**INTRODUCTION**

The importance of poultry products as a source of Salmonella infection has been investigated in several studies (Kimura et al., 2004; Braden, 2006). Human salmonellosis caused by Salmonella enterica serotypes commonly isolated from poultry products, including Salmonella enterica serovar Enteritidis, Montevideo, Infants, and Hadar, resulted in higher proportions of invasive disease than that of Salmonella Typhimurium, which is considered one of the most common Salmonella serotypes worldwide (Jones et al., 2008).

Salmonella may enter the production chain at stages of poultry production, and transmission may occur directly or indirectly from animal feed, at the farm, within the slaughterhouse or packing plant, in the manufacturing, processing, and retailing of food (Bailey et al., 1994; Rose et al., 1999). Healthy chickens can be infected with Salmonella without showing any clinical symptom of infection, so many farmers do not know that their chickens are infected (Beam et al., 2013). Therefore, to confirm whether a broiler flock is contaminated with Salmonella, it is necessary to detect Salmonella from the carcass or their environment.

In South Korea, chicken meat production was 720,000 tons a year (in 2012); it is the second largest source of animal protein, and about 30 million broilers are slaughtered and processed monthly. Most chicken meat is produced by integrated broiler operations. These broiler operations control and operate through all phases of the chicken industry, such as breeder flock management, hatchery operation, feed management, broiler slaughter, and retail distribution (Kim et al., 2007).

Previous studies have reported Salmonella in a Korean broiler supply chain, with a high rate of Salmonella reported in broiler chickens (Kim et al., 2007, 2012a; Lee et al., 2007), which was associated with increased isolation rate of Salmonella from various poultry products. However, the dissemination of Salmonella in the entire broiler supply chain including retail chicken meats in Korea has not previously been studied.

The aim of this study was to investigate the prevalence and distribution of Salmonella species in an integrated broiler supply chain in Korea. We investigated the prevalence of Salmonella species in a total number of 1,214 samples collected from broiler breeder farms, commercial broiler farms, slaughterhouse, commercial broiler trucks, and retail commercial chicken meats of a single integrated broiler company in Korea. In addition, the relationships between isolates were investigated by
molecular characterization by using pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

Sampling Methods

The swab method was performed as described previously (Swanenburg et al., 2001) with minor modifications. Briefly, swabs were made using disposable stockinetts (3M Animal Care Products, St. Paul, MN), which were cut into pieces (approximately 5 to 7 cm each), packed into aluminum foil, and sterilized for 15 min at 121°C. After sterilization, 4 swabs were packed into a sterile plastic bag under sterile conditions. Each plastic bag was soaked in 30 mL of buffered peptone water (BPW; Difco Laboratories, Detroit, MI). After swabbing the dust with a sterile glove or swabbing the floor by using the drag swab method (Zewde et al., 2009), the swabs were returned to the plastic bag and transported to the laboratory in a portable ice chest.

The carcass rinse method was performed as previously described by Kim et al. (2012a). Briefly, carcass or retail chicken meats was separately placed in a sterile plastic bag containing 400 mL of BPW (Difco Laboratories) and shaken for 2 min. The 200 mL of BPW rinse constituted the sample. Collected samples were transported in cold conditions to the Avian Disease Laboratory at Konkuk University for microbiological analyses.

Sample Collection

Broiler breeder farms, commercial broiler farms, broiler trucks, slaughterhouse, and retail chicken meats were investigated in this study. All samples were collected from a single integrated broiler company.

In total, 9 broiler breeder farms and 17 commercial broiler farms were sampled from November 2011 to March 2012. The number of broiler breeder houses on a farm ranged from 4 to 12, averaging 6.5. The number of broiler houses on a farm ranged from 1 to 6, averaging 3. There were 2 to 4 visits for each broiler breeder farms and 1 to 2 for commercial broiler farms. For every breeder and broiler house, 2 drag samples and 2 dust samples were taken using the swab method.

In total, 7 broiler trucks were investigated from November 2011 to February 2012. Every truck belonging to the investigated slaughterhouse was sampled. For each truck, after chickens were unloaded, 1 drag sample was taken using the swab method.

In March 2012, the slaughterhouse of the same integrated broiler supply chain with processing capacity of 200,000 birds per day was visited twice in a 1-wk interval. During each visit, 36 samples were collected. Samples included 13 environment collected before cleaning and disinfection (C&D), 10 carcass samples from the postchill step, and 13 environment samples collected after C&D per each visit. Environment samples were taken in the same way with broiler farms: from the lairage, hanging room, bleeding room, evisceration room, chiller tank room, and grading/packing room. Swabs from processing devices and drag swab samples were also taken from all areas, except for the lairage.

A total of 129 retail chicken meats were collected from one local supermarket located in Seoul. From January 2011, the supermarket was visited 30 times in 3-wk intervals and poultry meats produced by the same integrated broker chicken operation were collected. On each sampling day, 3 or 6 individually packed whole chicken meats were collected. All samples were stored and distributed in refrigerated conditions. Collected samples were transported on ice to the Avian Disease Laboratory at Konkuk University, and microbiological analyses were performed within 2 h after collection.

Salmonella isolation and Serotype Identification

For preenrichment, 200 mL of BPW was added to the swab samples from the dust and drags in sterile plastic jars with caps. The samples were then incubated at 37°C for 18 to 24 h. For the carcass rinse samples, rinsed BPW was transferred to a sterile plastic jar with cap and incubated at 37°C for 18 to 24 h.

For selective enrichment, 100 µL of enriched BPW broth culture was transferred to 9.9 mL of Rappaport-Vassiliadis broth (Difco Laboratories) and incubated at 42°C for 24 to 48 h. Following incubation, the Rappaport-Vassiliadis broth enrichment cultures were streaked onto xylose lysine deoxycholate agar (Difco Laboratories) and Brilliant Green agar (Difco Laboratories), and the plates were incubated overnight at 37°C. Two presumptive Salmonella colonies from each sample were transferred to MacConkey agar (Difco Laboratories). Colonies with a positive result were confirmed by Salmonella-specific PCR as previously described (Halatsi et al., 2006).

Serotyping was performed using antisera (Difco Laboratories) in agglutination tests on the basis of somatic O antigen and phase 1 and phase 2 flagella antigens according to the Kauffmann-White scheme.

PFGE

Molecular typing of the Salmonella Hadar isolates was performed using PFGE with the restriction endonucleases XbaI in accordance with the standardized protocols used by PulseNet Laboratories (http://www.pulsenetinternational.org; Ribot et al., 2006). Briefly, for the preparation of plugs, bacterial cells were suspended in TE buffer (100 mM Tris, 100 mM EDTA, pH 7.5) and mixed with an equal volume of 1.2% SeaKem Gold Agarose (FMC Bioproducts, Rockland, MA). The plugs were incubated with ES buffer (0.5 M EDTA, pH 9.0, 1% sodium laurylsarcosine) containing proteinase K to lyse the bacterial cells. For restriction endonuclease digestion, the lysed plugs were incubated at 37°C
for 3 h with 50 U of XbaI (Roche, Maylan, France). The PFGE was performed with 1% agarose gels and 0.5× Tris-borate-EDTA buffer at 14°C in a CHEF mapper apparatus (Bio-Rad, Richmond, CA) set at 6 V/cm with a linearly increased switching time of 2.16 to 62.38 s for 18 h. The DNA fingerprint patterns were interpreted using BioNumerics 5.1 software (Applied Maths, Austin, TX). The banding patterns were compared using Dice coefficients with a 1.5% band position tolerance. Clustering of the patterns was performed using the unweighted pair group method with arithmetic averages.

A total number of 15 Salmonella Hadar isolates were randomly chosen for PFGE. Four from breeder farms, 4 from broiler farms, 1 from commercial broiler transporting truck, 1 from the slaughterhouse environment, 1 from the carcass from slaughterhouse, and 4 from retail chicken meats. For samples from breeder farms and broiler farms, no more than 1 sample from the same farm was chosen.

### Statistical Analysis

Differences in the Salmonella isolation rate from various sampling sites and conditions were compared with Fisher’s exact test. A 2-sided P-value of <0.05 was considered statistically significant. Analyses were performed using GraphPad InStat software, version 3.0 (GraphPad Software, La Jolla, CA).

### RESULTS

#### Prevalence of Salmonella Through the Broiler Supply Chain

As shown in Table 1, Salmonella was isolated from 195 out of 1,214 total samples (16.6%), including 72 out of 682 samples from broiler breeder farms (10.56%), 52 out of 324 samples from commercial broiler farms (16.05%), 5 out of 7 samples from commercial broilers trucks (71.43%), 46 out of 72 samples from slaughterhouses (63.89%), and 20 out of 129 samples from retail chicken meats (15.5%).

#### Serotype Distribution

Isolates were assigned to 9 serovars of Salmonella enterica subspecies. Salmonella Hadar was the dominant serovar (162 isolates, 83.08%) in the Salmonella-positive samples, with Salmonella Virchow (10 samples, 5.13%) a distant second (Table 2). Serovars of the 7 other isolates included Salmonella Senftenberg (7 isolates, 3.59%), Salmonella London (2 isolates, 1.03%), Salmonella Typhimurium (1 isolates, 0.51%), Salmonella Havana (1 isolates, 0.51%), Salmonella Reading (1 isolates, 0.51%), and Salmonella Vellore (1 isolates, 0.51%). The serotype could not be determined for 8 isolates. Salmonella Hadar was the most dominant Salmonella serovar at every step of the integrated broiler supply chain investigated in this study.

#### Prevalence of Salmonella in the Slaughterhouse Environment Before and After C&D

Among 52 samples collected from the slaughterhouse environment, 59.62% were Salmonella positive with no significant difference between before and after C&D (Table 3). The lairage was the only site to be free of Salmonella-positive samples. Salmonella Hadar was the dominant serovar (77.42%) from the slaughterhouse environment. There was no significant difference in the isolation rate between drag and dust samples.

#### PFGE Profiles

Genomic DNA from Salmonella Hadar were analyzed by PFGE using the XbaI enzyme. The same pulsotype of all Salmonella Hadar isolates was detected from carcass and environment samples from commercial broiler breeder farms, broiler farms, broiler trucks, slaughterhouse, and retail chicken meats (Figure 1).

### DISCUSSION

This study shows that the prevalence of Salmonella in an integrated broiler supply system can be rather high. In particular, Salmonella was detected at every step along the integrated broiler supply chain from breeder farms to retail chicken products. Because an increased prevalence of Salmonella in retail chicken meats can lead to an increased risk of poultry-borne salmonellosis outbreak, effective control of Sal-

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>No. of visits</th>
<th>No. of total samples</th>
<th>No. of Salmonella-positive samples</th>
<th>Isolation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler breeder farms</td>
<td>26</td>
<td>682</td>
<td>72</td>
<td>10.56</td>
</tr>
<tr>
<td>Broiler farms</td>
<td>27</td>
<td>324</td>
<td>52</td>
<td>16.05</td>
</tr>
<tr>
<td>Broilers trucks</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>71.43</td>
</tr>
<tr>
<td>Slaughterhouses</td>
<td>2</td>
<td>72</td>
<td>46</td>
<td>63.89</td>
</tr>
<tr>
<td>Retail chicken meats</td>
<td>30</td>
<td>129</td>
<td>20</td>
<td>15.5</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>1,214</td>
<td>195</td>
<td>16.66</td>
</tr>
</tbody>
</table>
monella} is required through a farm-to-table approach at all stages of the supply chain.

Previous study showed that even one infected breeder flock is capable of causing widespread {Salmonella} contamination (van de Giessen et al., 1991). In addition, Davies et al. (1997) previously investigated a company that suffered repeated {Salmonella} infections in a broiler breeder farm and found a variety of routes by which the infection may have been recirculating within the company. In this study, {Salmonella} was recovered from 10.56% of samples from broiler breeder farms. This contamination can lead to the dissemination of {Salmonella} to breeder farms via vertical or horizontal transmission to day-old chicks. Furthermore, even if broiler breeder flocks were infected with {Salmonella}, the infection may be subclinical and hatchability may be unaffected (Cason et al., 1994). In this study, {Salmonella} infection was also subclinical. Therefore, continuous “silent” circulation of {Salmonella} in this broiler supply system poses a potential risk of spread of {Salmonella} and spillover to humans.

Because of the relatively poor quality of sanitation management and the short cycle of flocks, broiler farms usually have a higher prevalence of microorganisms (Jacobs-Reitsma et al., 1994). {Salmonella} was recovered from 16.05% of samples from commercial broiler farms in this investigation. This isolation rate was significantly higher than that of previous step of broiler supply chain, broiler breeder farms ($P = 0.0251$). {Salmonella} Hadar was the most frequently isolated {Salmonella} species in broiler breeder farms, slaughterhouses, and retail chicken meats. Analyzed 15 {Salmonella} Hadar isolates had single identical {XbaI} pulstype from every step along the broiler supply chain, suggesting that {Salmonella} Hadar strains isolated in this study could be closely related clones. In addition, less serotype diversity in breeder farms than broiler farms might be due to better management practice and biosecurity in breeder farms. Moreover, it is likely that higher serotype diversity in broiler farms than that of breeder farms might be caused by additional {Salmonella} contamination while day-old chicks were transported from the breeder farms to the broiler farms.

Previously, Corry et al. (2002) suggested that contaminated crates can lead to infection or contamination of birds with {Salmonella}. In this investigation, 5

<table>
<thead>
<tr>
<th>Location</th>
<th>Sampling site</th>
<th>Isolates detected at first visit</th>
<th>Isolates detected at second visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lairage Drag</td>
<td>—1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hanging room Device</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bleeding room Drag</td>
<td>Typhimurium</td>
<td>—</td>
<td>Hadar Hadar Hadar Hadar</td>
</tr>
<tr>
<td>Scalding room Device</td>
<td>Hadar</td>
<td>—</td>
<td>Hadar Hadar Virchow</td>
</tr>
<tr>
<td>Evisceration room Drag</td>
<td>Hadar</td>
<td>—</td>
<td>Hadar Montevideo Montevideo</td>
</tr>
<tr>
<td>Chiller tank room Device</td>
<td>Hadar</td>
<td>—</td>
<td>Hadar</td>
</tr>
<tr>
<td>Grading/packing room Device</td>
<td>Hadar</td>
<td>—</td>
<td>Hadar</td>
</tr>
<tr>
<td>Grading/packing room Drag</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Isolation rate (% in parentheses)</td>
<td>10/13 (76.92)</td>
<td>7/13 (53.85)</td>
<td>8/13 (61.54)</td>
</tr>
</tbody>
</table>

1Not detected.
Salmonella serovars were isolated from 7 broiler transporting trucks. Although the number of samples in this group was lower than that at other steps, there was a significant difference in the isolation rate between commercial broiler farms and broiler transporting trucks ($P = 0.0201$). Various serotypes were isolated from commercial broiler transporting trucks despite the small sample number. This may be because the trucks frequently visit many broiler farms including those that were not investigated in this study. By being exposed to the contaminated environment of various broiler farms and slaughterhouses, broiler transporting trucks may play a critical role in disseminating Salmonella between broiler farms and slaughterhouses. Further research is required to clarify the role of transporting trucks in disseminating Salmonella in the broiler supply chain by using a large number of samples. Also, trucks should be adequately cleaned and disinfected by poultry producers, such as by the application of disinfectant at a recommended concentration and temperature and removal of fecal soiling before disinfection.

Some Salmonella serotypes can survive in certain niches of the slaughterhouse environment and may become part of the resident flora. Contaminated slaughterhouse environments can result in subsequent carcass contamination of slaughter chickens passing along the slaughter line (Marin et al., 2011; Henry et al., 2012). In this investigation, the slaughterhouse showed a high prevalence of Salmonella species in both the environment (59.62%) and carcass samples (70%). Even after C&D, no significant decrease in the Salmonella isolation rate was observed (Table 3). This result echoes previous reports that Salmonella residing in the environment are not eradicated by C&D procedures (Marin et al., 2011). The high persistence of Salmonella in the slaughterhouse environment after C&D may be due to the high content of organic materials such as fats and proteins in this environment (Gradel et al., 2004), resistance of the Salmonella strains to applied disinfectants (Castelijn et al., 2013), or the development of biofilm (Rodrigues et al., 2011). In addition, Salmonella remaining after C&D will become part of the resident flora, thereby causing subsequent carcass contamination of chickens arriving next day, even chickens from Salmonella-free farms.

The prevalence of Salmonella in retail chicken meat (15.5%) in this study was relatively lower than that in previous investigations in Korea (Chung et al., 2003; Hyeon et al., 2011; Kim et al., 2012a). These differences may be due to different detection methods used and different time periods of investigation. However, we speculate that the reason for the different prevalence rates may be that our investigation is limited to products from a single integrated broiler supply chain.

For interpretation of our data, contamination by Salmonella in the broiler supply chain is integrated. Salmonella circulating at the broiler breeder level may be vertically transmitted to the commercial broiler level. These Salmonella may be part of the resident flora in the slaughterhouse environment and are difficult to eradicate in spite of daily C&D. Finally, subsequent Salmonella contamination of carcasses from slaughterhouse equipment may lead to outbreaks of poultry-borne nontyphoidal salmonellosis. Although there are numerous potential causes of Salmonella contamination, there is evidence that contamination can be con-
trolled in broiler operations (Davies et al., 2003). In addition to good management practice, hygiene on the farm and in the slaughterhouse is central to the reduction of *Salmonella* contamination (Van Immerseel et al., 2005). The current study suggests that control of *Salmonella* circulating at the broiler breeder level is a matter of utmost importance.

In previous study, *Salmonella* Hadar was isolated from fecal samples of patients with diarrhea and other symptoms of acute gastroenteritis in 2001, 2004, 2007, and 2009 (Kim et al., 2012b). The PFGE profiles of *Salmonella* Hadar strains isolated in this study were not identical or similar to those of human isolates of *Salmonella* Hadar in Korea. However, it is certain that investigated flocks in this study were contaminated with *Salmonella* Hadar and multiple reports described that there were frequent food poisonings caused by poultry products contaminated with *Salmonella* Hadar in several countries. Therefore, even though the PFGE profiles were not matched between *Salmonella* Hadar isolated from poultry products and humans, there is a high risk of *Salmonella* Hadar outbreak in humans caused by poultry products. These results suggested that *Salmonella* Hadar should be included into the list of *Salmonella* that are regularly monitored and controlled in the poultry industry.

In summary, this is the first study to show dissemination of *Salmonella* in the entire broiler supply chain in Korea, from broiler breeder farm to retail chicken meat. The results suggest that there is a critical need to control *Salmonella* in commercial breeder farms and slaughterhouses to decrease contamination in the broiler supply chain. Further studies are required to investigate the major source of *Salmonella* contamination in broiler breeder farms in Korea, the results of which may be critical to eradicate the source of massive *Salmonella* contamination throughout the broiler industry.

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