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The prevalence and load of *Salmonella*, and key risk points of *Salmonella* contamination in a swine slaughterhouse in Jiangsu province, China

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ABSTRACT

We investigated *Salmonella* contamination in a slaughterhouse in Jiangsu province (China) by analysing prevalences, loads, and serotypes of *Salmonella* isolates. In total, 480 samples were collected, with total prevalence of 25.4%. High *Salmonella* prevalence and load were observed at exsanguination and splitting stages (40.6% and 75.0%; 2.50 ± 0.94 and 2.24 ± 0.72 logMPN/m^2, respectively), with low prevalence and load at dehairing, flaming, and chilling stages (2.5%, 5.0%, and 15.0%; 1.39 ± 0.42, 1.36 ± 0.31, and 1.38 ± 0.30 logMPN/m^2, respectively). The *Salmonella* prevalence and load increased substantially from flaming to splitting stage. Six serovars were represented among 122 *Salmonella* isolates; *S.* Derby and *S.* Typhimurium were predominant and were subtyped using PFGE and CRISPR typing approaches. Fourteen PFGE clusters were identified, with discrimination indices (DI) of 0.929 and 0.976 for *S.* Derby and *S.* Typhimurium, respectively. Clusters A1, A2, B, C1, C3, C4, D1, F, I, J, L1, M2, and N2 indicated the isolates from same sampling visit were distributed in same cluster. Cluster D1 and K showed that the strains isolated from splitter and carcass swab-samples were highly resemble. *Salmonella* serovars with the same CRISPR type were mostly isolated after splitting (represented by Dercr 1, Dercr 2, Dercr 3, Tycr 1, Tycr 2, Tycr 3, Tycr 4, and Tycr 6). Our findings revealed that introduced *Salmonella* was the major source of swine carcass contamination; three slaughtering steps (polishing, rectal drilling, and evisceration) between flaming and splitting were important risk points for *Salmonella* release, post-splitting slaughtering processes were major contamination risk points, and the splitter was a contamination factor. Our data suggest routes for controlling *Salmonella* contamination in a swine slaughterhouse.

1. Introduction

Bacteria from the genus *Salmonella* are among the most common foodborne pathogens (EFSA and ECDC, 2015) and one of the major causes of human gastroenteritis and bacteraemia worldwide (Hendriksen et al., 2011). It has been estimated that 1.2 million human beings are annually infected by *Salmonella* in the United States (Scallan et al., 2011). Swine is a common carrier of *Salmonella* (EFSA, 2012; Fosse, Seegers, & Magras, 2009), and pork consumption is a major factor in bacterial ingestion associated with human salmonellosis (Zhao et al., 2008). *Salmonella* contamination in swine slaughterhouses should be addressed by the

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implementation of monitoring and control measures.

Official surveillance data for *Salmonella* in China is not available, but increasing numbers of studies are being conducted to investigate the basic contamination status in swine slaughterhouses. For example, the prevalence of *Salmonella* was found to be 10.7–71.8% (Cai et al., 2016; Li et al., 2013; Zhou et al., 2017), with *S.* Derby and *S.* Typhimurium being the common predominant serovars. Nevertheless, reports describing the enumeration of *Salmonella* in swine slaughterhouses in China are scarce. Quantifying *Salmonella* contamination would aid in understanding the dynamic changes of *Salmonella* loads in slaughtering lines and devising a quantitative microbial risk assessment to provide data supporting the control of *Salmonella*.

To control *Salmonella* contamination in swine slaughterhouses, the sources of bacteria should be first identified. Subtyping has identified house flora as the major source of swine contamination in some studies (Baptista, Dahl, & Nielsen, 2010; Visscher et al., 2011), while other studies have pointed to introduced *Salmonella* as the contamination source (Piras, Brown, Meloni, Mureddu, & Mazzette, 2011). Thus, the source of contamination might be slaughterhouse-specific.

Different types of molecular-based subtyping methods have been employed in epidemiological studies of *Salmonella* isolates, including pulsed-field gel electrophoresis (PFGE) (Li et al., 2016) and clustered regularly interspaced short palindromic repeats (CRISPR) (Zheng et al., 2016). PFGE typing is based on digesting chromosomal DNA with a rare-cutting restriction enzyme, followed by electrophoresis (Shariat et al., 2013). CRISPR typing, which entails sequence analysis of CRISPR arrays (Shariat & Dudley, 2014) is a new approach whose applicability in certain scenarios should be validated.

Here, we investigated *Salmonella* prevalences, loads, and serovars of *Salmonella* isolates in a single slaughterhouse to survey the contamination status. PFGE and CRISPR typing identified the source of *Salmonella* responsible for pig slaughterhouse contamination and possible contamination risk points.

2. Materials and methods

2.1. Sample collection and Salmonella detection

Salmonella contamination was evaluated in a slaughterhouse in Jiangsu, China. Approximately 2000 pigs are slaughtered at this slaughterhouse each day; the slaughtering process is shown in Fig. 1. Four sampling visits were separately conducted from October 2016 to April 2017, every one or two months. The carcasses, equipment, environment, and intestinal contents were probed as

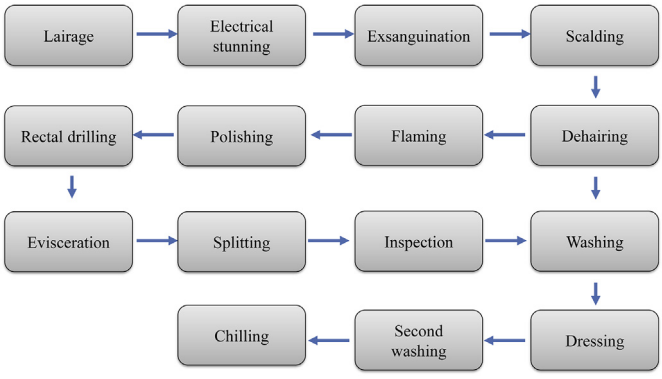

Fig. 1. Slaughtering processes in a swine slaughterhouse investigated in the current study.

potential contamination sources, as described previously (Zhou et al., 2017). Briefly, pig carcass swab samples were collected at six locations (trotter, snout, belly, kettleback, and both buttocks) before splitting and six locations after splitting (neck, belly, both buttocks, both transections) from eight steps, including exsanguination, dehairing, flaming, splitting, washing, dressing, second-washing, and chilling, which are identified as important in pig slaughterhouse (Busser, Zutter, & Dewulf, 2013; Swart, Evers, Simons, & Swanenburg, 2016). Each location was swabbed over a 100 cm^2 area using six cotton balls, which were then collected in sterile plastic bags. Environmental samples include lairage-area swab samples, slaughter floor swab samples, and scalding water. Lairage-area swab samples were obtained by swabbing the overshoes used in the slaughterhouse lairage by the authors. Slaughter floor swab samples were collected by swabbing a 1 m^2 floor area at the six sites for each slaughter step. For scalding water samples, 15 mL of water from a scalding tank was collected in sterile collection tube, and each sample was collected at different sites in the scalding tank. Equipment samples from the fleam, grainer, driller, and splitter were swabbed using cotton balls and immediately transferred to a sterile plastic bag. Approximately 5 g intestinal contents were collected for intestinal content sample. Cotton balls were prepared by soaking 200 cotton balls in 500 mL buffered peptone water (Difco, BD, Sparks, MD).

All samples were placed in an icebox, transported to Jiangsu Key Laboratory of Zoonosis (Yangzhou University), and processed within 10 h.

To isolate *Salmonella*, each sample was transferred to 100 mL of buffered peptone water (Difco, BD, Sparks, MD) and incubated at 37 °C for 24 h. Then, 0.1 mL of the broth culture was sub-cultured in 10 mL of Rappaport–Vassiliadis R10 broth (RVR10) (Difco, BD) and incubated at 42 °C for 24 h. The RVR10 broth culture was then streaked onto xylose lysine tergitol 4 (Difco, BD) agar plates and incubated at 37 °C for 20–24 h. Presumptive *Salmonella* isolates were selected and analysed using the API 20E assay system (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions. Confirmed *Salmonella* isolates were serotyped based on the Kauffmann–White scheme.

2.2. Salmonella enumeration

The most probable number (MPN) method (Hoek et al., 2011; Tadee, Boonkhot, & Patchanee, 2014) was used to enumerate *Salmonella* in some carcass swab samples, including 4 samples taken after exsanguination, dehairing, flaming, splitting, washing, and chilling. These samples were extruded and buffered peptone water was presented and served as primary liquid of 10-fold serial dilutions. Then, three consecutive 10-fold serial dilutions were prepared in buffered peptone water, in triplicate, and were enriched by incubating for 24 h at 37 °C. The enriched cultures were then sub-cultured in RVR10 broth and plated on the selective xylose lysine tergitol 4 agar plates, as described in section 2.1. Presumptive *Salmonella* isolates were selected and analysed using the API 20E assay system, according to the manufacturer's instructions.

Consequently, an array of positive numbers was obtained from the three serial dilutions per sample, and the MPN value of each sample was determined using an MPN table (Sutton, 2010). To estimate the MPN value per m^2, we assumed that the bacteria were distributed homogeneously over the surface of each pig carcass.

2.3. PFGE typing

PFGE typing with the *Xba* I restriction enzyme was employed to analyse *S.* Derby and *S.* Typhimurium isolates, as previously described (Ribot et al., 2006; Zhou et al., 2017). Gel images were