Epidemiological Analysis of Salmonella enteritidis Isolates from Humans and Broiler Chickens in Thailand by Phage Typing and Pulsed-Field Gel Electrophoresis

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To determine the phage types (PT) of Salmonella enteritidis found in Thailand and to clarify the potential for human infection by S. enteritidis in broiler chicken meat, human and poultry isolates taken from Thailand between 1990 and 1997 were phage typed and analyzed by pulsed-field gel electrophoresis (PFGE). Ten different PT were found among the 302 isolates phage typed, with PT 4 being the most frequent in human (73.9%) and poultry (76.2%) isolates, followed by PT 1 (8.0%), 8 (3.6%), and 7a (2.2%) in human isolates and by PT 7a (4.9%), 1 (3.7%), and 12 (2.4%) in poultry isolates. Of the 53 isolates analyzed by PFGE, 45 showed an indistinguishable pattern (pattern A) by BlnI-digested PFGE and the other 8 isolates showed a very similar pattern that differed by only a few bands. These results indicate the spread of a genetically identical clone of S. enteritidis in humans and poultry in Thailand.

Human infections with Salmonella enteritidis have been increasing worldwide since 1980 and have been shown to be related mainly to consumption of eggs and egg products (4, 18). On the other hand, S. blockley, S. Weltzienii, and S. Amsterdam have been identified as common serovars found in broilers, layers, and breeder parent stock, respectively, and Salmonella has been detected in eggs from layers, according to a Thai report (20). Furthermore, S. enteritidis has been isolated from chicken fettes and chicken meat in Thailand (1, 6). However, the relationship between human infections and isolates of S. enteritidis from broiler chicken meat remains obscure, as does the significance of chicken meat as a vehicle of infection, since epidemiological analysis, including phage typing and genetic analysis, has not been performed on these Thai isolates.

Phage typing has been used with great success to trace the source of S. enteritidis infection in humans (26). Pulsed-field gel electrophoresis (PFGE) based on analysis of the whole genome by restriction endonuclease digestion might also be useful for investigation of sources of salmonellosis (14). The objective of the present study was to analyze the phage types (PT) of S. enteritidis found in Thailand and to determine the significance of broiler chicken meat as a food vehicle for human infection by using the data obtained by phage typing and PFGE.

MATERIALS AND METHODS

Bacterial strains. A total of 302 strains of S. enteritidis were phage typed. This total comprised 138 strains isolated from native Thai patients with sporadic diarrhea between 1990 and 1997, 87 strains from broiler chicken meat taken from slaughterhouses between 1993 and 1997, 23 strains from retail broiler chicken meat samples taken in 1997, and 54 strains from broiler chicken fettes samples collected between 1993 and 1997. Fifty-three isolates were randomly selected from the 302 strains for PFGE analysis to be representative of all of the PT detected by origin. These consisted of 29 isolates from humans, 12 from broiler chicken meat from slaughterhouses, 2 from retail broiler chicken meat, and 10 from broiler chicken fettes.

Phage typing. Phage typing was done by the method of Ward et al. (26). Briefly, 24-h cultures of isolates on agar were inoculated into 3 ml of phage broth. After 2 h of incubation with vigorous shaking, the broth was poured directly onto a phage agar plate. After 30 min incubation overnight, the phage lysis pattern of each culture was compared with the published patterns. Phages were obtained from the Laboratory of Enteric Pathogens, Public Health Laboratory Service, in England. Strains showing a pattern that did not conform to any recognized PT were designated as “reacted but did not conform” (RDNC) (26). Strains that did not react with any of the typing phages were designated as “untypeable” (UT).

PFGE. The extraction of genomic DNA and the conditions for PFGE were as previously described (14), with minor modifications. In brief, bacterial cells on agar medium were directly embedded in low-melting-temperature agarose (Bio-Rad Laboratories, Richmond, Calif.). Solidified agarose gel plugs were first digested with 1 mg of lysozyme solution per ml at 37°C overnight and then with 1 mg of PMSF to inactivate the proteinase K at 5°C overnight. The plugs were then transferred to a tube containing 1 mM EDTA (pH 9.0) at 30°C overnight. The plugs were then washed with a tube containing 1 mM PMSF to inactivate the proteinase K at 5°C for 1 h twice. The plugs were equilibrated in Tris-EDTA at 37°C for 1 h twice, cut to an appropriate size, digested with 10 U of restriction endonuclease BglII or XbaI (Takara, Otsu, Shiga, Japan) at 37°C overnight. PFGE was performed with a 1% agarose gel by using a CHEF DRII apparatus (Bio-Rad Laboratories) in 0.5× Tris-borate-EDTA buffer at 13°C and 200 V. For separation of whole genomes, a linearly ramped switching time of 5 to 50 s was applied for 22 h. After PFGE, the gel was stained with ethidium bromide (0.2 μg/ml) and photographed under UV transillumination.

RESULTS

The PT distribution of S. enteritidis isolates from human and poultry specimens taken between 1990 and 1997 is shown in Table 1. PT 4 was the predominant PT in both human and poultry isolates, followed by PT 1 in humans (8.0%) and PT 7a in poultry (4.9%). PT 8 was third in humans (3.6%) but was not found at all in poultry, where the third most common PT was PT 1 (3.7%). Three and two isolates of PT 7a and 12, respectively, were also found in humans. Only one isolate from a human was identified as PT 9a, while four and two isolates of PT 12 and 4a,
respectively, and one isolate each of PT 6a, 9b, and 35 were found in poultry. Of the 320 isolates, 20 were designated atypical (RDNC) and 10 were UT. Although PT 1 was found to be the predominant PT in human isolates in 1995, it had already been identified in slaughterhouse chicken meat in 1994.

To discriminate further among isolates with the same PT, the PFGE method was applied to 53 isolates of *S. enteritidis* that were selected to represent all of the PT in both human and poultry isolates. Digestion with restriction enzyme *Bln*I demonstrated nine PFGE patterns that could be evaluated, ranging from approximately 50 to 1,000 kb (Fig. 1). It was found that 45 (84.9%) of 53 isolates consisting of eight different PT showed an indistinguishable pattern, which we named pattern A (Table 2). Although the other eight PFGE patterns of human and poultry isolates differed slightly from pattern A, on the whole, they appeared to be quite similar (Fig. 1). Furthermore, isolates showing PFGE pattern A had another indistinguishable pattern in common when digested with enzyme *Xba*I (data not shown).

### DISCUSSION

The present study showed that PT 4 was consistently the most common PT among those found in human and poultry isolates from Thailand between 1990 and 1997. PT 4 is known to be the most common PT in England (26), Germany (21), Italy (13), and Japan (7, 12, 22). In Japan, PT 4 has been detected in isolates from parents of layer chickens imported from England in 1988, and the detection of PT 4 in Japanese human isolates has increased since 1990 (12). Although parents of broiler chickens have been imported into Thailand since 1977 (personal communication), their infection with *S. enteritidis* has not been demonstrated. On the other hand, PT 8 is the most common PT in the United States (3, 25), Canada (8), and the Slovak Republic (9), and in the present study it was the third most common in Thai humans but was not found in Thai poultry. PT 1 was found here to be the second most common type among Thai human isolates and is known to be the second most common in Italy (13) but the fourth most common in the Slovak Republic (9) and only the fifth most common in Germany (21), England (26), and Canada (8).

The present study showed, interestingly, that the isolates of

![FIG. 1. PFGE patterns of *S. enteritidis* isolates collected from Thai humans and poultry from 1990 to 1997 and digested with *Bln*I. Lanes: L, lambda ladder used as molecular size markers; A, pattern A derived from 45 isolates of *S. enteritidis* from humans and poultry; B to E, patterns B to E from four human isolates collected in 1990; F and G, patterns F and G from isolates from chicken feces collected in 1993 and 1994; H and I, patterns H and I from isolates from retail chicken meat collected in 1996 and 1997.](http://jcm.asm.org/)
different PT produced the same PFGE pattern, A. In addition, Thong et al. (23) also observed the same phenomenon. Some studies have implicated poultry and poultry product (e.g., egg) contamination as the primary cause of increased \textit{S. enteritidis} infection in humans (4, 18). Although we did not examine the PT of poultry egg isolates in this study, we found that the PT distribution in isolates originating from the meat of broiler chickens was similar to that in human isolates. Furthermore, isolates of PT 1 were found in slaughtered chicken meat in Thailand, in 1994, 1 year prior to their isolation from human diarrheal patients in the present study. The digestion patterns of whole genomes of isolates from humans and poultry were quite similar. This suggests that some of the sporadic human \textit{Salmonella} infections in Thailand are due to the consumption of contaminated broiler chicken meat from Thailand. A previous report from England (18) has shown that the majority of \textit{S. enteritidis} isolates from humans in England, Scotland, and Wales are PT 4, as are the \textit{S. enteritidis} isolates from poultry and eggs. In this study, isolates originating from the feces of both 1- and 30-day-old chickens showed PT 4 as their predominant PT, and all had PFGE pattern A. This might suggest that the chicks in Thailand had been infected with \textit{S. enteritidis} by a transovarial route, as was demonstrated in layer chickens according to a previous report (5).

Because predominant PFGE pattern A was also shared by isolates with different PT other than 4, there might have been PT conversion among the isolates in this study. Such conversion has been shown to occur from PT 4 to 7, 9a, and 24 (15, 16, 24). Although in previous studies, genomic DNA has been treated with only one restriction enzyme and separated by PFGE (2, 11), it has been suggested by Murase et al. (10) that different genotypes might be resolved by digestion with a different restriction enzyme. Restriction enzyme XbaI was employed to check for subpopulations among isolates with PFGE pattern A when \textit{BlnI} was used, and it was confirmed that the isolates showing pattern A formed another indistinguishable PFGE pattern when \textit{XbaI} was used (data not shown). The other eight patterns obtained with \textit{BlnI} were quite similar to pattern A. These data, therefore, suggest a close correlation between isolates of \textit{S. enteritidis} from humans and those from broiler meat products in Thailand.

The use of antibiotics in feed or drinking water given to different species has been shown to affect the distribution of isolates originating from the meat of broiler chickens and humans. In a previous report, the use of antibiotics in feed or drinking water given to chickens has become common in Thailand. In the present study, plasmid patterns or antibiograms were not investigated. Because these data are known to be helpful in tracing where the strains originated, especially when the PFGE showed little variation among the isolates (17, 19), further studies are needed to investigate the plasmid patterns or antibiograms of \textit{S. enteritidis} isolates for future differentiation. Since the export of poultry products is one of the major businesses in Thailand, contamination of the products by pathogens such as \textit{Salmonella} is a serious matter not only for the Thai but for consumers worldwide. Therefore, efforts are needed to eliminate \textit{Salmonella} from poultry products intended for domestic consumption and export to the world market.


table 2: PFGE patterns and PT of 53 \textit{S. enteritidis} isolates collected from Thai humans and poultry from 1990 to 1997

<table>
<thead>
<tr>
<th>PT</th>
<th>No. of isolates with PFGE pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 (H; 2, CMS; 1; CF, 2)</td>
</tr>
<tr>
<td>4</td>
<td>25 (H; 2; CMS; 5; CF, 2)</td>
</tr>
<tr>
<td>4a</td>
<td>2 (CMS; 1; CF, 1)</td>
</tr>
<tr>
<td>6a</td>
<td>1 (CMS)</td>
</tr>
<tr>
<td>7a</td>
<td>5 (H; 2; CMS; 2; CF, 1)</td>
</tr>
<tr>
<td>8</td>
<td>1 (H)</td>
</tr>
<tr>
<td>9a</td>
<td>1 (H)</td>
</tr>
<tr>
<td>9b</td>
<td>1 (H)</td>
</tr>
<tr>
<td>12</td>
<td>5 (H; 2; CMS; 1; CF, 2)</td>
</tr>
<tr>
<td>35</td>
<td>1 (CMS)</td>
</tr>
</tbody>
</table>

\* The 53 strains consisted of 29 from human specimens, 12 from slaughtered chicken meat, 2 from retail chicken meat, and 10 from chicken feces.

\* The values in parentheses are numbers of isolates by origin. H, CMS, RCM, and CF: humans, slaughtered chicken meat, retail chicken meat, and chicken feces, respectively. All of the isolates originating from chicken feces showed pattern A, with the exception of two isolates collected in 1993 and 1994, which showed patterns F and G.

REFERENCES