Pulsed-field profile diversities of *Salmonella* Enteritidis, S. Infantis, and S. Corvallis in Japan

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Abstract

The diversity of pulsed-field profiles (PFPs) within non-typhoidal *Salmonella* subtypes influences epidemiological analyses of *Salmonella* outbreaks. Therefore, determining the PFP diversity of each *Salmonella* serovar is important when evaluating current circulating strains. This study examined the PFP diversity of three important public health *Salmonella enterica* sub-species *enterica* serovars, S. Enteritidis (n=177), S. Infantis (n=205), and S. Corvallis (n=90), using pulsed-field gel electrophoresis. Isolates were collected from several sources, primarily from chicken-derived samples, in the Kyushu-Okinawa region of Japan between 1989 and 2005. S. Enteritidis isolates displayed 51 distinct PFPs (E-PFPs), with 92 (52.0%) and 32 (18.1%) isolates displaying types E-PFP1 and E-PFP10, respectively. The 205 S. Infantis isolates showed 54 distinct PFPs (1-I-PFPs), with 87 (42.4%) and 36 (17.6%) isolates being I-PFP4 and I-PFP2, respectively. I-PFP18 was the dominant I-PFP of layer chicken isolates across a 5-year period. Fourteen distinct S. Corvallis PFPs were detected. Simpson’s index results for the genetic diversities of S. Enteritidis, S. Infantis, and S. Corvallis isolates were 0.70, 0.79, and 0.78, respectively. None of the E-PFPs or I-PFPs of layer chicken isolates overlapped with those of broiler chicken isolates, and the dominant clonal lines existed for >10 years. In conclusion, limited PFP diversities were detected amongst S. Enteritidis, S. Infantis, and S. Corvallis isolates of primarily chicken-derived origins in the Kyushu-Okinawa region of Japan. Therefore, it is important to take into account these limitations in PFP diversities in epidemiological analyses of *Salmonella* outbreaks.

Introduction

Pulsed-field gel electrophoresis (PFGE) subtype diversity within non-typhoidal *Salmonella* serovars can influence epidemiological analyses using pulsed-field profiling of *Salmonella* isolates, especially those using isolates from disease outbreaks. Pulsed-field profiling of non-typhoidal *Salmonella* strains is useful for public health as it enables detection of geographically dispersed outbreaks of this important diarrheal pathogen (Mishu Allos et al., 2004). However, this type of analysis is complicated by the fact that some pulsed-field profiles (PFPs) may be common and widely distributed (Lindqvist and Pelkonen, 2007; Swaminathan et al., 2001). For example, Pang et al. (2007) found limited genetic diversity amongst *Salmonella enterica* sub-species *enterica* serovar Enteritidis isolates in Taiwan and Germany. They observed that a single major worldwide clone of S. Enteritidis was present in several sources, including deer, pigs, fish, chickens, horses, other birds, rodents, eggs, corn, corn-mushrooms, soil, and water, when PFPs were determined using three restriction enzymes. Three serovars were chosen for analysis in the current study because these three serovars are relatively important for food hygiene. S. Infantis is the most common food-associated serovar in Japan, particularly in chicken meat (Murakami et al., 2001), and is also one of the most important serovars for public health (Murakami et al., 2007). S. Enteritidis is also commonly associated with human disease (NHD, 2006), and is related to layer chickens (Humphrey 2006). S. Corvallis is not a dominant serovar but is routinely isolated from chicken and poultry products (Murakami et al. 2001). While analysis of current PFP data is very important for public health, recording old PFP data is also important from a historical viewpoint. In addition, comparing historical PFP data with that from current isolates can provide important information, such as identification of recurring PFPs. PFGE data for the three chosen serovars can provide important information, including deer, pigs, fish, chickens, horses, other birds, rodents, eggs, corn, corn-mushrooms, soil, and water.

Three serovars were chosen for analysis in the current study because these three serovars are relatively important for food hygiene. S. Infantis is the most common food-associated serovar in Japan, particularly in chicken meat (Murakami et al., 2001), and is also one of the most important serovars for public health (Murakami et al., 2007). S. Enteritidis is also commonly associated with human disease (NHD, 2006), and is related to layer chickens (Humphrey 2006). S. Corvallis is not a dominant serovar but is routinely isolated from chicken and poultry products (Murakami et al. 2001). While analysis of current PFP data is very important for public health, recording old PFP data is also important from a historical viewpoint. In addition, comparing historical PFP data with that from current isolates can provide important information, such as identification of recurring PFPs. PFGE data for the three chosen serovars can provide important information, including deer, pigs, fish, chickens, horses, other birds, rodents, eggs, corn, corn-mushrooms, soil, and water.

Materials and Methods

Isolates

All isolates (n=472) belonging to the three serovars are listed in Table 1. Isolates were selected for long-term assessment of clonal lines, and included nearly all samples collected from several sources, mainly chicken-derived samples, in theKyushu-Okinawa region of Japan over a 17-year period.
from a collection of the three serovars from the Fukuoka Institute of Health and Environmental Sciences (FIHES), Dazaifu, Fukuoka Prefecture, in the Kyushu-Okinawa region of Japan. The Kyushu-Okinawa region consists of eight prefectures, including Japan’s third largest island, and is located southwest of the main island of Honshu. Although the majority of isolates and their original samples were obtained from the Kyushu-Okinawa region, nine isolates were obtained from other regions. Sample details are documented in Table 1.

S. Enteritidis isolates (n=177) from 173 samples were collected between 1989 and 2005 (Tables 2 and 3). S. Infantis isolates (n=205) from 184 samples, and S. Corvallis isolates (n=90) from 88 samples, were collected between 1995 and 2005 (Tables 2 and 3). S. Enteritidis PFP (E-PFP) data for 64 isolates collected between 1989 and 2004, S. Infantis PFP (I-PFP) data for 134 isolates collected between 1995 and 2005, and S. Corvallis PFP (C-PFP) data for 16 isolates collected from food handlers in 1999 and 2000 were previously reported in a different context (Murakami et al., 1999a, 1999b, 2001, 2007; Noda et al., 2010, 2011), and were re-analyzed in the current study by PFGE for comparison with the newly tested isolates.

Isolates belonged to the following five categories: human, food, slaughterhouse, farm and shell egg production environment, and environmental inspection. Human samples included outbreak isolates from symptomatic patients, food handler isolates, and sporadic case isolates from symptomatic patients (Table 1). The outbreak isolates were obtained from outbreaks in Fukuoka Prefecture, and were isolated at the FIHES. The food handler isolates were obtained from a clinical laboratory and serotyped at the FIHES (Murakami et al., 2007), except for one S. Infantis isolate, which was isolated at the FIHES. The sporadic case isolates were also obtained from a clinical laborato-

| S. enterica subsp. enterica serovar | Origin details | Isolates Samples (n) | Origin | Location | Year isolated |
|------------------------------------|----------------|----------------------|--------|----------|---------------|
| **Enteritidis** | Layer chicken-related | Pooled broken shell-eggs | 14  | 2 facilities | Fukuoka Prefecture* | 1996–1997 |
| | Liquid egg | 11  | 5 makers | Kyushu-Okinawa region | 1995–1997 |
| | Broiler chicken-related | Pooled feces of broiler chickens | 6  | 4 farms | Kyushu-Okinawa region | 1995–1996 |
| Human | Outbreaks (feces of symptomatic patients) | 37  | 37 outbreaks | Fukuoka Prefecture | 1988–1994, 1996–2001, 2003–2005 |
| | Sporadic cases (feces) | 93  | 93 cases | Kyushu-Okinawa region and Kinki region (3 isolates)* | 1999–2000 |
| | Food handlers (feces) | 14  | 14 handlers | Kyushu-Okinawa region | 1999–2000 |
| | River water | 1  | 1 river | Fukuoka Prefecture | 1996 |
| | Sewage | 1  | 1 facility | Fukuoka Prefecture | 1996 |
| | Subtotal | 177  | 173  |  |  |
| Infantis | Layer chicken-related | Pooled broken shell-eggs | 2  | 1 farm | Fukuoka Prefecture region | 1995, 1999, 2000 |
| | Swabs from shell-eggs and egg production environment | 5  | 2 facilities | Kyushu-Okinawa region | 1995, 1999, 2000 |
| | Broiler chicken-related | Broiler chicken meat | 88  | 71 shops | Fukuoka Prefecture region | 1995–1997, 1999–2005 |
| | Pooled feces of broiler chickens | 29  | 15 farms | Kyushu-Okinawa region | 1995–1996 |
| | Autopsy materials of broiler chickens | 6  | 4 farms | Kyushu-Okinawa region | 1995 |
| | Sporadic cases and outbreaks (symptomatic) (feces) | 10  | 1 outbreak and nine sporadic cases | Kyushu-Okinawa region and Kinki region (1 isolate)* | 2001, 2005 |
| | Food handlers (feces) | 56  | 56 handlers | Kyushu-Okinawa region | 1996, 1999–2000 |
| | Other | Chicken cases in a slaughterhouse (not identified as broiler- or layer-related) | 2  | 1 facility | Kyushu-Okinawa region | 1995 |
| | Pork | 1  | 1 shop | Fukuoka Prefecture | 2002 |
| | River water | 2  | 1 river | Fukuoka Prefecture | 1995 |
| | Sewage | 4  | 1 facility | Fukuoka Prefecture | 1995 |
| | Subtotal | 205  | 184  |  |  |

| Corvallis | Layer chicken-related | Pooled broken shell-eggs, swabs of shell-eggs, and egg production environment | 33  | 11 facilities | Kyushu-Okinawa region and Chugoku regions (5 isolates)* | 1996, 1998–2000, 2002, 2003–2005 |
| | Layer chicken slaughterhouse samples (chilling water and carcass) | 3  | 1 facility | Kyushu-Okinawa region | 1998–1999 |
| | Broiler chicken-related | Broiler chicken meat | 9  | 8 shops | Kyushu-Okinawa region | 1996, 1998, 2000, 2005 |
| | Broiler chicken slaughterhouse samples (chilling water and carcass) | 10  | 3 facilities | Fukuoka Prefecture | 1997–1999 |
| Human | Food handlers (feces) | 25  | 25 handlers | Kyushu-Okinawa region | 1998–2000 |
| | River water | 7  | 4 rivers | Fukuoka Prefecture | 1995, 1998–1999 |
| | Sewage | 1  | 1 facility | Fukuoka Prefecture | 1995 |
| | Beef | 2  | 1 shop | Fukuoka Prefecture | 1998 |
| Other | Subtotal | 90  | 88  |  |  |

*Fukuoka Prefecture is located in the Kyushu-Okinawa region, which consists of eight prefectures, including Japan’s third largest island, and is located southwest of the main island of Honshu; “Kinki and Chugoku regions are outside of Kyushu.”
Table 2. Pulsed-field profiles of *Salmonella* isolates and their origins.

| PFPs                        | Layer chicken-related | No. of isolates by origin | Humans | Others* | Total |
|-----------------------------|-----------------------|---------------------------|--------|---------|-------|
| *Salmonella enterica*       |                       |                           |        |         |       |
| subspecies                 |                       |                           |        |         |       |
| *S. enterica*               | E-PFPs found in       | E-PFP 1 (13)*             |        |         | 124   |
| several sources             | E-PFP 10 (3)          | E-PFP 10 (12)             |        |         | 53    |
| *S. Infantis*               | E-PFPs found          | E-PFP 2 (1)               |        |         |       |
| in a single source          | E-PFP 8 (1)           | E-PFP 8 (1)               |        |         |       |
| *S. Enteritidis*            | E-PFP 11 - E-PFP 14   | E-PFP 11 - E-PFP 14 (1 each) |       |         |       |
| (S.) Enteritidis-PFPs       | E-PFP 15 (3)          | E-PFP 15 (3)              |        |         |       |
| E-PFP 3 - E-PFP 8 (1 each) |                       |                           |        |         |       |
| E-PFP 18 - E-PFP 29 (1 each) |                     |                           |        |         |       |
| E-PFP 30 - E-PFP 38 (1 each) |                     |                           |        |         |       |
| E-PFP 39 (3)                |                       |                           |        |         |       |
| E-PFP 40 - E-PFP 41 (1 each) |                     |                           |        |         |       |
| E-PFP 42 (1)                |                       |                           |        |         |       |
| E-PFP 43 - E-PFP 45 (1 each) |                     |                           |        |         |       |
| E-PFP 47 (1)                |                       |                           |        |         |       |
| E-PFP 48 - E-PFP 69 (1 each) |                     |                           |        |         |       |
| E-PFP 50 - E-PFP 51 (1 each) |                     |                           |        |         |       |
| E-PFP 51 - E-PFP 55 (1 each) |                     |                           |        |         |       |
| I-PFP 40 (1)                |                       |                           |        |         |       |
| I-PFP 40 (1)                |                       |                           |        |         |       |
| I-PFP 40 (1)                |                       |                           |        |         |       |
| E-PFP 40 (1)                |                       |                           |        |         |       |
| C-PFPs identified in a single source |                       |                           |        |         |       |
| S. Infantis-PFPs            | I-PFPs identified in several sources | I-PFP 2 (21) |        |         | 145   |
| I-PFP 4 (59)                |                       |                           |        |         |       |
| I-PFP 9 (1)                 |                       |                           |        |         |       |
| I-PFP 20 (1)                |                       |                           |        |         |       |
| I-PFP 25 (7)                |                       |                           |        |         |       |
| I-PFP 37 (2)                |                       |                           |        |         |       |
| I-PFP 50 (1)                |                       |                           |        |         |       |
| I-PFP 40 (1)                |                       |                           |        |         |       |
| I-PFP 40 (1)                |                       |                           |        |         |       |
| I-PFP 40 (1)                |                       |                           |        |         |       |
| I-PFP 40 (1)                |                       |                           |        |         |       |
| E-PFP 3 (1)                 |                       |                           |        |         |       |
| E-PFP 5 - E-PFP 8 (1 each)  |                       |                           |        |         |       |
| E-PFP 11 (2)                |                       |                           |        |         |       |
| E-PFP 12 - E-PFP 14 (1 each) |                     |                           |        |         |       |
| E-PFP 15 (3)                |                       |                           |        |         |       |
| E-PFP 16 (1)                |                       |                           |        |         |       |
| E-PFP 21 (1)                |                       |                           |        |         |       |
| E-PFP 23 - E-PFP 24 (1 each) |                     |                           |        |         |       |
| E-PFP 26 - E-PFP 32 (1 each) |                     |                           |        |         |       |
| E-PFP 34 - E-PFP 36 (1 each) |                     |                           |        |         |       |
| E-PFP 41 - E-PFP 42 (1 each) |                     |                           |        |         |       |
| E-PFP 47 (1)                |                       |                           |        |         |       |
| E-PFP 51 (3)                |                       |                           |        |         |       |
| E-PFP 53 - E-PFP 55 (1 each) |                     |                           |        |         |       |
| I-PFP 56 (2)                |                       |                           |        |         |       |
| I-PFP 19 (1)                |                       |                           |        |         |       |
| I-PFP 21 - I-PFP 22 (1 each) |                     |                           |        |         |       |
| I-PFP 33 (1)                |                       |                           |        |         |       |
| Subtotal                    | 7                     | 123                       | 66     | 9       | 205   |
| *S. Corvallis-PFPs*         | C-PFPs identified in several sources | C-PFP 1 (2) |        |         | 78    |
| I-PFP 4 (23)                |                       |                           |        |         |       |
| C-PFP 5 (1)                 |                       |                           |        |         |       |
| C-PFP 6 (1)                 |                       |                           |        |         |       |
| C-PFP 7 (1)                 |                       |                           |        |         |       |
| C-PFP 9 (1)                 |                       |                           |        |         |       |
| C-PFP 11 (2)                |                       |                           |        |         |       |
| C-PFP 10 (3)                |                       |                           |        |         |       |
| C-PFP 12 (2)                |                       |                           |        |         |       |
| C-PFP 13 (1)                |                       |                           |        |         |       |
| C-PFP 14 (1)                |                       |                           |        |         |       |
| Subtotal                    | 36                    | 19                        | 25     | 10      | 90    |
and serotyped at the FIHES (Murakami et al., 2007). The sporadic case samples were obtained from periodic inspections of food handlers in the Kinki (three S. Enteritidis isolates and one S. Infantis isolate) and Kyushu-Okinawa regions. In the food category, isolates were collected from beef, broiler meat, liquid-egg, and pork (Table 1). These food isolates were obtained during a food hygiene inspection survey for foodborne pathogens in Fukuoka Prefecture, and were isolated at the FIHES. In the slaughterhouse category, isolates were collected from broiler slaughterhouse samples (chilling water and carcass), chicken samples from a slaughterhouse (not identified as broiler- or layer-derived), and layer slaughterhouse samples (chilling water and carcass) (Table 1). These isolates were obtained from five slaughterhouses in the Kyushu-Okinawa region and were isolated at the Fukuoka Prefectural Meat Safety Inspection Center, Chikushino, Fukuoka Prefecture, and at another laboratory in Fukuoka Prefecture.

In the farm and shell egg production environment category, isolates were grouped into those from swabs from egg shells and the egg production environment, pooled broken shell eggs, pooled feces of broiler chickens, and necropsy materials from broilers (Table 1). The swabs from egg shells and egg production environment isolates and the pooled broken shell egg isolates were obtained from 32 chicken farms and three egg-packing facilities. Thirty-one of the 32 farms that donated samples were located in the Kyushu-Okinawa regions, while one farm that donated four samples of S. Corvallis was outside of the region. Some of these isolates were provided by a live-stock hygiene service center. Two of the three egg-packing facilities (facilities A and B) provided isolates from swab samples obtained from eggshells and the egg production environment. Facility A packs 360,000 eggs per day, and these eggs are supplied from nine farms. Facility B has an integrated operation with 240,000 eggs supplied daily from their own farm. Retail data were not available from farms, or for the third egg-packing facility.

Finally, in the environment inspection category, isolates were obtained from river water and sewage samples (Table 1). The samples were obtained from Fukuoka Prefecture, and bacteria were isolated at the FIHES (Murakami et al., 2001). These isolates were re-categorized into the following four groups: layer chicken-related, broiler chicken-related, human, and other isolates (Table 2).

One isolate from each sample was analyzed, except in the case of four S. Enteritidis-containing samples, 21 S. Infantis-containing samples, and two S. Corvallis-containing samples for which two isolates from each sample were analyzed. In these samples, two representative isolates showed different PFPPs, indicating the presence of more than one strain. Therefore, both isolates were analyzed from these 27 samples (totaling 54 isolates).

### Table 3. Chronological appearance of each pulsed-field profile of Salmonella enterica subspecies enterica serovars Enteritidis (S. Enteritidis), S. Infantis, and S. Corvallis over a 17-year period.

|                | Isolation year          | Total (%) |
|----------------|-------------------------|-----------|
|                | 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 |
| S. Enteritidis (177 isolates) | E-PFPs observed for 2 or more years | The remaining 49 E-PFPs observed in a single year only |
| E-PFP 1 | 1 | 1 | 7 | 10 | 4 | 13 | 50 | 1 | 1 | 2 | 1 | 92 | (52.0) |
| E-PFP 10 | 2 | 2 | 5 | 22 | 1 | 1 | 2 | 1 | 2 | 1 | 32 | (18.1) |
| S. Infantis (205 isolates) | I-PFPs observed for 2 or more years | The remaining 44 I-PFPs observed in a single year only |
| I-PFP 2 | 4 | 4 | 2 | 15 | 2 | 6 | 1 | 2 | 36 | (17.6) |
| I-PFP 4 | 16 | 10 | 4 | 4 | 32 | 1 | 6 | 2 | 5 | 7 | 87 | (42.4) |
| I-PFP 9 | 1 | 1 | 1 | 2 | 3 | (1.5) |
| S. Corvallis (90 isolates) | C-PFPs observed for two or more years | The remaining 4 C-PFPs observed in a single year only |
| C-PFP 1 | 1 | 10 | 3 | 14 | (15.6) |
| C-PFP 3 | 2 | 3 | 5 | (5.6) |
| C-PFP 4 | 9 | 1 | 16 | 3 | 2 | 7 | 1 | 39 | (43.3) |
| C-PFP 5 | 1 | 1 | 2 | (2.2) |
| C-PFP 6 | 2 | 2 | 3 | (3.3) |
| C-PFP 7 | 7 | 1 | 8 | (8.9) |
| C-PFP 8 | 2 | 2 | 4 | (4.4) |
| C-PFP 9 | 1 | 1 | 3 | (3.3) |
| C-PFP 10 | 1 | 2 | 3 | (3.3) |
| C-PFP 11 | 2 | 1 | 6 | (6.7) |

PFPS, pulsed-field profiles.
Pulsed-field gel electrophoresis

Clonal lineages of the S. Enteritidis, S. Infantis, and S. Corvallis serovars were determined by PFGE analysis. PFGE was performed as described previously (Murakami et al. 1999b; Murakami et al. 2007), with the following modifications. After preparation for restriction endonuclease digestion, the DNA in each S. Corvallis plug was digested with 20 units of XbaI (Takara Bio, Otsu, Japan) at 37°C for 15 h, and then electrophoresed at 200 V for 22 h, with a switched pulse time of 5–50 s at 14°C. Plugs of S. Enteritidis and S. Infantis were then digested with 20 units of BlnI (Takara Bio) at 37°C for 15 h, and electrophoresis was performed at 200 V for 24 h with a switched pulse time of 2–43.1 s at 14°C. DNA fragment patterns were assessed visually, and different PFPs were assigned based on the presence or absence of bands. Similarity and cluster analyses were performed using the Dice coefficients of similarity and an unweighted pair group method with average linkage, respectively, using FPQuest Software (Bio-Rad Laboratories, Hercules, CA, USA). S. Enteritidis strain ATCC 13076, S. Infantis strain ATCC 51741, and S. Corvallis strain K54-1 were used as respective reference strains in all analyses.

Simpson’s index analysis

Simpson’s index of diversity (Hunter and Gaston 1988) was used to evaluate genetic diversity within each serovar. This index is given by the following equation:

\[ D = 1 - \frac{\sum (n - 1)}{N(N - 1)} \]

where \( n \) is number of isolates belonging to the \( n \)th type and \( N \) is total number of isolates in the same population. A value of 1 indicates infinite diversity, and a value of 0 indicates no diversity.

Statistical analysis

PFP associations between layer and broiler chicken isolates of each of the three serovars were evaluated using the Wilcoxon rank-sum test (Hollander and Wolfe, 1973) using SAS Software version 9.1.3 (SAS Institute, Cary, NC, USA) to assess population differences between the two host types.

Results

Pulsed-field gel electrophoresis

The three serovars showed limited PFP diversities. Although the S. Enteritidis isolates displayed 51 distinct PFPs (Tables 1 and 2), 92 (52.0%) and 32 (18.1%) isolates displayed E-PFP1 and E-PFP10, respectively. In particular, E-PFP1 was detected every year except one between 1990 and 2005 (Table 3), and no E-PFPs other than E-PFP1 and E-PFP10 were detected in more than one year (Table 3). No E-PFPs were shared between layer chicken isolates (pooled broken shell eggs and liquid-egg isolates) and broiler chicken isolates (pooled feces) (Tables 1 and 2). In addition, many E-PFPs associated with broiler-derived isolates grouped together in clades that were distinct from the layer chicken isolates (Appendix Figure 1).

Figure 1. Dendrogram of pulsed-field profiles for Salmonella enterica subspecies enterica serovar Infantis (I-PFPs) following BlnI digestion. Fifty-four different I-PFPs were obtained from 205 isolates. Numbers indicate fragment sizes. Origins of each I-PFP are indicated. I-PFPs with closed circles were observed in two or more years. Some I-PFPs were assigned numbers in our previous study, and thus are not numbered consecutively. The scale indicates the percentage similarity, as determined using Dice coefficients.
The 205 S. Infantis isolates showed 54 I-PFPs, with 87 (42.4\%) and 36 (17.6\%) isolates being I-PFP4 and I-PFP2, respectively (Table 1, Figure 1). I-PFP2 was found in isolates from both broiler chicken and human samples, while I-PFP4 appeared in isolates from pork, broiler chickens, and humans (Table 2). I-PFP18 was the dominant I-PFP of layer chicken isolates across a 5-year period (Table 3). Among the 54 I-PFPs, 10 were detected over multi-year periods, and 44 (51 isolates) were each found in a single year. Some I-PFPs were assigned numbers in our previous study, and thus are not numbered consecutively in this study. Layer and broiler chicken isolates shared no common I-PFPs (Tables 1 and 2).

Many layer chicken isolate-associated I-PFPs grouped together in clades that were distinct from those of the broiler chicken isolates (Figure 1).

Fourteen distinct S. Corvallis C-PFPs were detected (Tables 1 and 2, and Appendix Figure 2). C-PFP4 was the dominant C-PFP for over 10 years, and was found in 39 (43.4\%) of 90 isolates (Table 3). Among the 14 C-PFPs, nine were present over multi-year periods (Table 3).

Analyses using Simpson’s index

Simpson’s index results for the genetic diversities of S. Enteritidis, S. Infantis, and S. Corvallis were 0.70, 0.79, and 0.78, respectively.

Statistical analysis

PFP patterns of the S. Infantis isolates were significantly different (P<0.001) between isolates associated with layer and broiler chickens. No statistical differences between these groups were found for S. Enteritidis (P=0.147) and S. Corvallis (P=0.597).

Discussion

Our study had three major findings. First, Simpson’s index analysis indicated that there was little PFP diversity within the serovars. Second, dominant PFPs of S. Enteritidis, S. Infantis, and S. Corvallis were observed in both chicken- and human-derived strains. The dominant PFPs of S. Enteritidis and S. Infantis persisted for a relatively long time (>10 years). Finally, of the population structures of the PFPs for the three serovars, only the I-PFPs of broiler chicken isolates differed significantly from those of layer chicken isolates, based on the Wilcoxon rank-sum test. Moreover, none of the PFPs detected for S. Enteritidis and S. Infantis from layer chicken isolates overlapped with those from broiler chicken isolates. Based on these findings, we concluded that one E-PFP was dominant in the Kyushu-Okinawa region of Japan (Table 3), and that S. Infantis from several sources showed limited PFP diversity.

Comparing Simpson’s index results determined in the current analysis to those from other countries, the diversity of the S. Enteritidis isolates (0.70) was lower than that determined in previous studies: 0.76 for a general US survey (using BlnI) (Zheng et al., 2007), 0.79 in Minnesota, USA (using XbaI) (Rounds et al., 2010), and 0.79 in France (using XbaI) (Kérouanton et al., 2007). The value for S. Infantis (0.79) was also lower than those reported in other studies: 0.97 in Minnesota (using XbaI) (Rounds et al., 2010) and 0.88 in France (using XbaI) (Kérouanton et al., 2007). The differences between the current and previous studies may stem from physiological differences or clonalities of the serovars. We collected samples mainly from the Kyushu-Okinawa region, which may be limited in comparison with other studies. However, the values determined in all nontyphoidal Salmonella studies are lower than those for other salmonellae or foodborne pathogens such as S. Typhi (0.952) (Kubota et al., 2005) or Escherichia coli O157 (0.98) (Avery et al., 2002), illustrating the limited clonal populations of these three Salmonella serovars in the Kyushu-Okinawa region of Japan.

There are several explanations for the observed lower genetic diversity of the Salmonella serovars examined in the current study. Hauser et al. suggested genetic stability or broad dissemination of a recent ancestor as possible reasons for the high clonality of S. Infantis (Hauser et al., 2012). However, another reason might be the limited genealogical diversity of industrial poultry chickens. Almost all commercial layer and broiler chicken flocks in many developed countries, including Japan, are derived from a few great-grandparental flocks that are imported from limited countries (Leeson and Summers, 2000), likely limiting the impact of genealogical factors. Therefore, S. Enteritidis and S. Infantis serovars might have evolved to adapt to the limited chicken genetic population, as is described by the theory of co-evolution (Pfenning, 2001). If all members of a population adapt to an evolutionarily stable state, no mutations can evolve under the influence of natural selection according to Maynard-Smith (1982).

Conclusions

Limited PFP diversities were detected in S. Enteritidis, S. Infantis, and S. Corvallis isolates collected between 1989 and 2005 from primarily chicken-derived origins in the Kyushu-Okinawa region of Japan. It is important to account for these limited PFP diversities in epidemiological analyses of outbreaks caused by these Salmonella serovars.

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