academicJournals

Vol. 7(25), pp. 3272-3274, 18 June, 2013
DOI: 10.5897/AJMR2013.5630
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Short Communication

Estimation of the *Salmonella* spp. prevalence in pig farms with dry and wet feeding

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Accepted 5 June, 2013

Human salmonellosis originating from pork is an important zoonotic disease, and the objective of this study was to determine whether the *Salmonella* shedding was lower for pigs herds provided wet-feed compared to those on traditional dry rations. Four wet-feeding farms and six dry-feeding farms were selected. Individual faecal and feed samples were collected (faces 30, feed 10 from pigs per farm), and analysed for the presence of *Salmonella*. The results showed a low level of on-farm *Salmonella* shedding (overall prevalence from faecal samples 2.0% as well as 1.0% of the feed samples). The overall prevalence was 30% in studied farms (3 out of 10). Two of the dry-feeding farms (33.33%) tested positive compared to only one of the wet-feeding farms (25%). *Salmonella* was isolated in 5 of 180 faecal samples from farms with dry-feeding, compared to farms with wet-feeding where it was isolated in only one sample out of 120. *Salmonella* was also recovered from the feed on one dry-feeding farms but were not isolated from the farms using wet-feeding. These findings indicate that farms with wet-feeding are associated with lower ($p<0.01$) prevalence of *Salmonella*.

Key words: Pigs, *Salmonella,* feeding, prevalence, risk-factor.

INTRODUCTION

Pork and pork products are recognized as important sources of human salmonellosis (Smith et al., 2010). *Salmonella* is an important cause of food-borne (alimentary) health problems in humans (Hernandez et al., 2013). The risk of *Salmonella* might differ between the production systems, caused by components of the husbandry systems affecting disease development and pathogen shedding (Zheng et al., 2007), or differences in the level of resistance to the pathogen.

On-farm interventions to reduce *Salmonella* prevalence are difficult to implement; nevertheless they are important to reduce the risk of *Salmonella* on pig skin and therefore the risk of contamination of carcasses at the abattoir (Blagojevic et al., 2011). “Good hygiene practice” and “all-in all-out management” are not sufficient to reduce the spread of *Salmonella* in many circumstances. In addition to these steps, the feed is an important component of *Salmonella* control program, in particular, the type of feed appears to be strongly associated with the presence of *Salmonella*. Many studies indicate that pig farms that use dry-feeding have a higher prevalence of *Salmonella* (Lo FoWong et al., 2004; vanWinsen et al., 2002) than farms with wet-feeding system, possibly using a fermented diet (van Winsen et al., 2002). The objective of this study was to use faecal culture to determine whether wet-feeding is associated with a lower

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Abbreviations: BPW, Buffered peptone water; **RVB,** Rappaport Vassiliadis broth; **DSE,** delayed secondary enrichment; **BG,** brilliant green; **TSI,** triple sugar iron.

Table 1. Distribution and presence of *Salmonella* spp. in fecal and feed samples among 6 dry and 4 wet feeding swine farms.

Farm	A	B	C	D	E	F	G	H	I	J	Total
Dry-feeding	yes	yes	yes	yes	yes	yes					6
Wet-feeding							yes	yes	yes	yes	4
Number (%) of feed samples positive	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Number (%) of fecal samples positive	0 (0.0)	1 (3.3)	4 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	6 (2.0)

Salmonella shedding than dry-feeding in farms in Serbia.

MATERIALS AND METHODS

Farms

The initial selection of herds is based on a subset of 10 farms with intensive way of keeping pigs in Serbia. The intensive way of keeping the pigs means a closed cycle of production from conventional feed and modern genetics. These 10 farms were selected randomly based on herd size. Of the 10 farms in this survey, 4 farms had wet-feeding and 6 farms had dry-feeding system for grower–finisher pigs (Table 1). The faecal and feed samples were collected during 2011 and 2012 from farrow-to-finish herds.

Sampling and laboratory analysis

Faecal and feed samples were collected from all 4 wet-feeding farms and 6 dry-feeding farms. Samples were collected into sterile containers and transported on ice packs to the laboratory within 2–4 h and cultured immediately. From each farm, 30 faecal samples and 10 feed samples were collected. Samples were cultured using 25 g of feces and a double enrichment step (Davies et al., 2000). Twenty-five grams of feces were added to 225 ml of buffered peptone water (BPW) and incubated for 24 h at 37°C. A 0.1 ml of sample was added to 9.9 ml of Rappaport Vassiliadis broth (RVB) and incubated for 24 h at 41.5°C. Cultures were kept at room temperature for 96 h. After that, 0.1 ml of RVB culture was inoculated into 9.9 ml of RVB and again incubated for 24h at 41.5°C. Finally, a loopful of the delayed secondary enrichment (DSE) broth was plated out on xylose–lysine–tergitol 4 (XLT-4) agar and brilliant green (BG) agar and incubated at 37°C for up to 72 h and examined every 24 h for growth.

Salmonella spp. colonies were selected and tested on triple sugar iron (TSI), lysine decarboxylase, citrate and urease agar, and examined with a slide agglutination test employing polyvalent anti-*Salmonella* antisera to determine the presence of *Salmonella*-specific somatic or O antigen. The isolates were stored at -28°C in cryovials containing 0.3 ml tryptic soy broth (TSB), 0.3 ml glycerol, and 0.6 ml of a 2 h culture of the isolate. A farm was classified as positive when *Salmonella* spp. was isolated from at least one faecal sample.

Data analysis

Data were entered into an Excel spreadsheet (Microsoft Excel 2010) and imported into Stata (Stata 8 Intercooled for Windows 9x) in which data were analyzed. Descriptive analysis was done in MiniTab version 14 (MiniTabR14b) and Excel (Microsoft Excel 2010). The data were processed using analysis of variance (ANOVA) and Post Hoc Test was used for comparison of the means of treatments. Statistical significance of differences between means was determined at the level of $p<0.01$.

RESULTS AND DISCUSSION

Salmonella spp. were isolated from 5 of 180 faecal samples (2.78%) from dry-feeding farms compared to 1 of 120 samples (0.83%) from wet-feeding farms, furthermore, two farms (33.33%) using dry-feeding had at least one positive sample compared to only one wet-feeding farms (25%). On a farm basis, of the two dry-feeding farms with positive faecal samples, one was positive for *Salmonella* on feed. On farms that use wet-feeding, *Salmonella* was not isolated from feed samples. One positive farm that uses wet-feeding had only one positive pig's faecal sample, and two positive farms with dry-feeding had 1-4 positive samples (Table 1).

The results of this study clearly indicate that wet-feeding was associated with a lower risk ($p<0.01$) of being culture positive for *Salmonella* compared to dry-feeding. The association between wet-feeding and lower *Salmonella* prevalence in swine farms has been reported previously (Farzan et al., 2006; Lo FoWong et al., 2004; van Winsen et al., 2002). Several explanations have been offered, including that during a natural fermentation process in wet feed, the pH is lowered due to the production of lactic acid and acetic acid by lactic-acid producing bacteria and the growth of yeasts (which inhibits growth of *Salmonella* on the feed) (van Winsen et al., 2001) or at least reduces the numbers beyond the detection limit. Gastro-intestinal tract microflora modification with lactic acid-producing bacteria is a mechanism for *Salmonella* exclusion (Canibe and Jensen, 2003; van Winsen et al., 2002). Likely, this protective effect in this research is also based on the same principle. The

Salmonella shedding prevalence in the Denmark study (Bonde and Sørensen, 2012) and in the Switzerland study (Wacheck et al., 2012; Ledergerber et al., 2003) is similar to that in our study (2.3%). The amount of faeces, secondary and delayed enrichment, may affect the sensitivity of bacterial culture (Šišak et al., 2011; Funk et al., 2000). In this study, we used 25 g of faeces for faecal sample and applied two enrichment steps in order to increase the sensitivity of the test. Certainly, the overall *Salmonella* prevalence, 33.3% of the surveyed farms and 6% of all examined samples obtained in our study, is probably lower than the true prevalence of *Salmonella* in Serbia.

Feed samples indicate that *Salmonella* was present on one of the 10 (10.0%) farms. A total of 100 feed samples were cultured and the rate of isolation was 1.0% (one of 100 positive for *Salmonella*). Similar results have been confirmed in studies by Fedorka-Cray et al. (1997) (0.7%). Our estimates of prevalence of *Salmonella* on these particular farms may be low because we obtained only a small number of samples and visited the herds only once.

ACKNOWLEDGEMENT

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No. TR31034.

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