strain P1/7 was used as the positive control for the presence of *mrp*, *epf* and *sly* genes. Five different virulence-associated gene profiles (genotypes) were obtained from 40 *S. suis* isolates (Table 2). It was found that the *mrp+/epf–/sly–* genotype (18 of 40, 45%) was the predominant genotype among the pig isolates tested. This is consistent with previous research [2]. This genotype comprises strains of serotype 3, three probably unencapsulated typable strains (serotypes 17, 21 and 22) identified by multiplex PCR as found by Okura *et al.* [16] and nine of the remaining non-typable strains. No variant phenotype of *mrp* was found in the isolates, whereas the *epf* variant (*epf**) was detected in one isolate of serotype 4. All serotype 2 isolates presented the same genotype, *mrp−/epf–/sly+*, in contrast to strains isolated from humans in the same geographical region that carried the *mrp+/epf− /sly−* genotype (80.6%) [22]. These results indicate a difference within serotype 2; strains from humans presented only the *mrp* gene, while strains from pigs presented only the *sly* gene. While this present research was conducted using strains from pig carcasses, serotype 2 isolated from diseased pigs in China was found to have the *mrp+/epf+/sly+* genotype [24]. Unfortunately, no data on the serotype and genotype of *S. suis* from diseased pigs in Thailand are available, which makes comparisons impossible. Studies with additional strains as well as analysis of the strains by multilocus sequence typing will significantly contribute to the knowledge about serotype 2 pig and human strains isolated in this part of the country. The *mrp−/epf–/sly−* genotype was distributed in many serotypes, including serotypes 3, 4, 5, 9 and 31. This genotype is the most prevalent in the isolates from Chinese slaughtered pigs. This could imply great genetic heterogeneity and geographical variation of *S. suis*.

In conclusion, vastly different serotypes were firstly found from *S. suis* isolates originally isolated from pig carcasses sold in wet markets in Chiang Mai, Northern Thailand. The important finding is that serotypes isolated from human patients were also present in pig carcasses, which are used in local raw pork dishes. The results of this study should have a significant positive impact on the prevention of human *S. suis* infection from the traditional habit of consuming raw pork dishes and provide essential information about the *S. suis* serotypes and genotypes present in pigs in Thailand.

ACKNOWLEDGMENT. The study was supported by a grant-in-aid from the Endowment Fund for Medical Research, Faculty of Medicine, Chiang Mai University, Thailand.

REFERENCES

1. Callejo, R., Prieto, M., Salamone, F., Auger, J. P., Goyette-Desjardins, G. and Gottschalk, M. 2014. Atypical *Streptococcus suis* in man, Argentina, 2013. *Emerg. Infect. Dis.* **20**: 500–502. [Medline] [CrossRef]
2. Chatellier, S., Gottschalk, M., Higgins, R., Brousseau, R. and Harel, J. 1999. Relatedness of *Streptococcus suis* serotype 2 isolates from different geographic origins as evaluated by molecular fingerprinting and phenotyping. *J. Clin. Microbiol.* **37**: 362–366. [Medline]
3. Fittipaldi, N., Segura, M., Grenier, D. and Gottschalk, M. 2012. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. *Future Microbiol.* **7**: 259–279. [Medline] [CrossRef]
4. Gottschalk, M., Segura, M. and Xu, J. 2007. *Streptococcus suis*

infections in humans: the Chinese experience and the situation in North America. *Anim. Health Res. Rev.* **8**: 29–45. [Medline] [CrossRef]
5. Gottschalk, M., Xu, J., Calzas, C. and Segura, M. 2010. *Streptococcus suis*: a new emerging or an old neglected zoonotic pathogen? *Future Microbiol.* **5**: 371–391. [Medline] [CrossRef]
6. Gottschalk, M., Lacouture, S., Bonifait, L., Roy, D., Fittipaldi, N. and Grenier, D. 2013. Characterization of *Streptococcus suis* isolates recovered between 2008 and 2011 from diseased pigs in Québec, Canada. *Vet. Microbiol.* **162**: 819–825. [Medline] [CrossRef]
7. Gomez, E., Kennedy, C. C., Gottschalk, M., Cunningham, S. A., Patel, R. and Virk, A. 2014. *Streptococcus suis*-related prosthetic joint infection and streptococcal toxic shock-like syndrome in a pig farmer in the United States. *J. Clin. Microbiol.* **52**: 2254–2258. [Medline] [CrossRef]
8. Gustavsson, C. and Ramussen, M. 2014. Septic arthritis caused by *Streptococcus suis* serotype 5 in pig farmer. *Emerg. Infect. Dis.* **20**: 489–490. [Medline] [CrossRef]
9. Ip, M., Fung, K. S., Chi, F., Cheuk, E. S., Chau, S. S., Wong, B. W., Lui, S., Hui, M., Lai, R. W. M. and Chan, P. K. S. 2007. *Streptococcus suis* in Hong Kong. *Diagn. Microbiol. Infect. Dis.* **57**: 15–20. [Medline] [CrossRef]
10. Kerdsin, A., Akeda, Y., Hatrongjit, R., Detchawna, U., Sekizaki, T., Hamada, S., Gottschalk, M. and Oishi, K. 2014. *Streptococcus suis* serotyping by a new multiplex PCR. *J. Med. Microbiol.* **63**: 824–830. [Medline] [CrossRef]
11. Kerdsin, A., Dejsirilert, S., Puangpatra, P., Sripakdee, S., Chumla, K., Boonkerd, N., Polwichai, P., Tanimura, S., Takeuchi, D., Nakayama, T., Nakamura, S., Akeda, Y., Gottschalk, M., Sawanpanyalert, P. and Oishi, K. 2011. Genotypic profile of *Streptococcus suis* serotype 2 and clinical features of infection in humans, Thailand. *Emerg. Infect. Dis.* **17**: 835–842. [Medline] [CrossRef]
12. Kerdsin, A., Dejsirilert, S., Sawanpanyalert, P., Boonnark, A., Noithachang, W., Sriyakum, D., Simkum, S., Chokngam, S., Gottschalk, M., Akeda, Y. and Oishi, K. 2011. Sepsis and spontaneous bacterial peritonitis in Thailand. *Lancet* **378**: 960. [Medline] [CrossRef]
13. Kerdsin, A., Oishi, K., Sripakdee, S., Boonkerd, N., Polwichai, P., Nakamura, S., Uchida, R., Sawanpanyalert, P. and Dejsirilert, S. 2009. Clonal dissemination of human isolates of *Streptococcus suis* serotype 14 in Thailand. *J. Med. Microbiol.* **58**: 1508–1513. [Medline] [CrossRef]
14. Maneerat, K., Yongkiettrakul, S., Kramomtong, I., Tongtawe, P., Tapchaisri, P., Luangsuk, P., Chaicumpa, W., Gottschalk, M. and Srimanote, P. 2013. Virulence genes and genetic diversity of *Streptococcus suis* serotype 2 isolates from Thailand. *Transbound. Emerg. Dis.* **60** Suppl 2: 69–79. [Medline] [CrossRef]
15. Nghia, H. D. T., Hoa, N. T., Linh, D., Campbell, J., Diep, T. S., Chau, N. V. V., Mai, N. T. H., Hien, T. T., Spratt, B., Farrar, J. and Schultsz, C. 2008. Human case of *Streptococcus suis* serotype 16 infection. *Emerg. Infect. Dis.* **14**: 155–157. [Medline] [CrossRef]
16. Okura, M., Lachance, C., Osaki, M., Sekizaki, T., Maruyama, F., Nozawa, T., Nakagawa, I., Hamada, S., Rossignol, C., Gottschalk, M. and Takamatsu, D. 2014. Development of a two-step multiplex PCR assay for typing of capsular polysaccharide synthesis gene clusters of *Streptococcus suis*. *J. Clin. Microbiol.* **52**: 1714–1719. [Medline] [CrossRef]
17. Okwumabua, O., Abdelmagid, O. and Chengappa, M. M. 1999. Hybridization analysis of the gene encoding a hemolysin (suilysin) of *Streptococcus suis* type 2: evidence for the absence of the gene in some isolates. *FEMS Microbiol. Lett.* **181**: 113–121. [Medline] [CrossRef]
18. Okwumabua, O., O'Connor, M. and Shull, E. 2003. A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase. *FEMS Microbiol. Lett.* **218**: 79–84. [Medline] [CrossRef]
19. Schultsz, C., Jansen, E., Keijzers, W., Rothkamp, A., Duim, B., Wagenaar, J. A. and van der Ende, A. 2012. Differences in the population structure of invasive *Streptococcus suis* strains isolated from pigs and from humans in The Netherlands. *PLoS ONE* **7**: e33854. [Medline] [CrossRef]
20. Silva, L. M., Baums, C. G., Rehm, T., Wisselink, H. J., Goethe, R. and Valentin-Weigand, P. 2006. Virulence-associated gene profiling of *Streptococcus suis* isolates by PCR. *Vet. Microbiol.* **115**: 117–127. [Medline] [CrossRef]
21. Takeuchi, D., Kerdsin, A., Pienpringam, A., Loetthong, P., Samerchea, S., Luangsuk, P., Khamisara, K., Wongwan, N., Areeratana, P., Chiranairadul, P., Lertchayanti, S., Petcharat, S., Yowang, A., Chaiwongsaen, P., Nakayama, T., Akeda, Y., Hamada, S., Sawanpanyalert, P., Dejsirilert, S. and Oishi, K. 2012. Population-based study of *Streptococcus suis* infection in humans in Phayao Province in northern Thailand. *PLoS ONE* **7**: e31265. [Medline]
22. Tharavichitkul, P., Wongsawan, K., Takenami, N., Pruksakorn, S., Fongcom, A., Gottschalk, M., Khanthawa, B., Supajatura, V. and Takai, S. 2014. Correlation between PFGE Groups and *mrp/epf/sly* Genotypes of Human *Streptococcus suis* Serotype 2 in Northern Thailand. *J. Pathogens* **2014**: 350416. [Medline] [CrossRef]
23. Wang, K., Fan, W., Wisselink, H. and Lu, C. 2011. The cps locus of *Streptococcus suis* serotype 16: development of a serotype-specific PCR assay. *Vet. Microbiol.* **153**: 403–406. [Medline] [CrossRef]
24. Wang, K., Zhang, W., Li, X., Lu, C., Chen, J., Fan, W. and Huang, B. 2013. Characterization of *Streptococcus suis* isolates from slaughter swine. *Curr. Microbiol.* **66**: 344–349. [Medline] [CrossRef]
25. Wangsomboonsiri, W., Luksananun, T., Saksornchai, S., Ketwong, K. and Sungkanuparph, S. 2008. *Streptococcus suis* infection and risk factors for mortality. *J. Infect.* **57**: 392–396. [Medline] [CrossRef]
26. Wei, Z., Li, R., Zhang, A., He, H., Hua, Y., Xia, J., Cai, X., Chen, H. and Jin, M. 2009. Characterization of *Streptococcus suis* isolates from the diseased pigs in China between 2003 and 2007. *Vet. Microbiol.* **137**: 196–201. [Medline] [CrossRef]
27. Wertheim, H. F., Nguyen, H. N., Taylor, W., Lien, T. T., Ngo, H. T., Nguyen, T. Q., Nguyen, B. N. T., Nguyen, H. H., Nguyen, H. M., Nguyen, C. T., Dao, T. T., Nguyen, T. V., Fox, A., Farrar, J., Schultsz, C., Nguyen, H. D., Nguyen, K. V. and Horby, P. 2009. *Streptococcus suis*, an important cause of adult bacterial meningitis in northern Vietnam. *PLoS ONE* **4**: e5973. [Medline] [CrossRef]
28. Wisselink, H. J., Reek, F. H., Vecht, U., Stockhofe-Zurwieden, N., Smits, M. A. and Smith, H. E. 1999. Detection of virulent strains of *Streptococcus suis* type 2 and highly virulent strains of *Streptococcus suis* type 1 in tonsillar specimens of pigs by PCR. *Vet. Microbiol.* **67**: 143–157. [Medline] [CrossRef]
29. Zhang, C. -P., Ning, Y. -B., Zhang, Z.-Q., Song, L., Qiu, H. -S., Gao, H. -Y. and Fan, X. -Z. 2009. Prevalence of *Streptococcus suis* isolated from clinically healthy sows in China. *Agric. Sci. China* **8**: 638–642. [CrossRef]