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Features of *Salmonella* serovars among food handlers in Kyushu, Japan

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SUMMARY

Salmonella were isolated from 106 (0.032%) of 331,644 fecal samples from food handlers, and from 144 of 11,478 fecal samples from symptomatic patients in Japan to determine the incidence and features of *Salmonella* serovars among food handlers. *S. enterica* subspecies *enterica* serovar Infantis (*S.* serovar Infantis) was the dominant serovar (accounting for 48.1%), followed by *S.* serovar Corvallis, which showed poor genetic diversity, and *S.* serovar Enteritidis among food handlers. The former two serovars were not dominant among symptomatic patients. The present study demonstrates the need for education on the sanitary handling of chicken eggs and chicken meat, which are possible infectious sources of these *Salmonella* serovars.

KEY WORDS: Salmonella, *S.* serovar Enteritidis, food handler, food hygiene

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Salmonella species are currently the most common cause of food-borne infections in Japan (National Institute of Infectious Diseases, 2000), and are organisms for which humans as carriers pose potential problems as sources of outbreaks (Cruickshank and Humphrey, 1987). Therefore, consideration of the significance of fecal carriage of *Salmonella* by food handlers is important to public health. However, there have been few studies estimating the general carrier rate, or serotyping the *Salmonella* carried asymptomatically by such workers in wider areas such Kyushu or Japan as a whole. The serotyping of isolates from food handlers is important in estimating the prevalence of *Salmonella* strains harbored by food handlers in the wider area. The present study was performed to determine the incidence and features of *Salmonella* among food handlers compared with serovars from symptomatic patients in the same period in Kyushu, Japan. *Salmonella* serovars were isolated from 106 (0.032%) of 331,644 fecal samples from food handlers; *S. enterica* subspecies *enterica* serovar Infantis (*S.* serovar Infantis) was the dominant serovar, followed by *S.* serovar Corvallis and *S.* serovar Enteritidis. This study provides information on these serovars, and demonstrates the need for further education on food hygiene, including methods of sanitary handling of chicken eggs and chicken meat, which are possible infectious sources of *S.* serovar Infantis, *S.* serovar Corvallis and *S.* serovar Enteritidis.

From November 1999 to May 2000, fecal samples (331,644) were collected from food handlers working in food factories, hotels, restaurants, supermarkets or companies that provide food services for offices, factories, hospitals, schools, daycare centers and other facilities, in eight pre-

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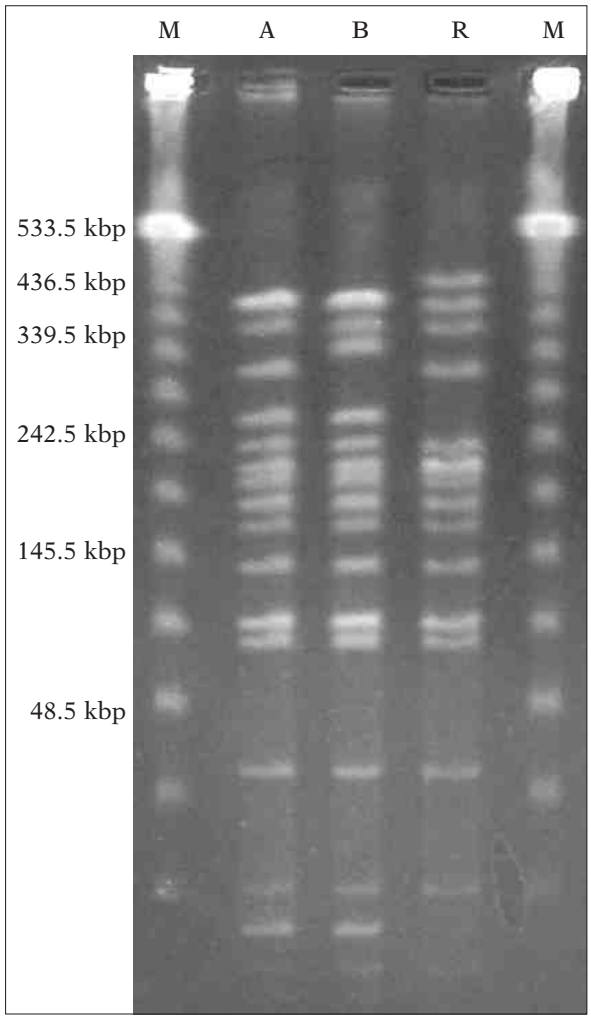

FIGURE 1 - *Profiles of 18 isolates of* Salmonella *serovar Corvallis, obtained using pulsed-field gel electrophoresis. Eleven isolates of food handlers showed pulsed-field profile A (lane A) and five isolates showed pulsed-field profile B (lane B). Lane M shows the DNA size standard, a bacteriophage lambda consisting of concatemers starting at 48.5 kilobase pairs. Lane R shows the reference pulsed-field profile of an isolate obtained from river water.*

fectures in Kyushu, Japan. Fecal samples (11,478) from patients with diarrhea were collected from 800 clinical facilities in eight prefectures in Kyushu and Kinki in Japan during the same period. These symptomatic patients ranged in age from 1 to 84 years (patients who were 1 to 10 years old accounted for 59% of the total, while those who were 11 to 84 years old accounted for 41%).

Samples were cultured in tubes with 10 ml of modified Rappaport broth for 18 h at 37°C, then streaked for isolation on differential plating media, using *Salmonella-Shigella* agar plates, and incubated for 24 h at 37°C. Potential *Salmonella* colonies were then identified as *Salmonella*, and were serotyped using somatic (O) antisera and flagella (H) antisera as described previously (Murakami *et al.*, 2001).

Sixteen isolates of *S.* serovar Corvallis in this survey were characterized by pulsed-field gel electrophoresis (PFGE) analysis. PFGE analysis was performed as described previously (Murakami *et al.*, 1999b), with brief modifications. After appropriate preparations for restriction endonuclease digestion, the DNA in each plug was digested with 20 U *Xba* I (Takara Bio Inc., Otsu, Japan) at 37°C for 15 h. Electrophoresis was performed at 200 V for 22 h with a switched pulse time of 5-50 s at 14°C. DNA fragment patterns were assessed visually. The presence and absence of a band were assigned different pulsed-field profiles (PFPs). *Xba* I digestion is more advantageous for distinguishing *S.* serovar Corvallis from isolates with different genotypes than PFGE with *Bln* I digestion (data was not shown). Thus, we used *Xba* I digestion in this study.

Salmonella were isolated from 106 (0.032%) of the 331,644 fecal samples from the food handlers, and from 144 (1.25%) of the 11,478 fecal samples from symptomatic patients. The monthly numbers of isolates of *Salmonella* from food handlers were as follows: 23 (0.066%) from 34,838 samples in November 1999; nine (0.020%) from 44,669 samples in December; six (0.014%) from 41,900 samples in January 2000; five (0.012%) from 43,513 samples in February; nine (0.020%) from 44,604 samples in March; 14 (0.024%) from 59,459 samples in April; and 40 (0.064%) from 62,661 samples in May. Table 1 shows the numbers of isolates from food handlers and symptomatic patients. The dominant serovar among 16 serovars and two untypable isolates from the food handlers was *S.* serovar Infantis, followed by *S.* serovar Corvallis and *S.* serovar Enteritidis. Among the 18 serovars and one untypable isolate from the patients, the dominant serovar was *S.* serovar Enteritidis. From both groups of samples, 10 serovars of *Salmonella* were isolated in common. *S.* serovar Corvallis comprised 16 (15.1%) of 106 isolates from food handlers and none from symptomatic patients.

TABLE 1 - *Isolation incidences of* Salmonella *serovars, especially* S. *serovar Infantis,* S. *serovar Corvallis and* S. *serovar Enteritidis, from food handlers and symptomatic patients".*

Serovar	*No. of isolates (%)*						
O-group	*Name*	*Food handlers*		*Symptomatic patients*		*Total*	
C_1	*S. enterica* serovar Infantis	51	(48,1%)	9	(6,3%)	60	(24,0%)
C_3	*S. enterica* serovar Corvallis	16	(15,1%)			16	(6,4%)
D	*S. enterica* serovar Enteritidis	13	(12,3%)	99	(68,8%)	112	(44,8%)
B	*S. enterica* serovar Agona	6	(5,7%)	2	(1,4%)	8	(3,2%)
	S. enterica serovar Haifa			1	(0,7%)	1	(0,4%)
	S. enterica serovar Paratyphi B	1	(0,94%)	2	(1,39%)	3	(1,20%)
	S. enterica serovar Stanley	1	(0,9%)	1	(0,7%)	2	(0,8%)
	S. enterica serovar Typhimurium			4	(2,8%)	4	(1,6%)
	Untypable (b : -)	2	(1,9%)			2	(0,8%)
C_1	*S. enterica* serovar Montevideo	3	(2,8%)	1	(0,7%)	4	(1,6%)
	S. enterica serovar Oranienburg			1	(0,7%)	1	(0,4%)
	S. enterica serovar Tennessee	2	(1,9%)			2	(0,8%)
	S. enterica serovar Thompson	1	(0,9%)	2	(1,4%)	3	(1,2%)
	Untypable			1	(0,7%)	1	(0,4%)
C_2	*S. enterica* serovar Breda	1	(0,9%)			1	(0,4%)
	S. enterica serovar Hadar	1	(0,9%)	1	(0,7%)	2	(0,8%)
	S. enterica serovar Litchfield	3	(2,8%)			3	(1,2%)
D	*S. enterica* serovar Dublin			2	(1,4%)	2	(0,8%)
	S. enterica serovar Javiana			10	(6,9%)	10	(4,0%)
	S. enterica serovar Miyazaki	1	(0,9%)	3	(2,1%)	4	(1,6%)
	S. enterica serovar Onarimon			1	(0,7%)	1	(0,4%)
	S. enterica serovar Panama	1	(0,9%)			1	(0,4%)
E_1	*S. enterica* serovar Anatum			1	(0,7%)	1	(0,4%)
	S. enterica serovar London	1	(0,9%)			1	(0,4%)
	S. enterica serovar Weltevreden	2	(1,9%)	1	(0,7%)	3	(1,2%)
F	*S. enterica* serovar Aberdeen			2	(1,4%)	2	(0,8%)
Total		106	(100%)	144	(100%)	250	(100%)

S. enterica serovar Infantis: *S. enterica* subspecies *enterica* serovar Infantis

PFGE analysis of the fragments obtained by *Xba* I digestion of genomic DNA from 16 isolates of *S.* serovar Corvallis showed only two distinct PFPs; 11 isolates showed PFP A and five isolates showed PFP B, with 16 resolvable fragments, ranging from approximately 10 to 582 kilobase pairs.

According to a previous study (Yamada *et al.*, 1999), *S.* serovar Corvallis accounted for 28.0% of all isolates (1,125) from human and other sources, and many isolates of this serovar (93.7%) were obtained from food handlers in Miyazaki Prefecture, Kyushu, Japan, between 1996 and 1999. In the present study, *S.* serovar Corvallis was frequently isolated only from food handlers, not from symptomatic patients. Clearly, the prevalent *Salmonella* serovars found in the general population differ between countries, residential areas and human groups, depending on a large number of factors including age bracket, occupation, dietary preferences, eating habits, food processing, distribution practices, and so on. Therefore, we cannot explain why *S.* serovar Infantis and serovar Corvallis, which were not dominant serovars among symptomatic patients, were often harbored by food handlers in Kyushu, Japan in this period of time.

S. serovar Enteritidis, the dominant serovar among symptomatic patients, is most commonly associated with chicken egg production, and *S*. serovar Infantis, the dominant serovar among food handlers, is commonly associated with broiler meat production (Humphrey, 1994; Murakami *et al.*; 1999a, Murakami *et al.*, 1999b), but there have been few reports on the ecology or virulence of *S*. serovar Corvallis. In our previous study, *S*. serovar Corvallis was isolated from raw chicken meat, chicken eggs, sewage and river water samples (Murakami *et al.*, 2001), and possible reservoirs of this organism include hens and broilers. On PFGE analysis, isolates of *S*. serovar Corvallis had only two PFPs, demonstrating poor genetic diversity among *S*. serovar Corvallis isolates. Poor genetic diversity shows that the sources of infection for food handlers were not varied. Since chicken eggs and chicken meat are possible infectious sources of *S*. serovar Corvallis, *S*. serovar Infantis and *S*. Enteritidis for food handlers, further education on food hygiene, including methods of sanitary handling of chicken eggs and chicken meat, is important.

Previous studies have shown that asymptomatic carriers in sensitive professions, such as food handlers and caregivers, are rarely involved in the transmission of *Salmonella* (Buchwald and Blaser 1984; Janda and Abbott, 1998). There have been few outbreaks in which asymptomatic carriers who were food handlers or healthcare personnel were clearly shown to be the source of infection. Roberts reported that, in most instances, food handlers are the victims rather than the source, and become infected from frequent contact with contaminated raw food, from tasting during preparation, or from eating leftover contaminated cooked food (Roberts, 1982). However, there have been a few reports in which food handlers, with or without symptoms, or who had children with gastrointestinal disease, were implicated as sources of infection (Khuri-Bulos *et al.*, 1994; Maguire *et al.*, 2000). Thus, it is important to note that contamination of food by handlers is an unusual occurrence, and such workers should be given further instruction in aspects of food hygiene, such as improvement of premises and practices in food handling, as described previously (Charles, 1982).

The present data suggest that *S*. serovar Infantis, *S*. serovar Corvallis and *S*. serovar Enteritidis are harbored by food handlers in Kyushu, Japan, with a relatively high frequency. Therefore, further education of food handlers is required regarding food hygiene, including information on the sanitary handling of chicken eggs and chicken meat, and the eradication of *S*. serovar Infantis, *S*. serovar Corvallis and *S*. serovar Enteritidis.

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