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Surveillance of Mainly Pathogenic Bacteria Contaminant Impacting Pork Safety During Pig Slaughtering

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To understand the pathogenic factors that impact pork safety and quality during pig slaughtering, so that set out control specification of pathogenic bacteria available for different slaughter scale level. 1712 samples were selected at 6 key slaughtering link such as good-rest before slaughter, showering, slaughtering process, precool and pork storage. *Salmonella spp.*, *E. coli* O157: H7 and *L. monocytogene* were tracking monitored so as to analyse contaminant factors from different slaughtering process. Total detection rate of mainly pathogenic bacteria in 6 slaughtering process was 26.2%, including *Salmonella spp.* 16.3%, *E. coli* O157: H7 1.0% and *L. monocytogene* 4.2%. The superiority serotypes of *Salmonella spp.* were *S. derby*, *S. typhimurium* and *S. thompson*. Detection rate of both *Salmonella spp.* and *E. coli* O157: H7 increased after showering during slaughtering, and reached peak while half-chop finished. The detection rate of *Salmonella spp.* was lowest (5.4%) while fresh pork was stored in refrigerator house. Highly pathogenic bacteria contaminant or cross-contaminant were observed during pig slaughtering, especially in the lower slaughter scale level. Pathogenic bacteria control measures or good slaughter practice should be set out according to different slaughter process so as to ensure pork safety.

Key words: Pathogenic bacteria; surveillance; bacteria contaminant; slaughtering; animal origin food safety.

Foodborne pathogenic bacteria is one of the important factors impacting animal derived product quality and safety. According to the European food safety authority (EFSA) 2013 annual report[1], with the toxic substances such as veterinary drugs and heavy metal residue under control, pathogenic microorganisms such as *Salmonella spp.*, *E.coli* O157: H7 and *L. monocytogenes* has become main factors of animal origin food safety in European. China, as a consumption power country of pigs and pork products in the world, pathogenic bacteria contaminant status of pig products in most areas of China was not optimistic. Preliminary surveillance studies revealed that general detection rate of pathogenic bacteria such as *Salmonella spp.*, *E.coli* O157: H7 and *L. monocytogenes* in some regional commercial fresh pork , and *Salmonella spp.* contamination detection rate from pig carcass were 17.82 and 54.45%, respectively[2, 3].

Quality and safety control for fresh pork has always been the focus of animal origin food safety management in China. Since June 2013, the pig slaughter management functions that took on more than 60 years by the Ministry of Commerce were allotted to the Ministry of Agriculture in order to further rationalize pig slaughtering and pork quality security relations[4]. From the slaughter process, pig slaughtering link control of pathogen bacteria involved consecutive links such as pig

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breeding, slaughter quarantine, slaughtering and pork processing before storage. This study was designed to track monitoring the main pathogenic bacteria carrier status such as *Salmonella spp.*, *E. coli* O157: H7 and *L. monocytogenes* by detection of samples from good-rest before slaughter, showering, slaughtering process, precool and pork storage, so as to find out pathogen factors impacting pig slaughtering pork quality and safety. Furthermore, basic data were prepared so as to further assess pork contaminant risk from pathogenic bacteria and there toxin, as well as searching for reduce and control measures of bacteria contaminant.

MATERIALS AND METHODS

Sample collection

A total of 1712 samples were collected during the period from March 2013 to December 2014. Six main pig slaughterhouse links such as anal swab before slaughtering, showering, carcass surface before and after splitting, precooling area fresh pork and cold storage pork surface were sampled in different slaughterhouse scale in Shandong province, China. Among them, anal swabs before slaughtered and cotton swabs from pig body surface after showering was collected samples of 100 and 212, respectively. The remaining links each was sampled surface swabs 350. All fresh samples collected were put in 2ml sterile tubes containing Luria-Bertani (LB) medium (30% glycerol added), and transported to laboratory within 12 hours. Analyses began immediately after the arrival at the laboratory.

Separation and identification of Salmonella isolates

Salmonella spp. was separation of cultivation and preliminary identification[5]. Presumptive colonies from the positive culture and biochemical test results were confirmed as *Salmonella* by using PCR to assay for the presence of the invA gene[6]. Each putative *Salmonella* colony was placed in 20 µL of DNasefree water (Invitrogen) and boiled to lyse the cells. The lysate was centrifuged to sediment cell debris, and 2 µL of the lysate was used as a template in invA PCR. Each PCR mixture consisted of 22 µL of PCR SuperMixm (Invitrogen) and a 0.2 µL Mconcentration of each primer (139F and 141R)[7]. Cycling conditions included an initial denaturation step of 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s and a final extension step at 72°C for 7 min. PCR products were separated in 1.5% agarose gels and examined by UV transillumination using a UVgel imager (Bio-Rad, Hercules, CA). All confirmed *Salmonella* isolates were analysed by serum credits type analysis in Luminex 200 test system (Luminex, USA). And then further characterized with *Salmonella* belong to O antigen diagnosis further determine serum[8].

Separation and identification of *E. coli* O157 isolates

All samples were identified to be *E. coli O157* using API 20E biochemical test strips (bioMérieux, France). Sorbitol fermentation characteristic was examined using sorbitol-MacConkey agar (SMAC) (Oxoid, UK). The determination of O antigens was firstly carried out by testing for specific *E. coli* O groups of interest targeting group specific genes within the O-antigen gene cluster described by Deb Roy[9]. The entire coding sequence of the *fliC* gene was amplified by PCR with the primers *fliC*-F (5'-ATGGCACAAGTCATTAATACCCAAC-3') and *fliC*-R (5'-CTAACCCTGCAGCAGAGACA-3') reported by Qing M[10]. Serotypes of each isolate were determined by agglutination tests with anti-*Escherichia coli* sera (SSI Denmark)[11].

Separation and identification of *L. monocytogenes*

Swab samples were pre-enriched in 10 ml BPW for 24± 2h at 30°C with shaking. Then 0.1ml BPW pre-enrichment broth was used to inoculate a selective Fraser enrichment broth for Listeria detection. After incubation, *L. monocytogenes* isolation was performed on chromogenic media agar (Oxoid, UK) and PALCAM Listeria selective agar containing supplement (Merck-Germany). The suspicious isolates were further confirmed with API Listeria (API, Appareil et Procédé d'Identification, bioMérieux, Marcy l'Etoile, France)[12].

RESULTS AND DISCUSSION

The results showed that *Salmonella* overall detection rate from slaughter pigs, slaughtering and pork storage link was 16.3% (279/1712). *Salmonella* isolate separation from anal swab

of before slaughtered pigs were 29.0% (29/100), while pig surface after showering was 11.8% (25/212). After entering slaughter lines, *Salmonella* contamination differences were significant in different links. Among which carcass surface detection rate after half-cutting was the highest (32.0%, 112/350), followed by carcass surface before scalped (18.0%, 63/350), surface of pre-cooling pork (11.7%, 41/350) and surface of storage pork (5.4%, 19/350). Among 279 strains of *Salmonella* isolated, 37 strains were not typed with Luminex-xMAP method. Other 242 strains of *Salmonella* belong to 13 serotypes, of which the first three superiority serotype was *S. Derby*, *S. Typhimum* and *S. Thomphson*, with the proportion of 45.0% (109/242), 24.4% (59/242) and 13.6% (33/242), respectively. Other serotypes, such as *S. Agama*, *S. Agona*, *S. Tsevie* were also isolated in Fig. 1. [17] *E. coli* O157: H7 positive isolates were detected from six selected link samples. The overall contaminant rate was 1.0% (17/1712), with carcass surface before scalped 6 positive samples and a single link contaminant rate was 1.7% (6/350), carcass surface after half-cutting 11 positive samples and single link contaminant rate was 3.1% (11/350). Specimens from other sampled links were all negative for *E. coli* O157: H7. Results showed that positive samples of *L. monocytogenes* was 72, accounting for 4.2% of the total samples(72/1712). Compared with *Salmonella* contaminant, *L. monocytogenes* contaminant was lower at slaughter link. It was not detected before the link of scalped. However, surface of storage pork contamination was relatively higher with the contamination rate 10.9% (38/350). From the monitoring results of three main pathogenic bacteria contaminant, contaminant after half-cutting was most severe with the average contaminant rate 38%. All the three monitored pathogenic bacteria were existed and had different degree of contaminant. Followed by carcass surface before scalped with contaminant rate 19.7, and mixed contaminant was observed for both salmonella and *E. coli* O157: H7, as shown in Fig. 2. The main pathogenic bacteria overall detection rate was 26.2% (448/1712). From Fig. 3, salmonella detection rate before slaughtered was higher, followed by *L. monocytogenes*, but without *E. coli* O157: H7 checked out. Once entering the slaughter line after showering, both *Salmonella* and *E. coli* O157: H7 detection rate was increased, peaking after half-cutting. Then, *Salmonella* detection rate

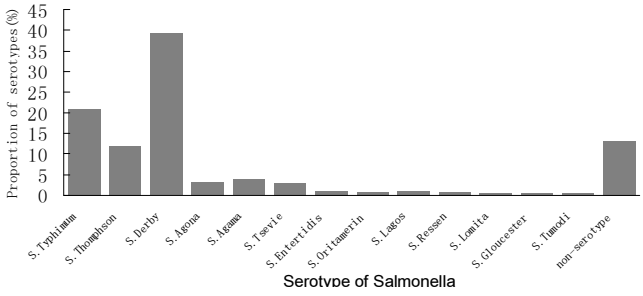

Fig. 1. The distribution of *Salmonella* serotypes isolated in the link of before slaughtered, slaughter process and fresh pork storage

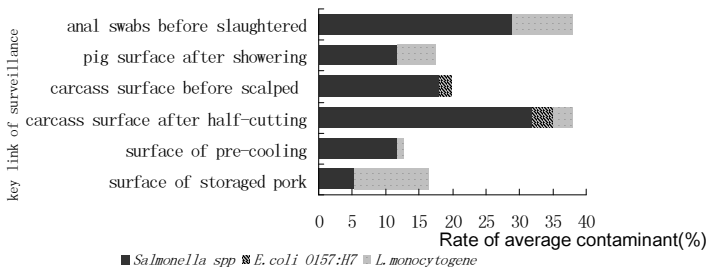

Fig. 2. The separation rate of key pathogen contamination in pig slaughtering and pork storage link

decreased and reached contaminant minimum level at storage pork phase(5.4%). At this link, *E.coli* O157: H7 was no longer checked out. *L. monocytogenes* contaminant was relatively irregular, but obvious change trend was observed at storage pork link with detection rate reaching its highest detection level, as shown in Fig. 3.

Pig slaughter links is one of the key risk links of pork contamination by pathogenic bacteria. This study chose slaughter capacity of 300 to 20, 000pigs per day of different scale slaughterhouse and sampled from 6 main slaughter link that impacting pork safety. Total 1712 samples were collected from such links as pig anal swab before slaughtered, pig surface after showering, carcass surface before scalped, carcass after half-cutting, pre-cooling and cold storage so as to assess the contaminant status of three main pathogenic bacteria. From the monitoring results, both *L. monocytogenes* and *Salmonella* were carried in slaughter pigs. Before slaughtered, pigs carrying high level pathogenic bacteria may cause contaminant to pork while slaughtering. Serious cross contamination maybe present between pigs, pig and slaughter appliance, pork and premises. However, pathogenic bacteria level decreased significantly after showering. It indicated that showering before slaughter was effective measures to reduce pathogenic bacteria contaminant to pork[13].

After showering, in the process of slaughter, *Salmonella spp.*, *E. coli* O157: H7 and *L. monocytogenes* average contaminant rate were 25.0%, 2.4% and 1.5%. While at the precooling link all the three kind of bacteria detection rate was 11.7%, 0% and 0.9% respectively. In addition, *E. coli* O157: H7 was only isolated from the link of carcass surface before scalped and after half-cutting. It maybe meant that the slaughter processing might be an important pathogen contaminant origin to pork. From pathogen monitoring at different slaughtering process, pathogen contaminant rate was highest at phage of carcass surface after half-cutting. In addition, scalp tools also carried higher contaminant. The reason of small scale slaughterhouse higher contaminant rate, for their scalp tools disinfection fewer or no more disinfected to those tools and causing serious contaminant. *L. monocytogenes* isolate rate was higher in storage pork link in refrigerator than the other two kinds of bacteria. Meanwhile, the contaminant rate was significantly higher than the slaughter links and pre-cooling pork link. On one hand, it showed that *L. monocytogenes* characteristic could proliferate at low temperature[14], on the other hand, contaminant of *L. monocytogenes* might exist in some fresh pork storage environment. In this study, *E. coli* O157: H7 was detected in the process of slaughter, but absent at the pork surface of pre-cooling and cold storage link. However, it was isolated frequently from sampling pork collected at retail or supermarkets[2, 13, 15], it might be for the machining process clean at late period and the lower temperature at the link of pre-cooling and pork storage. Contrarily, bacteria contaminant might exist at transportation, transport tools and storage environment.

Compare pathogenic bacteria contaminant status to scale of slaughterhouses and sampling seasons, HACCP was implemented better in large-scale slaughtering enterprises than small one. Furthermore, large scale slaughterhouse established good slaughter practices according to HACCP and national standard. But part of small or medium scale slaughter enterprises were not

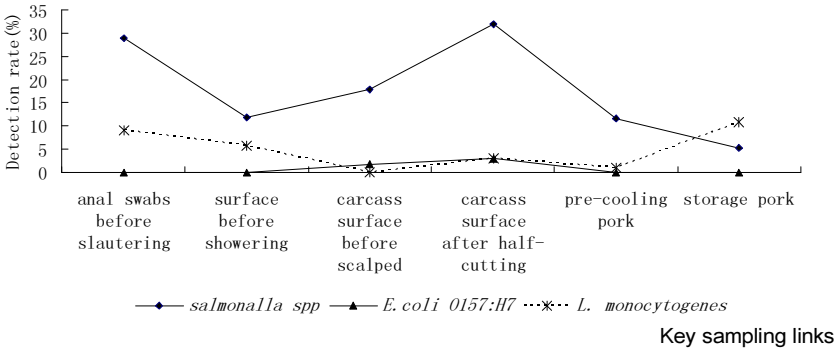

Fig. 3. Detection of the main pathogenic bacteria in different link

strictly comply with good practices, and in these premises pathogenic bacteria contaminant were some severe or not optimistic. Pathogen detection rate was also higher than the national standard limitations and requirements of management specification[16, 17]. Interestingly, sampling season also influenced pathogenic bacteria contaminant which was fewer contaminant rate of slaughter link in winter and higher in summer and autumn, followed by spring.

Superiority serotypes of *salmonella spp.* isolated from pig slaughterhouses in this study was *S. derby* (45.0%), *S. typhimurium* (24.4%) and *S. Thompson*(13.6%). This was similar to the result of pork producing link *salmonella spp.* surveillance in Sichuan province of China which was *S. derby* (58.0%), *S. typhimurium* (12.5%) and *S. Potsdam* (6.25%)[18], but differences with the superiority serotypes of *S. anatum* (32.38%), *S. derby* (29.05%) and *S. typhimurium* (6.19%) respectively in Yingkou city in Liaoning province of north-eastern China[3], as well as existing large discrepancy with slaughter link surveillance data in Kaifeng city of Henan province, central China which was *S. Agona* (34.1%) and *S. derby* (22.7%) was the superiority serotype in this region[19]. Thus, it furtherly indicated that complicated distribution of pig origin *Salmonella spp.* serotype existed in different regional in China.

Consuming pork contaminated by pathogenic bacteria of animal origin is one of the most common factors of food poisoning. Understanding the mainly pathogenic bacteria contaminant status of pigs before slaughtered, slaughtering link and stored pork, can not only timely grasp the area of foodborne pathogens contaminant status and distribution, but also have vital benefit for epidemiological investigation and porcine derived pathogenic bacteria monitoring.

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