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ARTICLE

Seroprevalence of Swine Salmonellosis in Korean Swine Herds

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Abstract

Salmonellosis is one of the most important wasting diseases that leads to economic damage in the swine industry. Many risk factors have been reported to increase the spread of *Salmonella* infection; therefore, it is important to understand how to treat the risk factors of *Salmonella* to effectively prevent salmonellosis in commercial pig farms. To accomplish this, we conducted a study to determine if the seasons and porcine production stages affected the serological response to *Salmonella* in Korea. A total of 1,592 serum samples submitted to the School of Veterinary Medicine of Kangwon National University between Jan. 2001 and Dec. 2004 from commercial farms were tested by ELISA. The overall apparent seroprevalence of salmonellosis was 38.1% (95% CI, 38.0-38.2), while the prevalence of *Salmonella* according to seasons and production stages ranged from 17.9% to 62.8% for the former (24.6% in spring, 17.9% in summer, 38.5% in autumn, and 62.8% in winter) and from 16.1% to 68.3% for the latter (17.9% in suckling pigs, 16.1% in weaning pigs, 37.50% in growers, 41.9% in finishers, 48.0% in gilts, and 68.3% in sows). In this study of seroprevalence by production stage, most pigs were naturally infected by *Salmonella* during the weaning stage. Also, seroprevalences were found to have a seasonal pattern in which most pigs were infected in autumn to winter.

Key words: swine salmonellosis, seroprevalence, ELISA, Korean swine herd

Introduction

Salmonella Enterica is an important and well-recognized food-borne pathogen in humans, but it also induces severe economic losses in terms of swine mortality (Kishima *et al.*, 2008; Thorns *et al.*, 2000). Although the more than 2,400 *Salmonella* serotypes categorized according to Kouffman-white scheme have various host ranges and are spread extensively, the primary clinical disease in pigs is caused by *S.* Choleraesuis or *S.* Typhimurium belonging to serogroups B and C, respectively (Griffith *et al.*, 2006; Yoshida *et al.*, 2007). Pigs can be infected with a variety of *S.* enterica serovars and infected pigs can be healthy carriers, spreading infection from both the intestinal tract and the mesenteric lymph nodes for extended periods of time (Griffith *et al.*, 2006).

The status and types of feed, housing contamination, antibacterial usage, presence of rodents and cats, herd health status, etc are considered as risk factors affecting the high prevalence of salmonellosis (Funk and Gebreyes, 2004). It is difficult to determine common risk factors due to differences in production, industry structure, and regulatory organization (Funk and Gebreyes, 2004). However, in a number of advanced countries, especially European countries, *Salmonella* reduction programs have been applied and proven to be effective (Christensen and Rudemo, 1998). Others, such as Italy, are investigating the epidemiology of salmonellosis for the future implementation of a reduction program (Merialdi *et al*., 2008). Nevertheless, some countries are continuously conducting risk factor studies as monitoring the prevalence of salmonellosis is important to prevent from salmonellosis and to make decisions on control strategies (Sanchez *et al*., 2007).

However, in Korea, there have been few reports of salmonellosis risk factors to achieve reasonable accuracy over long periods of time. The development of efficient strategies to control swine salmonellosis would help to decrease the presence of *Salmonella*. Therefore, we have conducted intensive longitudinal studies via ELISA, with the aim of determining and describing the correlation

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between the degree of infection with porcine production stages and seasons.

Materials and Methods

Study population

A total of 1,592 serum samples submitted to the School of Veterinary Medicine of Kangwon National University between Jan. 2001 and Dec. 2004 from commercial farms were evaluated for the prevalence of *Salmonella*. The samples were divided into groups of different production stages including suckler (<22 d old), weaner (22-70 days old), grower (71-119 d old), finisher (>120 days old), gilt and sow, and of different seasons, including spring (March-May), summer (June-August), autumn (September-November) and winter (December-Feburary). All serums submitted were heated for 30 min at 56°C for complement inactivation. And then the individual samples were stored below -20°C until use.

ELISA

The samples were slowly defrosted in a refrigerator between 4 and 7°C in preparation for the *Salmonella* ELISA (HerdCHek* Swine *Salmonella* Test Kit, IDEXX, USA). The coating antigens in this ELISA include LPS (lipopolysaccharide) of serogroup B, C1 and D (O-antigens 1, 4, 5, 6, 7, and 12). The ELISA protocol was carried out according to the manufacturer's instructions (wavelength: 650 nm). OD values of samples, positive and negative control were determined by measuring at 650 nm. An S/P > 0.5 or greater according to below calculation was considered to be a positive serum antibody response. The ELISA test has a specificity of 99.4%.

$$\text{S/P ratio} = (\text{Sample OD} - \text{Negative OD}) / (\text{Positive OD} - \text{Negative OD})$$

Statistical analysis

Data gathered from the survey were entered into a Microsoft Excel (Redmond, WA, USA) spreadsheet. The analysis was performed using the glimmix macro in Statistical Analysis System (SAS Institute, Inc., Cary, NC, USA) and the provincial differences for seroprevalence of salmonellosis in Korean pigs were compared using the chi-square test according to season and production stages ($p<0.05$).

Results

Prevalence by season

The relationship between *Salmonella* seroprevalence and season in Korea from 2001 to 2004 was shown in Table 1. A total of 607 pigs (38.1%; 95% CI, 38.0-38.2) out of 1,592 tested were positive for *Salmonella* by ELISA. The seasonal sero-prevalence of *Salmonella* spp. ranged from 17.86% to 68.29%; 24.6% (95% CI, 24.0-25.2) in spring, 17.9% (95% CI, 17.2-18.6) in summer, 38.5% (95% CI, 38.1-38.9) in autumn and 62.8% (95% CI, 62.6-63.0) in winter. Seroprevalence of *Salmonella* in serum decreased gradually between spring and summer, and increased considerably from 17.9% which is the lowest rate in summer until it reached a peak of 62.8% in winter (X = 209.2317, p<0.0001).

Prevalence by production stages

The results of Table 2 revealed *Salmonella* seroprevalence in the following age categories: 0 to 21 d (suckler), 21 to 70 d (weaner), 70 to 119 d (grower), 120 d and above (finisher), gilt and sow. The seropositivity rates in suckler, weaner, grower, finisher, gilt, and sow are 23.7%

Table 1. Seroprevalence of *Salmonella* by season in Korean pigs

Season	Individual survey		
	Selected samples (A)	Positive samples (B)	B/A (%) (95% CI[1)])
Spring	370	91	24.6 (24.0-25.2)
Summer	336	60	17.9 (17.2-18.6)
Fall	413	159	38.5 (38.1-38.9)
Winter	473	297	62.8 (62.6-63.0)
Total	1592	607	38.1 (38.0-38.2)

[1)] 95% confidence interval.
χ^2 = 209.2317, p<0.0001.

Table 2. Seroprevalence of *Salmonella* according to production stages in Korean pigs

Production stages	Individual survey		
	Selected samples (A)	Positive samples (B)	B/A (%) (95% CI[1)])
Suckler	270	64	23.7% (23.5-23.9)
Weaner	311	50	16.1% (16.0-16.2)
Grower	347	130	37.5% (37.3-37.7)
Finisher	217	91	41.9% (41.6-42.2)
Gilt	177	85	48.0% (47.6-48.4)
Sow	270	187	69.3% (69.1-69.5)
Total	1,592	607	38.1% (38.0-38.2)

[1)] 95% confidence interval.
χ^2 = 207.5812, p<0.0001.

(95% CI, 23.5-23.9), 16.1% (95% CI, 16.0-16.2), 37.5% (95% CI, 37.3-37.7), 41.9% (95% CI, 41.6-42.2), 48.0% (95% CI, 47.6-48.4), and 69.3% (95% CI, 69.1-69.5), respectively. Seroprevalence dropped between suckler (23.7%; 95% CI, 23.5-23.9) and weaner (16.1%; 95% CI, 16.0-16.2) stages, but then increased dramatically to the highest peak of 69.3% at the sow stage, 4 times higher than the weaner stage (X = 207.5812, p<0.0001).

Discussion

In Korea, Hazard Analysis Critical Control Point (HACCP) has established performance standards to reduce the prevalence of *Salmonella* in swine through on-farm interventions (Kim *et al*., 2008). However, there is no useable data on the prevalence of *Salmonella* and official control program in pigs in Korea.

The estimate of *Salmonella* prevalence for a specific period could be a good indicator of how well salmonellosis control measures are working on farms (Funk *et al*., 2000). Here, we performed ELISA to measure the prevalence of *Salmonella* spp. because the prevalence of *Salmonella* has never been correlated with sero-positivity in Korea. The advantages of ELISAs for detecting pigs infected with salmonellosis over other methods include greatly improved sensitivity, the low cost per sample, and the ability to test large numbers of samples (Leotides *et al*., 2003). However, the ELISA kit used in this study could only detect pigs infected with serogroups B, C1 and D of *Salmonella* spp. or pigs that had already produced antibodies from a prior *Salmonella* infection before sampling (van der Heijden, 2001).

The overall seroprevalence in our study was estimated at 38.13%, even though ELISA could only detect the antibody for serogroups B, C1, and D of *Salmonella*. The incidences of *Salmonella* spp. detected from swine fecal samples in the USA and Japan were only 4.9% and 3.1%, respectively (Bahnson *et al*., 2006; Kishima *et al*., 2008). In one Korean study, *Salmonella* incidence was reported at 9.5% from fecal samples (Kim *et al*., 2001). Therefore, sampling design might be an important characteristic related to *Salmonella* prevalence documentation (Sanchez *et al*., 2007). In a previous Korean study, *Salmonella* was isolated from 139 (21%) of 662 fecal samples as the result of bacterial culture on pigs with diarrhea or a history of diarrhea, which were lower results than the present study (Futagawa-Saito *et al*., 2008). This may be explained by a report comparing ELISA with bacterial culture, which indicated that a farm with a 50% prevalence determined by the blood ELISA showed only as 39% prevalence based on fecal culture sample methods, and an even lower value of 16% prevalence based on cecum analysis (Sanchez *et al*., 2007). Therefore, it is probable that the difference between detecting methods might affect the prevalence results.

Suh and Jung (2005) reported the highest prevalence of *Salmonella* was in suckling/nursery pigs. However, David *et al*. (1998) reported that prevalence reached the highest level in breeding pigs. Our study revealed that antibodies of *Salmonella* were extensively distributed throughout all stages of swine growth, with seroprevalence reaching a peak in the sow stage and being lowest in weaner pigs.

The early period after birth is important for the health and development of swine. During this period, the susceptibility to infectious diseases is not only affected by a variety of factors, including maturity of the immune system and susceptibility to tolerogenic signals, but maternal antibodies, especially IgA, also plays a critical role to protect animals from the diarrheal diseases (Offit and Clark, 1985). In our results, the swine maternal antibody was responsible for the weaner pigs having the lowest level of S*almonella* seropositivity. In addition, the dramatic increase of seroprevalence from weaner to grower may suggest that the highest number of infections break out between day 22 and day 70 of life. Improvement and application of health control during this period might be an important key point in controlling the spread of *Salmonella*. However, the continued increase of seroprevalence throughout all growth stages of swine showed that *Salmonella* infection was not limited only to the younger swine.

Jette *et al*. (1998) demonstrated that Danish swine had higher seroprevalence in winter than summer, which was similar to our results. Our results clearly indicated a seasonal pattern in salmonellosis outbreaks. In winter, it is not only difficult to control the ventilation and the temperature on farms, but the unpredictability of weather conditions makes proper setting of ventilation systems difficult, to create conditions that can cause stress and encouraging bacterial infection. The ventilation of swine barns is a compromise between maintaining adequate air exchange while conserving heat (Funk and Gebreyes, 2004). Swine farms equipped with the wrong ventilation system have reported a severe economic loss by dint of wasting diseases and faulty ventilation.

ELISAs have been proven to be a useful tool for the detection of farms contaminated with *Salmonella*, but it cannot give information on the infectious status of individual swine at a specific point in time (Leotides *et al*., 2003). The results indicated by ELISA are different when polymerase chain reaction or bacterial cultures are used. Therefore, we will conduct further studies in the future regarding the difference of prevalence data collecting using these different detection tools.

In conclusion, the results of the prevalence pattern in this study indicate that the things associated with season and production stages could act as the risk factors provoking the increase of *Salmonella* seroprevalence.

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References

1. Bahnson, P. B., Fedorka-Cray, P. J., Ladely, S. R., and Mateus-Pinilla, N. E. (2006) Herd-level risk factors for *Salmonella enterica* subsp. Enterica in U.S. market pigs. *Prev. Vet. Med.* **76**, 249-262.
2. Christensen, J. and Rudemo, M. (1998) Multiple changepoint analysis applied to the monitoring of *Salmonella* prevalence in Danish pigs and pork. *Prev. Vet. Med.* **36**, 131-143.
3. David, P. R., Bovie, F. G. M., Funk, J. A., Morrow, W. E. M., Jones, F. R., and Deen, J. E. (1998) Isolation of *Salmonella* serotypes from feces of pigs raises in multi-site production system in NC, USA. *J. Am. Med. Assoc.* **212**, 1925-1929.
4. Funk, J. and Gebreyes, W. A. (2004) Risk factors associated with *Salmonella* prevalence on swine farms. *J. Swine Health Prod.* **12**, 246-251.
5. Funk, J. A., Davies, P. R., and Nichols, M. A. (2000) The effect of fecal sample weight on detection of *Salmonella* Enterica in swine feces, *J. Vet. Diagn. Invest.* **12**, 142-148.
6. Futagawa-Saito, K., Hiratsuka, S., Kamibeppu, M., Hirosawa, T., Oyabu, K., and Fukuyasu, T. (2008) *Salmonella* in healthy pigs: Prevalence, serotype diversity and antimicrobial resistance observed during 1998-1999 and 2004-2005 in Japan. *Epidemiol. Infect.* **136**, 1118-1123.
7. Griffith, R. W., Schwartz, K. J., and Meyerholz, D. K. (2006) *Salmonella*. In Diseases of Swine ed. Straw, B. E., Zimmerman, J. J., D'Allair, S., and Taylor, D. J. pp. 739-754. Ames: Blackwell Publishing Professional.
8. Kim, S. H., Chun, S. G., Park, M. S., Park, J. H., and Lee, B. K. (2008) Finding the sources of Korean *Salmonella enterica* Serovar Enteritidis PT4 isolates by pulsed-field gel electrophoresis. *J. Microbiol.* **46**, 209-213.
9. Kim, S. Y., Lee, H. M., Kim, S., Hong, H. P., and Kwon, H. I. (2001) Epidemiological aspects of *Salmonella* spp. infections of domestic animals in Gyeongbuk province. *Korean J. Vet. Serv.* **24**, 43-50.
10. Kishima, M., Uchida, I., Namimatsu, T., Osumi, T., Takahashi, S., Tanaka, K., Matsuura, K., and Yamamoto, K. (2008) Nationwide surveillance of *Salmonella* in the faeces of pigs in Japan. *Zoonoses Public Health*, **55**, 139-44.
11. Leotides, L. S., Grafanakis, E., and Genigeorgis, C. (2003) Factors associated with the serological prevalence of *Salmonella* Enterica in Greek finishing swine herd. *Epidemiol. Infect.* **131**, 599-606.
12. Merialdi, G., Barigazzi, G., Bonilauri, P., Tittarelli, C., Bonci, M., D'incan, M., and Dottori, M. (2008) Longitudinal study of *Salmonella* infection in Italian farrow-to-finish swine herds. *Zoonoses Public Health*, **55**, 232-236.
13. Offit, P. A. and Clark, H. F. (1985) Protection against rotavirus induced gastroenteritis in a murine model by passively acquired gastrointestinal but no circulating antibodies. *J. Virol.* **54**, 58-64.
14. Sanchez, J., Dohoo, I. R., Christensen, J., and Rajic, A. (2007) Factors influencing the prevalence of *Salmonella* spp. in swine farms: A meta-analysis approach. *Prev. Vet. Med.* **81**, 148-177.
15. Suh, D. K. and Jung, S. C. (2005) Epidemiological characteristics of *Salmonella* spp. isolated from different stages of commercial swine farms. *Korean J. Vet. Serv.* **45**, 179-183.
16. Thorns, C. J. (2000) Bacterial Foodborne Zoonoses, *Rev. Sci. Tech*. **19**, 226-239.
17. van der Heijden, H. M. (2001) First international ring trial of ELISAs for *Salmonella*-antibody detection in swine. *Berl. Munch. Tierarztl. Wochenschr.* **114**, 389-392.
18. Yoshida, C., Franklin, K., Konczy, P., McQuiston, J. R., Fields, P. I., Nash, J. H., Taboada, E. N., and Rahn, K. (2007) Methodologies towards the development of an oligonucleotide microarray for determination of *Salmonella* serotypes. *J. Microbiol. Methods* **70**, 261-271.

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