

Analyses of prevalence and molecular typing of *Salmonella* in the goose production chain

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ABSTRACT This study investigated the prevalence of *Salmonella* and the molecular typing of all isolates in a goose production chain including hatchery, farm, slaughterhouse, and market. A total of 350 *Salmonella* isolates was detected from 1,030 samples, and 13 serotypes were recovered. The highest *Salmonella* contamination frequency was observed at the hatchery, which 51.8% (188/363) of samples were *Salmonella* positive. *S. Potsdam* and *S. Typhimurium* were the 2 most common serotypes. *S. Potsdam* was most frequently found in the hatchery, while *S. Typhimurium* was widely distributed in the goose production chain. In general, the antibiotic resistance of *Salmonella* isolates is low, which isolates from the market is comparatively higher than from other production links indicating a possibility of *Salmonella* cross-contamination in the market. By the multilocus sequence typing (MLST) analysis, 7 different ST types were identified. ST2039 was the most common ST type, which was mostly found from *S. Potsdam*

isolates in hatchery indicating that *S. Potsdam* might have been long existed in hatchery. The pulsed-field gel electrophoresis (PFGE) analysis of *S. Potsdam* indicated that *S. Potsdam* could be transmitted along the production chain. The PFGE analysis of *S. Typhimurium* showed that PFGE pattern 29 (PF29) was distributed in hatchery, and also in farm and from humans indicating the risk of *S. Typhimurium* transmitting to humans by the food supply chain. Our study provided the evidence of *Salmonella* cross-contamination in the slaughterhouse and the retail market of goose production chain, and specific serotypes existed for a long time at a particular production link. The spread of *Salmonella* along the production chain, might cause harm to humans through cross-contamination. Further studies would be needed to control the *Salmonella* contamination in hatchery and prevent the transmission of the pathogen during the goose production.

Key words: *Salmonella*, goose production chain, PFGE, MLST, food safety

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INTRODUCTION

Salmonella has been recognized as a major and important foodborne pathogen for humans and animals for more than a century, causing human foodborne illness as well as high medical and economic cost (Lee et al., 2015). It was estimated that non-typhoidal *Salmonella* causes about 93.8 million illnesses and 155,000 deaths

each year worldwide (Majowicz et al., 2010). The main sources of infection for humans include meat products, especially the consumption of contaminated poultry meat. The incidence of salmonellosis continues to increase, even in countries with a well-developed public health surveillance system. Therefore, *Salmonella* has extensive public attention and research worldwide (Antilles et al., 2015).

Salmonella is closely related to food safety. The prevalence of *Salmonella* associated with poultry and poultry meat products has been well-documented and this prevalence has both public health and economic implications (Cosby et al., 2015). Poultry has accounted

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for a higher percentage of *Salmonella* outbreaks of infection than other food commodities. It has been reported that 29 percent of *Salmonella* infections are caused by poultry (Scallan et al., 2011). However, many reports on *Salmonella* in poultry mainly focused on chickens and turkeys (Barrow et al., 2012). Recent studies have shown that waterfowl such as geese and ducks are also important sources of *Salmonella* (Grigar et al., 2017). *Salmonella* infection in geese and ducks is a recessive infection, but sometimes it can also have serious clinical symptoms with high mortality, and become the hidden danger of human health (Martelli et al., 2017).

China is the world's largest goose farming and consuming country. Every year, a large number of geese and goose eggs are purchased by Chinese consumers. However, there are few reports on *Salmonella* research on the goose production chain (including hatchery, farm, slaughterhouse, and market) in China. In the whole production chain of poultry, *Salmonella* contamination in any production link may lead to *Salmonella* contamination in the downstream production link, thereby increasing the potential for harm to human health. Enterprises such as avian product processing have evolved into a safety-centric industry involving all production areas (Leiva et al., 2018). Studying the contamination and prevalence of *Salmonella* in the whole production chain of goose is of great significance to food safety. Therefore, the purpose of this study was to investigate the prevalence of *Salmonella* in goose production chain of a large-scale goose farm in Yangzhou city, Jiangsu province, China. Serotyping and molecular typing were used to determine the relationship between the goose isolates and the relationship among the goose isolates and human isolates (8 strains of *S. Typhimurium* isolated from human by Yangzhou Center for Disease Control and Prevention). Provide data support for *Salmonella* prevention and control in the goose production chain.

MATERIALS AND METHODS

Sample Collection

In this study, 1,030 samples were collected from a large-scale goose farm between April, 2017 and December, 2018. The large-scale goose farm includes hatchery, farm, slaughterhouse, and with the market to form a complete production chain. It has 2 farms, with 20,000 parent geese and 100,000 commercial geese.

Hatchery Sample Collection

A total of 363 samples were collected from the hatchery. We collected swab samples from the outer wall, inner wall, ground, and egg tray of 15 incubators in hatchery, and randomly extracted the fertilized eggs, dead embryos, and Gosling. Incubator samples are wipes

from different areas of the incubator with a sterile buffered peptone water (BPW; Difco, BD, Sparks, MD) infiltrated cotton swab. These swabs were placed in a sterile sampling bag for labeling. The contents of the fertilized egg were mixed, and 10 g was moved into another sterile sampling bag for labeling. After anatomizing the dead embryo and gosling samples, the yolk sac and liver were removed, and placed in a sterile sampling bag, and marked.

Farm Sample Collection

A total of 364 samples were collected from the farm. We randomly collect stool samples, goose egg samples (goose eggs not yet in the hatchery) and feed samples in 15 goose houses on 2 farms and randomly sampled the water source. Approximately 10 g of fresh stool was picked up with sterile disposable gloves, placed in a sterile sampling bag and marked. Feed samples were taken from the 10 g of feed in goose trough and placed in a sterile sampling bag for marking. Water source samples were collected from the water in the goose house pool. After filtration, the filter was placed in a sterile sampling bag for marking. The method of collecting the goose egg samples was the same as the method of collecting the fertilized egg samples in the hatchery.

Slaughterhouse Sample Collection

A total of 126 samples were collected from the slaughterhouse. We collected swab samples from the goose carcass in the polishing, visceral removal, and freezing of the slaughterhouse, and randomly sampled the of the removed goose internal organs. All samples were collected using sterile sponges that were pre-moistened with BPW as described previously. To prevent cross-contamination, gloves were worn during sampling and changed after each sample (Bonardi et al., 2013).

Market Product Sample Collection

A total of 127 samples of goose meat products were collected from 5 downstream markets of the large-scale goose farm in Yangzhou. All samples are randomly selected and purchased. The samples were put in a disposable sterile sampling bag and marked.

Finally, all samples were transported in an icebox to the Jiangsu Key Laboratory of Zoonosis, Yangzhou University, and cultured for the isolation and identification of *Salmonella* (within 24 h of sample collection).

Isolation and Identification of *Salmonella*

Stool Samples There are many other intestinal bacteria in stool samples, and they interfere with the isolation of *Salmonella*. Therefore, we used the modified semi-solid Rappaport Vassiliadis (MSRV; Difco, BD, Sparks, MD) to isolate *Salmonella* (Soria et al., 2012).

Each sample was added 10 times the volume of BPW. Each sample was kept at 37°C, for 16 to 18 h of incubation, for the preliminary enrichment. BPW (300 µL) was added to MSR/V semi-solid medium in 3 aliquots and statically incubated for 24 h at 42°C for selective enrichment. One loopful of each MSR/V was then streaked onto xylose lysine tergitol 4 (XLT4; Difco, BD, Sparks, MD) agar plates, which were incubated at 37°C for 24 h. Suspicious colonies (circular, black, with a transparent annulus around) were picked for serotype identification. Biochemical identification was performed according to the API-20E Biochemical Reagent Guide. Colonies that were positive for biochemical identification were selected and purified again on XLT4 plates, and cultured at 37°C for 24 h. The suspected colonies were added to 4 mL liquid LB medium with a disposable sterile inoculating loop, and cultured for 12 to 16 h at 37°C on a constant temperature shaker for PCR biochemical identification.

Other Samples A water sample is first filtered and the filter is collected for the experiment. For swab and water samples, the pre-enrichment step was performed by suspending each sample in 50 mL BPW, and incubating the samples at 37°C for 16 to 18 h. For fertilized egg samples, goose egg samples, dead embryo samples, and gosling samples, the pre-enrichment step was performed by weighing 10 times the volume of BPW. After weighing, the samples were incubated at 37°C for 16 to 18 h. BPW pre-incubation droplets (1 mL) was added to 10 mL of Rappaport-Vassiliadis R10 broth (RVR10; Difco, BD, Sparks, MD) and statically incubated for 24 h at 42°C for selective enrichment. One loopful of each RVR10 broth culture was then streaked onto XLT4 agar plates, which were incubated at 37°C for 24 h. Suspicious colonies were picked for serotype identification. Biochemical identification was performed according to the API-20E Biochemical Reagent Guide. Colonies that were positive for biochemical identification were selected and purified again on the XLT4 plates, and cultured at 37°C for 24 h. The suspected colonies were picked up in the 4 mL liquid LB medium with a disposable sterile inoculating loop, and cultured for 12 to 14 h at 37°C on a constant temperature shaker, for PCR biochemical identification.

For market samples from retail markets, each sample (25 ± 0.5 g) was aseptically weighed and transferred into 225 mL of BPW and incubated at 37°C for 18 h. The pre-enrichment and following isolation and identification were performed as described above (Cai et al., 2016).

Antimicrobial Susceptibility Testing A total of 183 strains of *Salmonella* isolates (including 8 strains of human *S. Typhimurium* isolates) were tested for antimicrobial susceptibility testing. The Kirby–Bauer disk diffusion method was used to determine the isolates' antimicrobial susceptibility (Li et al., 2014). A total of 16 antimicrobial agents were applied: ampicillin (AMP, 10 µg), amoxicillin (20 µg), meropenem (10 µg), cefazolin (30 µg), aztreonam (ATM, 30 µg), nalidixic acid

(NAL, 30 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), tetracycline (TET, 30 µg), chloramphenicol (CHL, 30 µg), kanamycin (20 µg), amikacin (AK, 30 µg), gentamicin (10 µg), streptomycin (STR, 10 µg), trimethoprim-sulfamethoxazole (SXT, 10 µg), and nitrofurantoin (30 µg).

Multilocus Sequence Typing (MLST) We randomly selected 1/3 (104) *Salmonella* isolates and 8 human *Salmonella* isolates for multilocus sequence typing (MLST). Confirmed isolates were grown aerobically in LB broth with shaking overnight at 37°C. Genomic DNA was extracted with a TIAN amp Bacteria DNA Kit (Tiangen, Beijing, China) in strict accordance with the manufacturer's protocol. MLST was performed as described online (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/documents/primersEnterica_html). All polymerase chain reaction products were purified and sequenced by Nanjing GenScript Biotech Co. (Nanjing, China). The alleles and sequence type (STs) were assigned according to the MLST scheme at <http://mlst.warwick.ac.uk/mlst/dbs/Senterica>. A minimum spanning tree was generated using BioNumerics software, version 7.5 (Applied Maths, Kortrijk, Belgium) to analyze the distribution of STs in the goose production chain.

Pulsed Field Gel Electrophoresis (PFGE) A total 55 strains of *S. Typhimurium* (including 8 strains of human *S. Typhimurium* isolates) and 34 strains of *S. PotSDam* in different links of the goose production chain were analyzed by pulsed field gel electrophoresis (PFGE). PFGE was performed according to the protocol of the Centers for Disease Control and Prevention with some modifications (Ribot et al., 2006). In brief, *Salmonella* isolates were streaked onto LB plates and incubated overnight at 37°C. The pathogen concentration was modulated with bacterial suspensions until a McFarland turbidity of 4.0~4.5 was attained. DNA was digested with 50 U *Xba*I (Takara, Dalian, China) at 37°C for 3 h. The digested DNA was separated by electrophoresis in 0.5 × TBE buffer at 14°C for 20 h using a CHEF Mapper electrophoresis system (Bio-Rad, Hercules, CA). The pulse time was ramped from 2.16 to 63.8 s. In addition, a control strain of *S. Braenderup* (H9812), which served as a molecular weight standard, was processed with each batch of isolates. The gels were stained with ethidium bromide, and DNA patterns were visualized on a UV trans-illuminator (Bio-Rad, Hercules, CA). Dendrograms were created by BioNumerics software version 7.5 (Applied Maths, Kortrijk, Belgium), using the unweighted pair group method with arithmetic mean. The band-matching settings with optimization of 0.5% and position tolerance of 1.5% were applied.

Data Analysis

Data on the prevalence of *Salmonella* isolates from this study were analyzed using the statistical software program, SPSS (version 16.0, SPSS, Chicago, IL). The

data was compared using the chi-square test, with $P < 0.05$ regarded as being statistically significant.

RESULTS

Salmonella Isolation Rate and Serotype Distribution

As shown in [Table 1](#), a total of 1,030 samples were collected in this study, and 350 strains of *Salmonella* were isolated. The total positive rate of *Salmonella* isolates was 33.9%. In all the links of production chain, the highest *Salmonella* contamination frequency was observed at the hatchery, which 51.8% (188/363) of samples were *Salmonella* positive, followed by 43.8% (77/176) in the slaughterhouse. The positive rate of *Salmonella* isolates of the farm was 17.6% (64/364). The positive rate of *Salmonella* in market was 17.3% (21/127). The isolation rate of *Salmonella* in the hatchery and slaughterhouse was significantly higher than that of the farm and market ($P < 0.05$). In the hatchery, the isolation rate of *Salmonella* in dead embryo and gosling samples is significantly higher than that in other samples ($P < 0.05$). In the farm, the isolation rate of *Salmonella* in water samples is significantly higher than that in the other samples ($P < 0.05$). In the slaughterhouse, the isolation rate of samples (internal organs, polished, and visceral removal) during processing is higher than that of frozen samples ($P < 0.05$).

Thirteen different serovars were identified among the 350 positive *Salmonella* isolates ([Table 2](#)). The hatchery isolates contain 8 serotypes, the farm contains 6 serotypes, the slaughterhouse contains 5 serotypes, and the market contains 3 serotypes. In this study, the most common serotype was *S. Potsdam*, with an isolation rate of 49.7%. However, *S. Potsdam* is mainly from the hatchery ([Table 2](#)). Followed by *S. Typhimurium*, with an isolation rate of 32.6%, which was widely distributed in all links of the production chain ([Table 2](#)). Other serotypes were isolated in small amounts and concentrated in 1 or 2 links ([Table 2](#)), for example, the *S. Hadar* was isolated only on market.

Antimicrobial Resistance Phenotypes

The susceptibility to 16 antibiotics of 175 strains of *Salmonella* from the goose production chain, and 8 strains from human is shown in [Supplementary Table S1](#). The overall resistance of *Salmonella* isolates in this study is low. Resistance to NAL was the most commonly observed resistance in this study (8.2%). Resistance rates for STR (6.6%), AMP (6.0%), and TET (4.9%) were relatively high. All strains were not resistant to both ATM and AK ([Supplementary Table S1](#)).

Individual strains of *Salmonella* isolate from hatchery and farm are resistant to 1 type of antibiotic and have a lower overall resistance. The *Salmonella* isolates in the slaughterhouse were not resistant to 16 antibiotics. The isolates of *Salmonella* in the market were highly resistant to NAL and STR, reaching 100% and 75%, respectively. That human *Salmonella* isolates were the most severely resistant, the resistance to AMP and TET reached 100%. The resistance to SXT (50%) and CHL (50%) was also higher ([Supplementary Table S1](#)). As shown in [Table 3](#), *S. Typhimurium* has the most severe multidrug resistance, and the remaining serotypes have low multidrug resistance.

MLST Analysis

A total of 112 strains of *Salmonella* were analyzed by MLST. An interlinked dataset with partial sequencing of the 7 housekeeping genes from 399 bp to 501 bp revealed that 104 strains of *Salmonella* isolates from this sample were divided into 7 ST types (ST33, ST39, ST367, ST19, ST99, ST3975, and ST198). A minimum spanning tree of all ST types from both sources was generated using BioNumerics version 7.5 ([Figure 1](#)). Most of the hatchery samples were ST2039, but there was 1 strain of ST3975. Human isolates of *S. Typhimurium* and market samples of *S. Typhimurium* isolates belong to ST19, and slaughterhouse and farm isolates of *S. Typhimurium* belong to ST99. There is only 1 housekeeping difference between ST3975 and ST19, and there are 2 housekeeping differences between ST3975 and ST99. The STs in this study were correlated with specific serovars such as ST2039 with *S. Potsdam*, and ST33 with *S. Hadar*.

Table 1. Isolation of *Salmonella* from different links.

Link	Sample	Number of samples	Isolate (%)	Link separation rate (%)
Hatchery	Dead embryo	104	68 (65.4)	51.8
	Incubator	191	81 (42.4)	
	Fertilized egg	30	15 (50.0)	
	Gosling	38	24 (63.2)	
Farm	Stool	291	49 (16.8)	17.6
	Water	10	4 (40.0)	
	Goose egg	48	11 (22.9)	
	Feed	15	0 (0)	
Slaughterhouse	Internal organs	25	17 (68.0)	43.8
	Polished	90	41 (45.6)	
	Visceral removal	31	15 (48.4)	
	Frozen	30	4 (13.3)	
Market	Goose meat products	127	21 (17.3)	17.3
Total		1,030	350 (33.9)	

Table 2. Serotype distribution of *Salmonella* in different points of the different links in goose chain samplings.

Serotype	Hatchery				Farm				Slaughterhouse				Market	
	Dead embryo	Incubator	Fertilized egg	Gosling	Stool	Water	Goose egg	Feed	Internal organs	Polished	Visceral removal	Frozen	Goose meat	Total (% ratio)
<i>S. Potsdam</i>	65 (37.6)	58 (33.5)	14 (8.1)	16 (9.2)	2 (1.2)	0	10 (5.8)	0	6 (3.5)	2 (1.2)	0	0	0	173 (49.4)
<i>S. Typhimurium</i>	1 (0.9)	6 (5.2)	0	0	39 (33.9)	4 (3.5)	0	0	4 (3.5)	33 (28.7)	4 (3.5)	10 (8.7)	0	115 (32.9)
<i>S. Kottbus</i>	0	8 (32.0)	0	6 (24.0)	2 (8.0)	0	1 (4.0)	0	3 (12.0)	5 (20.0)	0	0	0	25 (7.1)
<i>S. Hadar</i>	0	0	0	0	0	0	0	0	0	0	0	10 (100)	0	10 (2.9)
<i>S. Newport</i>	0	6 (85.7)	0	0	1 (14.3)	0	0	0	0	0	0	0	0	7 (2.0)
<i>S. Agona</i>	0	0	0	0	0	0	0	0	4 (80.0)	1 (20.0)	0	0	0	5 (1.4)
<i>S. Konstanaz</i>	2 (40.0)	2 (40.0)	0	1 (20.0)	0	0	0	0	0	0	0	0	0	5 (1.4)
<i>S. Tallahassee</i>	0	1 (25.0)	0	0	3 (75.0)	0	0	0	0	0	0	0	0	4 (1.1)
<i>S. Indiana</i>	0	0	0	0	2 (100)	0	0	0	0	0	0	0	0	2 (0.6)
<i>S. Cerro</i>	0	0	0	1 (20.0)	0	0	0	0	0	0	0	0	0	1 (0.3)
<i>S. Paris</i>	0	0	1 (100)	0	0	0	0	0	0	0	0	0	0	1 (0.3)
<i>S. Korbol</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (0.3)
<i>S. Kentucky</i>	0	0	0	0	0	0	0	0	0	1 (100)	0	1 (100)	0	1 (0.3)
Total	68	81	15	24	49	4	11	0	17	41	15	4	21	350

PFGE Analysis

A total of 55 *S. Potsdam* and 34 *S. Typhimurium* (8 strains are human isolates) were characterized using PFGE. Figure 2 shows the PFGE fingerprint profiles of the *S. Potsdam* isolates, there were 22 PFGE patterns (PF1 to PF22) were characterized, which grouped into 5 clusters (A to E). PF13 contains samples from farms and slaughterhouses, and PF20 contains samples from the hatchery, farm, and slaughterhouse. The other pulse types are from the same links, for example, PF17 is only from the hatchery sample. Among the *S. Typhimurium* isolates (Figure 3), 18 PFGE patterns (PF23 to PF40) were characterized, and they were grouped into 9 clusters (F to N). Among them, the 3 pulse types PF29, PF30, and PF31 all contained *Salmonella* isolates from different link samples, of which PF29 contains human, farm, and hatchery isolate. PF30 contains *S. Typhimurium* isolates from the hatchery, farm and slaughterhouse. PF31 contains isolates from slaughterhouse and farm. Other pulse types are from the same link sample.

DISCUSSION

In the goose production chain, the separation rate of *Salmonella* was 33.9%, higher than the prevalence of *Salmonella* of geese (8.4%) in the EU reported in 2013 (Boelaert et al., 2015). In the 3 production links, the prevalence of *Salmonella* in the hatchery was the most severe, whose isolation rate reached 51.8%, which was significantly higher than the 24.5% isolation rate of *Salmonella* in a goose hatchery in 2007 (Chao et al., 2007). The *Salmonella* isolation rate in the farms was 17.6%, which was similar to the 14% isolation of *Salmonella* from goose farms in Anhui provinces, lower than the 26% in Shandong provinces, and higher than the 11% in Jiangsu provinces (Gong et al., 2014). The isolation rate of *Salmonella* in the slaughterhouse was 43.8%. For the slaughterhouse, the isolation rate of *Salmonella* was significantly higher than the 21.7% reported in the South Korean duck slaughterhouse (Lee et al., 2016). The *Salmonella* isolation rate of the market samples in this study was 17.3% and similar to the 12.8% reported in a market from Iran (Jamali et al., 2015). We found that the isolation rate of *Salmonella* in the slaughterhouse was significantly higher than that in the farm ($P < 0.05$). Similar result also appeared in the study of Djeffal et al. (2018). This may indicate cross-contamination during slaughter. According to many surveys, the contamination of poultry products with *Salmonella* may take place at different links of the production chain. After contamination of birds at the farm, bacteria colonize the intestines and can contaminate carcasses during slaughtering, and cross-contamination is also possible (De Busser et al., 2013). Therefore, it is essential to strengthen the disinfection and sanitation management during the slaughtering process, maintain the hygienic cleanliness of the operation room, and prevent cross-contamination of *Salmonella* (Denagamage et al., 2016).

Table 3. Multidrug resistance of different *Salmonella* serovars from geese.

Serotype (number)	Number of resistant strains (%)				Total (%)
	1 to 3	4 to 6	7 to 9	>9	
<i>S. Potsdam</i> (102)	3 (2.9)	0	0	0	3 (2.9)
<i>S. Typhimurium</i> (67)	13 (19.4)	7 (10.4)	0	0	20 (29.9)
<i>S. Kottbus</i> (11)	1 (9.1)	0	0	0	1 (9.1)
<i>S. Hadar</i> (2)	2(100)	0	0	0	2 (100)
<i>S. Newport</i> (1)	0	0	0	0	0

A total of 13 *Salmonella* serotypes were identified in this study. *S. Potsdam* is mainly isolated from the hatchery. Su et al., found that *S. Potsdam* may be the main serotype of waterfowl hatcheries, and *S. Potsdam* may be a specific isolate of geese and ducks (Su et al., 2011). This study is consistent with it. We isolated a large amount of *S. Potsdam* in the dead embryo samples during the incubation period, indicating that *S. Potsdam* may affect the hatching of goose fertilized egg. In this study, *S. Typhimurium* is a serotype shared by all links, EFSA reports that *S. Typhimurium* is the most extensive serotypes in ducks and geese (Boelaert et al., 2015).

Antimicrobial resistance in *Salmonella* is a threat to human public health. Our results indicate that the antimicrobial resistance of *Salmonella* isolates on the goose farm is not serious. After investigation, it was found that the large-scale goose farm used less antibiotics in the production process, which may be the reason for the low resistance of *Salmonella* isolates in the hatchery, farm and slaughterhouse. However, in market samples,

antimicrobial resistance to NAL is as high as 100% (Supplementary Table S1). This may be due to cross-contamination of *Salmonella* in the market. This phenomenon deserves our attention because resistance to this antimicrobial agent may lead to the delay or failure of fluoroquinolone therapies, and could have serious consequences (Marquez-Ruiz et al., 2008). We also found that the multi-antimicrobial resistance of *S. Typhimurium* in this study was significantly higher than that of *S. Potsdam*. These strains are resistant to the principal drugs for the treatment of invasive *Salmonella* infections. Because the highly resistant strains may have propagated, intensive surveillance and control measures are required in the China goose market (Hong et al., 2018).

MLST has become a fundamental technique for classifying bacterial isolates into strains. It has been applied in many contexts, especially those related to pathogen outbreak surveillance (Krongdang et al., 2017). MLST results revealed that a total of 7 STs were identified in this study (Figure 1). ST2039 was the most common STs recovered in this study, mostly from the hatchery, belonging to *S. Potsdam*. Therefore, we speculate that this ST type exists for a long time in hatchery. ST19 contains both *Salmonella* isolates from the market and human. This suggests that *Salmonella* contamination in the market has a high potential to eventually harm humans, as ST19 has been reported to cause human salmonellosis in recent years (Garvey et al., 2013). Additionally, isolates that were characterized as ST19 and ST99 belong to the same serotype (*S. Typhimurium*), and these 2 STs were also in great diversity in the

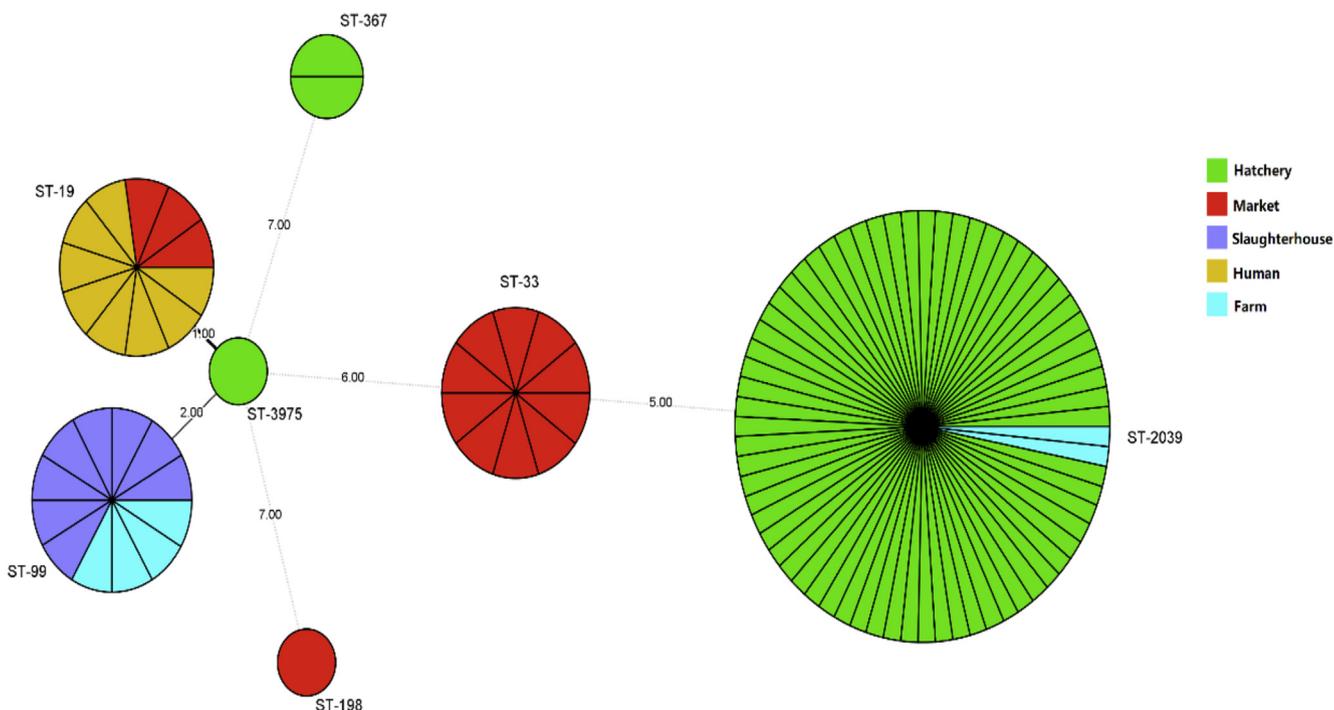


Figure 1. Minimum spanning tree analysis of STs for *Salmonella* isolated from breeding, hatching, and slaughtering links. Each circle represents 1 ST, and the area of the circle corresponds to the number of isolates. Green stands for hatchery isolates, red represents market isolates, dark blue represents slaughterhouse isolates, yellow represents human isolates of *S. Typhimurium*, and sky blue represents farm isolates.

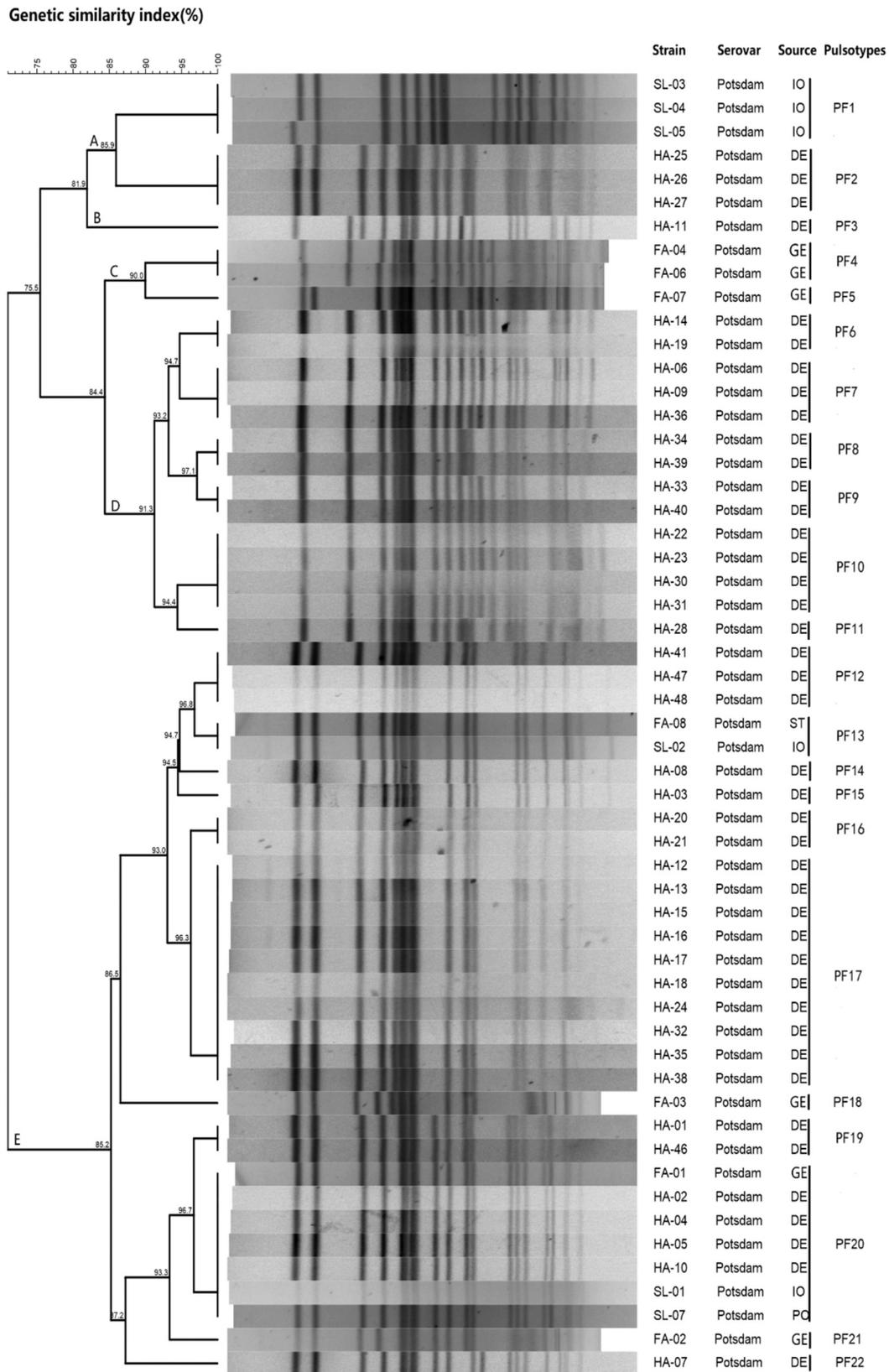


Figure 2. Dendrogram of PFGE patterns of *S. Potsdam*. A total of 5 clusters (A to E) were identified in the “strain” column. There was a total of 22 PFGE patterns (PF1 to PF22). The letter indicates the production link of the source of the strain. The number is the bacteria number. HA is for hatchery, SL is for Slaughterhouse, and FA is for farm. In the “source” column, letters represent samples from different sources. IO is for internal organ samples, and DE is for dead embryo samples. GE is for goose egg samples, and PO is for polished samples.

sources. We note that ST198 has been isolated from a market sample, and ST198 is *S. Kentucky*. This serotype has been associated with the chicken industry in the

USA. Now, its distribution is worldwide, especially as ST198 (Le Hello et al., 2013). This may indicate that goose meat is highly likely to be cross-contaminated by

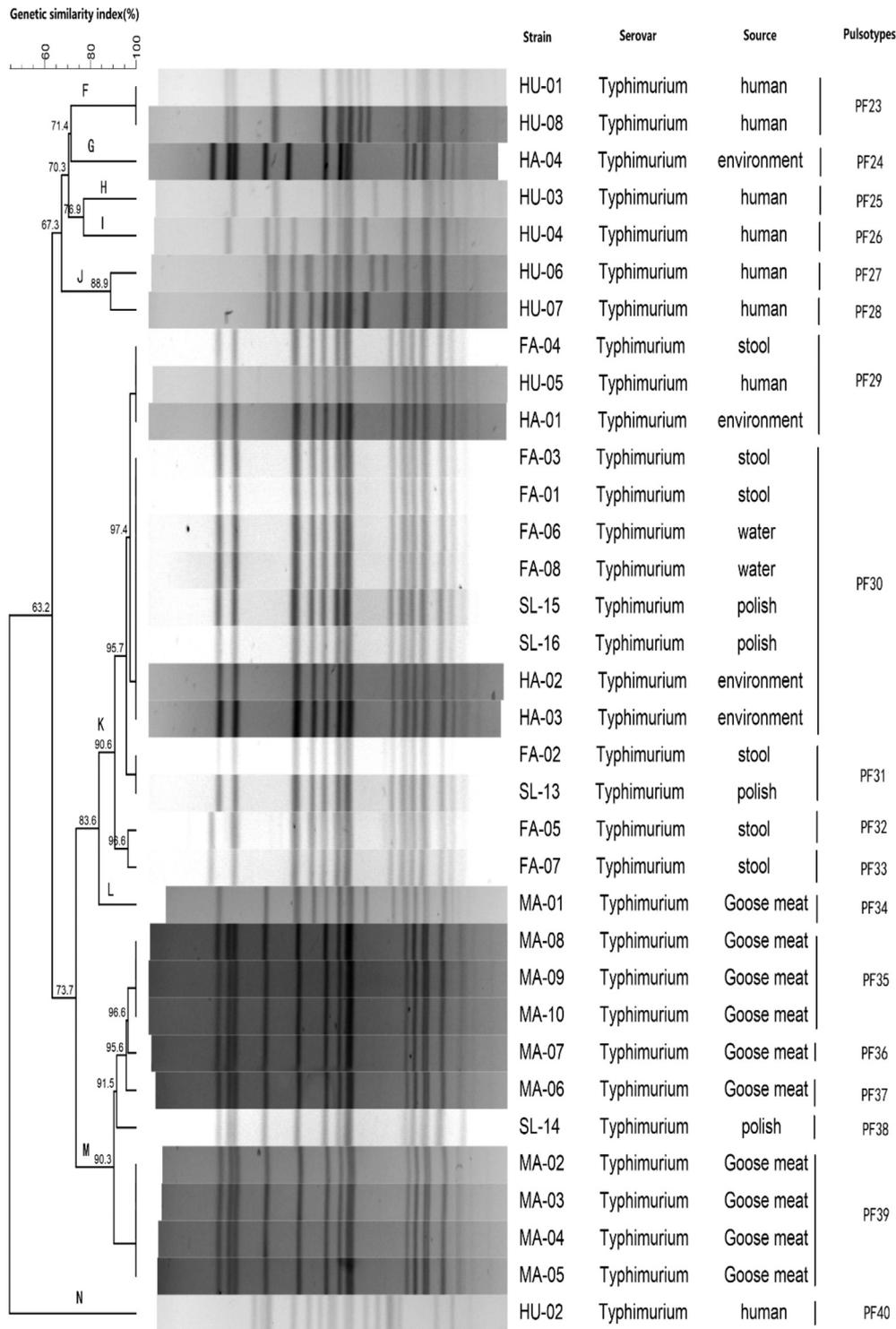


Figure 3. Dendrogram of PFGE patterns of *S. Typhimurium*. A total of 9 clusters (F to N) were identified, in the “strain” column. There was a total of 18 PFGE patterns (PF22 to PF40). The letters indicate the production link of the strain source, and the numbers represent the bacterial number. The human isolate is HU, the hatchery is HA, the slaughterhouse is SL, the farm is FA, and the market is MA. The source column, represents different types of samples.

Salmonella in the market, which needs everyone’s attention.

In our study, 55 strains of *S. Potsdam* isolates (ST2039) and 34 strains (including 8 isolates of human) *S. Typhimurium* (ST19 or ST99) were characterized into 22 and 18 pulse types, respectively, by PFGE. *S. Potsdam* (55 strains) was grouped into 5 clusters (A to E).

The E cluster contains isolates from the production links of hatchery, farm, and slaughterhouse (Figure 2), suggesting that *S. Potsdam* could be transmitted along the production chain and eventually may enter the market, causing harm to human. The E cluster contains samples of dead embryos and fertilized eggs from the hatchery, stool and goose eggs of the farm. This shows

that vertical transmission is an important mode of transmission for *S. Potsdam*. There have been reports of health hazards caused by *S. Potsdam*. There has been a recent case report that *S. Potsdam* caused a high fever with severe lower back pain and limited movement and was eventually diagnosed as lumbar vertebral osteomyelitis in a 29-year-old woman (Cheng et al., 2018). Therefore, monitoring and prevention of *S. Potsdam* in the goose production chain should be strengthened.

S. Typhimurium (34 strains) was grouped into 9 clusters. The M cluster contains samples from slaughterhouses and markets (Figure 3). The F cluster contains isolates from farms, hatcheries, slaughterhouses, and human, where strains FA-04, HU-05, and HA-01 belong to 1 pulse type (PF29). This indicates that *S. Typhimurium* may spread along the production chain and entered the market to harm human health. In addition, pulse PF30 also includes environmental samples from the hatchery, polished samples from the slaughterhouse, and water and stool samples from the farm. The isolation rate of *S. Typhimurium* in water samples is as high as 40%. The research did by Djeffa et al. shows that water sources are an important source of *Salmonella* contamination in poultry farming. (Djefal et al., 2018). Therefore, it is necessary to clean up the manure in farms over time and to disinfect farms and water sources regularly to reduce *Salmonella* contamination.

CONCLUSION

In general, our study found that the goose production chain had relatively serious *Salmonella* contamination. *S. Potsdam* may be the specific serotype of the goose hatchery, and *S. Typhimurium* was widely distributed in various production processes. *Salmonella* will spread along the production chain and eventually flow into the market to harm human health. There is a risk of cross contamination of *Salmonella* in slaughterhouse and market. Therefore, in the hatchery, the disinfection of the incubator environment should be strengthened to eliminate the long-term presence of *Salmonella*; in the farm, the stool should be cleaned up in time, and the disinfection of the water source should be strengthened; preventing cross-contamination of *Salmonella* in slaughter process and market are important for the prevention and control of *Salmonella* in the goose production chain.

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SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

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