TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Short Communication

Turk J Vet Anim Sci
(2013) 37: 94-96
© TÜBİTAK
doi:10.3906/vet-0911-237

Isolation of *Streptococcus* species from the tonsils of slaughtered pigs

Arzu Funda BAĞCIGİL*, Serkan İKİZ, Kemal METİNER,
Naciye Yakut ÖZGÜR, Seyyal AK, Ahmet Atila ILGAZ
Department of Microbiology, Veterinary Faculty, İstanbul University, Avcılar, İstanbul, Turkey

Received: 19.11.2009 • **Accepted:** 10.04.2012 • **Published Online:** 22.01.2013 • **Printed:** 22.02.2013

Abstract: The palatine tonsils of 220 pigs were examined for the occurrence of *Streptococcus suis* and other *Streptococcus* species. Samples were cultured onto Colombia agar plates with 5% defibrinated horse blood and selective antibiotic supplement and were incubated at 37 °C for 24–48 h aerobically. Presumptive colonies were identified using API20 Strep (bioMérieux). *S. suis* was not detected in any of the 220 palatine tonsils examined. *S. porcinus* was the most common streptococcus recovered and was isolated from 68 (31%) pigs. From the samples, *S. agalactiae* was isolated from 19 (8.6%), *S. equi* subsp. *zooepidemicus* from 10 (4.5%), *S. uberis* from 6 (2.7%), *S. dysgalactiae* subsp. *equisimilis* from 4 (1.8%), *Enterococcus faecium* from 4 (1.8%), and *S. pyogenes* from 1 (0.5%). The absence of *S. suis* among the pigs examined is hopeful. However, further studies on this disease and other infectious diseases should continue. Additionally, this is the first report of the isolation of *S. porcinus* in swine in Turkey.

Key words: *Streptococcus* spp., isolation, pig, palatine tonsil

Several *Streptococcus* species can be found in the tonsils of clinically healthy pigs; however, some of them, such as *Streptococcus suis*, *S. porcinus*, and group C streptococci, are potential pathogens. Katsumi et al. (1) reported that the causes of endocarditis in 51.5% of 495 pigs were *Streptococcus* species, particularly *S. suis*, *S. dysgalactiae*, and *S. porcinus*. In another study, the same research group reported that the main bacterial species in slaughtered pigs with endocarditis, arthritis, or lymphadenitis was *S. dysgalactiae* (2).

Among the *Streptococcus* species, *S. suis* is a particularly important pathogen for the swine industry. It causes meningitis, arthritis, endocarditis, pericarditis, bronchopneumonia, abortions, septicemia, and sudden death in pigs (1,3,4). Based on specific capsular polysaccharide antigens, 35 different serotypes (serotypes 1 to 34 and serotype ½) have been identified. Wide variations have been reported in the virulence between serotypes, as well as within each serotype (5–7). *S. suis* colonizes the upper respiratory tract, particularly the palatine tonsils and nasal cavities, as well as the genital tracts of both infected and clinically healthy swine. It is usually transmitted nasally or orally (5,8–10). The diagnosis of the infection is performed by isolation of the agent by conventional methods and serotyping based on polysaccharide capsular antigens (5,6,11). It has been indicated that sampling from palatine tonsils is the most reliable way for the detection of both infected and carrier pigs (8,11). *S. suis* is a conspicuous agent for public health because of its well-known zoonotic potential. It causes meningitis, followed by septicemia and endocarditis in humans. It has been indicated that those people in close contact with pigs or pig meat are at risk of *S. suis* infections (8,12).

In Turkey, pig farms and slaughterhouses are not routinely tested for the presence of pathogens such as *S. suis*, *Actinobacillus pleuropneumoniae*, or *Mycoplasma hyopneumoniae* due to limited facilities and the inadequacy of knowledge and experience of veterinarians in the swine industry. However, recent developments in this industry in Turkey showed the necessity of detailed studies on this species. The aim of this study was to investigate the occurrence of *S. suis* and its predominant serotypes in Turkey, as well as those of other streptococci. The palatine tonsils from pigs at the slaughterhouse were used for the isolation of the bacteria.

The palatine tonsils of 220 pigs from 10 different pig farms, aged between 3 months and 3 years old, were removed aseptically after slaughter and transported to the laboratory within sterile bags in a cold chain. The tonsil samples were submerged for 7 s in boiling water in order to avoid possible contaminations, placed in polyethylene

* Correspondence: fucigil@istanbul.edu.tr

bags, and macerated in a Stomacher (IUL Instruments, Barcelona, Spain) (13). The homogenate were cultured on Colombia agar plates (Oxoid CM331, Hampshire, UK) with 5% defibrinated horse blood and selective antibiotic supplement (Oxoid SR126), and were incubated at 37 °C for 24–48 h aerobically. Gram-positive cocci and catalase- and oxidase-negative colonies were identified using API20 Strep (bioMérieux, Marcy l'Etoile, France) (7,8,14).

S. suis was not detected among the 220 palatine tonsils tested. However, various *Streptococcus* spp. were isolated from 96 tonsil samples. *S. porcinus* was the most common streptococcus recovered and was isolated from 68 (31%) pigs. From the samples, *S. agalactiae* was isolated from 19 (8.6%), *S. equi* subsp. *zooepidemicus* from 10 (4.5%), *S. uberis* from 6 (2.7%), *S. dysgalactiae* subsp. *equisimilis* from 4 (1.8%), *Enterococcus faecium* from 4 (1.8%), and *S. pyogenes* from 1 (0.5%).

Although streptococcal and enterococcal bacteria are found in the tonsils, intestines, or feces of clinically healthy pigs, *S. suis*, *S. porcinus*, and group C-belonging *S. equi* subsp. *zooepidemicus* and *S. dysgalactiae* subsp. *equisimilis* are potential pathogens (5,10).

Aarestrup et al. (9) reported that 265 *S. suis* samples were recovered from pigs within a 2-year period in a Danish veterinary laboratory. Clifton-Hadley et al. (15) isolated *S. suis* from a proportion of pigs from 12 herds with a history of the disease and from 3 herds believed to be free from the disease. In Australia, tonsils collected at slaughter from 188 pigs originating from 3 herds known to be infected with *S. suis* type 2 were examined and the agent was isolated in 76% of the samples (14). Kataoka et al. (16) examined 291 palatine tonsils of clinically healthy pigs brought into a slaughterhouse for the presence of *S. suis* serotype 2, and it was isolated from 40 (13.7%) of them. A total of 200 nasal and tonsil swabs from pigs slaughtered and sold in the Philippines were examined for the presence of pathogenic streptococci. Out of the 200 pigs, 136 *E. faecalis* and 24 *S. dysgalactiae* samples were isolated, while *S. suis*, *S. porcinus*, and *S. pyogenes* were not isolated (17).

In the present study, *S. suis* was not recovered from the 220 pigs examined. This result offers indications that it may not be present in pigs in Turkey. In Turkey, there are a limited number of pig farms, and these farms are separated from each other and contain very small pig populations. According to the Turkish Statistical Institute, the number of raised pigs was 1558 in 2010 (18). Therefore, it is suggested that the negative isolation result might be due to these conditions. The farms were located mostly in the western part of Turkey. The farmers informed us that there was no transportation from other countries and that all of the animals originated from Turkey. Furthermore, there is no information on the occurrence of *S. suis* infection in neighboring countries such as Greece (19), Bulgaria, or Georgia.

In Turkey, there are no pure-bred pigs and no studies of the species or genotypic properties of the current pigs. Therefore, it is possible that resistance factors originating from genotypic properties might be acting in the protection against disease. Additionally, since this study was not focused on specific serotypes, conventional isolation procedures were followed and a specific hyperimmune sera addition or immune magnetic separation could not be used. In most countries, all of the pigs pass through a scald tank, in which the water is at approximately 60 °C, and it has been reported that *S. suis* could survive for up to 10 min at this temperature (15). Cleaning processes are performed manually and the temperatures of the scald tanks are kept at about 70 °C or higher in Turkey. The high-temperature processing in this study might have influenced the isolation chance negatively.

Another reason for the lack of isolation might be preventive antibiotic usage at the farms. Unfortunately, it is almost impossible to discuss this possibility due to the lack of information about the antibiotic usage for any health problems of the pigs at the farms sampled. The only definite information about the management was that no vaccination protocol had been performed at any of the farms.

S. porcinus can be isolated from the tonsils, pharynx, and nasal cavities of clinically healthy pigs; however, it causes contagious streptococcal lymphadenitis in pigs characterized with abscesses in the cervical, mandibular, and cephalic lymph nodes. Carrier pigs harbor *S. porcinus* in their tonsils and readily transmit the organisms to susceptible pigs via nose-to-nose contact and by contamination of water and feed. It has been isolated from pyogenic infections in humans (10). The isolation of *S. porcinus* from pigs, with a 30% isolation rate in the current study, indicates that *S. porcinus* is common among the pigs in Turkey.

Streptococcus equi subsp. *zooepidemicus* has been isolated from a wide range of animal species, such as horses, cats, dogs, and pigs, and it could cause endocarditis, pneumonia, meningitis, septic arthritis, and cervical adenitis in humans (10). In Hong Kong, the chromosomal DNA of *S. equi* subsp. *zooepidemicus* isolates from humans and pigs was compared by HindIII restriction endonucleases, and it was found that the human and pig isolates were identical (20). The *S. equi* subsp. *zooepidemicus* isolation rate (4.5%) in this study was found to be meaningful among the pigs in Turkey. In this study, the presence of *S. dysgalactiae* subsp. *equisimilis*, which causes sporadic septicemia, arthritis, and endocarditis in pigs, was determined with a low isolation rate (1.8%).

In conclusion, although the results of the study suggest the absence of *S. suis* infection in pigs in Turkey, further studies on this disease and other infectious diseases should

continue. We were able to confirm for the first time the presence of *S. porcinus* in pigs bred in Turkey. This is an important finding, taking into account that *S. porcinus* is a potential pathogen both for humans and pigs.

Acknowledgment
This work was supported by the Research Fund of İstanbul University, project numbers 393/03062007 and UDP-2045/23012008.

References

1. Katsumi, M., Kataoka, Y., Takahasmi, T., Kikuchi, N., Hiramune, T.: Bacterial isolation from slaughtered pigs associated with endocarditis, especially the isolation of *Streptococcus suis*. J. Vet. Med. Sci., 1997; 59: 75–78.
2. Katsumi, M., Kataoka, Y., Takahasmi, T., Kikuchi, N., Hiramune, T.: Biochemical and serological examination of beta-haemolytic streptococci isolated from slaughtered pigs. J. Vet. Med. Sci., 1998; 60: 129–131.
3. Chanter, N., Jones, P.W., Alexander, T.J.L.: Meningitis in pigs caused by *Streptococcus suis* — a speculative review. Vet. Microbiol., 1993; 36: 39–55.
4. McCaw, M.B.: *Streptococcus suis*: Nagging nursery nightmare. Proceedings of the North Carolina Healthy Hogs Seminar, North Carolina Swine Veterinary Group; 1993.
5. Staats, J.J., Feder, I., Okwumabua, O., Chengappa, M.M.: *Streptococcus suis*: past and present. Vet. Res. Commun., 1997; 21: 381–407.
6. Wisselink, H.J., Reek, F.H., Vecht, U., Stochofe-Zurwieden, N., Smits, M.A., Smith, H.E.: 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., 1999; 67: 143–157.
7. Madsen, L.W., Aalbaek, B., Nielsen, O.L., Jensen, H.E.: Aerogenous infection of microbiologically defined minipigs with *Streptococcus suis* serotype 2. A new model. APMIS, 2001; 109: 412–418.
8. Arends, J.P., Hartwig, N., Rudolphy, M., Zanen, H.C.: Carrier rate of *Streptococcus suis* capsular type 2 in palatine tonsils of slaughtered pigs. J. Clin. Microbiol., 1984; 20: 945–947.
9. Aarestrup, F.M., Jorsal, S.E., Jensen, M.E.: Serological characterization and antimicrobial susceptibility of *Streptococcus suis* isolates from diagnostic samples in Denmark during 1995 and 1996. Vet. Microbiol., 1998; 60: 59–66.
10. Higgins, R., Gottschalk, M.: Streptococcal diseases. In: Straw, B.E., Allaire, S., Mengeling, W.L., Taylor, D.J., Eds. Diseases of Swine. 8th edn., Iowa University Press, Iowa, USA. 1999; 563–578.
11. Clifton-Hadley, F., Alexander, T.J.L.: Diagnosis of *S. suis* infection in pigs. Practice, 1988; 10: 185–187.
12. Perch, B., Kristjansen, B., Skadhauge, K.: Group R streptococci pathogenic for man. Two cases of meningitis and one fatal case of sepsis. Acta Pathol. Microbiol. Scand. B, 1968; 74: 69–76.
13. van Leengoed, L.A.M.G., Vecht, U., Verheyen, E.R.M.: *Streptococcus suis* type 2 infections in pigs in the Netherlands (part two). Vet. Q., 1987; 9: 111–117.
14. Davies, P.R., Ossowicz, C.J.: Evaluation of methods used for detecting *Streptococcus suis* type 2 in tonsils, and investigation of the carrier state in pigs. Res. Vet. Sci., 1991; 50: 190–194.
15. Clifton-Hadley, F., Alexander, T.J.L., Enright, M.R., Guise, J.: Monitoring herds for *Streptococcus suis* type 2 by sampling tonsils of slaughtered pigs. Vet. Rec., 1984; 115: 562–564.
16. Kataoka, Y., Sugimoto, C., Nakazawa, M., Kashiwazaki, M.: Detection of *Streptococcus suis* type 2 in tonsils of slaughtered pigs using improved selective and differential media. Vet. Microbiol., 1991; 28: 335–342.
17. Benguet State University. Animal Health and Production. Benguet, Philippines. http://www.bsu.edu.ph/key_offices/rde/content/animal_production.php. Retrieved 6 June 2008.
18. T.C. Başbakanlık Türkiye İstatistik Kurumu. Ankara, Turkey. http://www.tuik.gov.tr/VeriBilgi.do?tb_id=46&ust_id=13. Retrieved 9 September 2011.
19. Papatsiros, V.G., Vourvidis, D., Tzitzis, A.A., Meichanetsidis, P.S., Stougiou, D., Mintza, D., Papaioannou, P.S.: *Streptococcus suis*: an important zoonotic pathogen for human – prevention aspects. Vet. World, 2011; 4: 216–221.
20. Yuen, K.Y., Seto, W.H., Choi, C.H., Ng, W., Ho, S.W., Chau, P.Y.: *Streptococcus zooepidemicus* (Lancefield group C) septicemia in Hong Kong. J. Infect., 1990; 21: 241–250.