Monitoring of Layer Feed and Eggs for Salmonella in Eastern Japan between 1993 and 1998

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ABSTRACT

In order to investigate contamination of chicken farms with Salmonella, feed and eggs were sampled from 16 commercial layer farms in eastern Japan between 1993 and 1998 and cultured for salmonellae. Salmonella enterica subsp. enterica isolates belonging to 19 serovars were obtained from the feed. Six of the 19 serotypes, including Salmonella serovar Enteritidis, were observed in isolates recovered from the eggs. Salmonella serovar Enteritidis strains obtained from a feed sample and egg contents in a layer farm showed pulsed-field gel electrophoresis patterns that were genetically related and belonged to a single phage type, suggesting that the contamination of the farms was linked to the occurrence of salmonellae in feed.

Salmonellae are one of major etiologic agents that cause foodborne infections all over the world (1, 7, 13, 17, 21). It is well known that the incidence of foodborne salmonellosis has increased in association with consumption of eggs infected with Salmonella enterica subsp. enterica serovar Enteritidis (11, 13, 17, 29). Although previous reports revealed that contamination of the internal contents of eggs with Salmonella is relatively rare (10, 11, 12, 15, 24), a significant portion of foodborne outbreaks caused by Salmonella serovar Enteritidis in recent years has been attributed to transovarian contamination of eggs with this organism (26). It is important to clarify the factors that introduce salmonellae into layer farms so that contamination of chickens and eggs with this organism can be prevented. Chicks (15, 23), pullets (15, 23), rodents (8), and dirty environment (15, 23) have all been considered as major sources for contamination with Salmonella serovar Enteritidis, although there have been only a limited number of reports of Salmonella contamination in Japanese chicken farms (6, 23). In this investigation, layer feed on the farms and eggs produced there were sampled between 1993 and 1998 and cultured for salmonellae.

MATERIALS AND METHODS

Isolation of salmonellae from feed. Feed was sampled from 16 farms located in eastern Japan once or twice a month between July 1993 and August 1998. Five to 10 tons of feed were transported to the farms by a lorry belonging to the feed company. When the layer feed arrived in each of the farms, a portion (500 to 1,000 g) of the feed was ladled out from the storeroom of the lorry and was collected into a sterilized plastic bag. A total of 10,418 samples were obtained from the 16 layer farms. A 30-g sample of the feed was preenriched in 225 ml of phosphate-buffered peptone water (Oxoid) for 48 h at 37°C. One milliliter of the preenrichment culture was poured into 10 ml of Hajna tetradionate broth (Eiken Chemical Co., Ltd., Tokyo, Japan) and incubated for 48 h at 42°C. The cultures were streaked on desoxycholate hydrogen sulfide lactose agar (Nissui-Seiyaku Co., Ltd., Tokyo, Japan) for 20 h at 37°C. Suspect colonies were identified as Salmonella by standard procedures (16), and all colonies were serologically typed with antisera to O- and H-antigens (Denka-Seiken Co. Ltd., Tokyo, Japan).

Isolation of salmonellae from eggs. Egg samples were obtained in the same farms stated above between 1995 and 1998. The shell and the contents of 30 eggs were pooled into a sterilized plastic bag (60 × 100 cm). A total of 3,740 cultures (112,200 eggs) were carried out. The bags containing the wholes eggs were sealed and incubated for 48 h at 37°C. One milliliter of the incubated contents was poured into 10 ml of the Hajna tetradionate broth, and salmonellae were isolated as described above. To investigate contamination of salmonellae in the egg contents, 1,000 eggs were collected from each of the chicken houses and subjected to bacterial examination because the number of Salmonella organisms is suspected to be very few (10–12, 15, 24). More than 1,000 eggs obtained from a chicken house at a time were used for bacteriological culture in the previous study (23). The shell surface of the eggs was carefully disinfected with cotton wool immersed in 70% ethanol, and the egg contents were divided into three sterilized plastic bags, each containing 333 or 334 eggs (60 by 100 cm). A total of 855 cultures from 285 chicken houses were investigated. The bags containing the egg contents were sealed and incubated for 48 h at 37°C. One milliliter of the incubated contents was poured into 10 ml of the Hajna tetradionate broth, and salmonellae were isolated as described above.

Typing of Salmonella serovar Enteritidis. Several isolates of Salmonella serovar Enteritidis were characterized by pulsed-field gel electrophoresis (PFGE) patterns of BlnI- and XbaI-digested chromosomal DNA (18, 19). Phage typing was carried out...
TABLE 1. Results of isolation of Salmonella from layer feed and eggs

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<tr>
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<td>1,232</td>
<td>1,771</td>
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<td>2,106</td>
<td>2,466</td>
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<td>21</td>
<td>5</td>
<td>3</td>
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<td>53</td>
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<tr>
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<tr>
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<td>ND</td>
<td>1,072</td>
<td>853</td>
<td>907</td>
<td>908</td>
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<td>17</td>
<td>2</td>
<td>2</td>
<td>25</td>
<td>46</td>
</tr>
<tr>
<td>Egg contents</td>
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<tr>
<td>No. of sample tested</td>
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<td>ND</td>
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<td>216</td>
<td>213</td>
<td>855</td>
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<tr>
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<td>13</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>22</td>
</tr>
</tbody>
</table>

*ND,* not done.

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RESULTS

A total of 53 strains of salmonellae were isolated from 10,418 feed samples. Isolation rates in 1995 (1.19%) and 1998 (0.69%) were high, whereas no salmonellae were isolated in 1993 (Table 1). The *Salmonella* isolates obtained from feed samples could be divided into more than 19 serovars, i.e., 6 *Salmonella* serovar Enteritidis, 4 *Salmonella* serovar Orion, and 3 each of *Salmonella* serovar Amersfoort, *Salmonella* serovar Derby, *Salmonella* serovar Infantis, and *Salmonella* serovar Tennessee (Table 2). *Salmonella* serovar Enteritidis was isolated only in 1995 and 1998. Forty-six strains of salmonellae were isolated from the mixed samples of egg contents and shell. *Salmonella* serovar Enteritidis was isolated in 1995 (10 strains) and 1998 (2 strains), when the isolation rate for salmonellae was high. Twenty-two *Salmonella* strains, including 6 *Salmonella* serovar Enteritidis, were isolated from the contents of the eggs. The serovars of salmonellae isolated from the egg contents were similar to those isolated from the mixture of contents and shell.

Isolation of salmonellae from feed and eggs each month was compared between January 1995 and September 1998. As shown in Figure 1, *Salmonella* isolates with identical serovars, such as *Salmonella* serovar Enteritidis were obtained from both feed and egg contents between October and November 1995 and August and September 1998, and *Salmonella* serovar Infantis, *Salmonella* serovar Bareilly, and *Salmonella* serovar Derby between May and July 1998. Similarly, identical serotypes such as *Salmonella* serovar Enteritidis and *Salmonella* Bareilley were recovered from both feed and egg contents in the periods of August to September 1995 and May to July 1998, respectively.

*Bl*I-digested PFGE patterns of two isolates obtained from whole eggs in farm C in October 1998 (Fig. 2, lanes 7 and 8) and that of an isolate from this farm in September 1998 (Fig. 2, lane 9) were interpreted as genetically related according to the established criteria of bacterial strain typing by PFGE (28). One of the strains from the whole eggs and the strain from the feed belonged to phage type 6. *Xba*I-digested patterns were identical, except for the one that was different by two DNA bands from the others (data not shown).

DISCUSSION

This is the first investigation that has monitored *Salmonella* contamination of both the feed consumed and the eggs produced in the same layer farms for long periods. The present investigation demonstrated that commercial chicken feed widely traded in Japan is not free from salmonellae and that contamination with *Salmonella* serovar
Enteritidis in feed should be a concern. Several investigations have been generated on Salmonella in poultry feeds (2, 3, 5, 29), although only limited investigations described isolation of Salmonella serovar Enteritidis from layer feed (5). Geue and Schluter (5) indicated possible contamination of feed stuff with this organism. Intrusion of wild animals (15) and rodents (8) is considered to cause salmonellae contamination of feed. It is known that mice excrete this organism for a long time (4). In the case of a Salmonella serovar Enteritidis infection that occurred in a commercial layer flock in southern California, Kinde et al. (14) suggested that mice could have contaminated feed bins in the layer houses.

The data obtained in this investigation suggest that the Salmonella contamination in chicken farms is linked to the occurrence of Salmonella in feed that were brought to farms and consumed by layer flocks. The identical serovars of salmonellae were isolated at the same time from feed consumed and eggs produced in the farms investigated. In particular, PFGE patterns of Salmonella serovar Enteritidis strains obtained from both feed and whole eggs in a farm belonged to the same phage type and were genetically related. It is possible that egg-producing chickens harbored salmonellae because they had eaten feed contaminated with salmonellae and that the eggs were contaminated by transovarian transmission (24) or penetration of the shell by organisms present in chicken feces (25). It was reported that the birds became infected with Salmonella serovar Enteritidis horizontally (8, 9, 30) or through vertical transmission from the parent (20). However, no salmonellae were isolated from 6,972 chicks younger than 4 days old hatched in the layer breeder farms where breeders have been given heated feed (unpublished data). Thus, we suggest the importance of feed contaminated with salmonellae as a source of contamination with this bacterium in the poultry industry. It is of interest that phage types determined in the present strains are usually found in strains from patients with gastrointestinal illness in Japan (21).

The isolation rate of Salmonella in the chicken feed varied each year, although the isolation method was not changed throughout this investigation. It should be noted that the raw materials of each lot of commercial feed are not of the same quality, because almost all raw materials
used for chicken feed in Japan are imported from various countries.

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REFERENCES


