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Salmonella Serovars from Foodborne and Waterborne Diseases in Korea, 1998-2007: Total Isolates Decreasing Versus Rare Serovars Emerging

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Salmonella enterica has been one of the most widespread foodborne pathogens in Korea. Between 1998 and 2007, a total of 9,472 *Salmonella* isolates were identified from foodborne and waterborne illness patients. During that time, Korea was transitioning into a developed country in industry as well as in its hygiene system. Although the isolation number of total *Salmonella* including serovar Typhi has decreased since 1999, the isolation of rare *Salmonella* serovars has emerged. Three most prevalent serovars during 1998-2007 were *S. enterica* Typhi, *S. enterica* Enteritidis, and *S. enterica* Typhimurium. There were remarkable outbreaks caused by rare serovars such as *S. enterica* Othmarschen, *S. enterica* London and *S. enterica* Paratyphi A, and overseas traveler-associated infections caused by *S. enterica* Weltevreden and *S. enterica* Anatum. *Salmonella* serovars from overseas travelers made a diverse *Salmonella* serovar pool in Korea. This study is the first review of the status of the human *Salmonella* infection trend in a developing country during 1998-2007. Newly emerging rare *Salmonella* serovars should be traced and investigated to control new type pathogens in the developed world.

Key Words: *Salmonella*; Serovar; Foodborne Diseases

INTRODUCTION

Salmonella has been a major foodborne and waterborne pathogen in Korea (1, 2). In 2006, *Salmonella* was a major foodborne bacterial pathogen in the United States, causing more deaths than any other foodborne pathogen (3). *S. enterica* Typhi, a causative agent of typhoid fever, has especially threatened Korean health (4). There were 1,921 deaths resulting in 17% mortality because of typhoid fever during 1945-1960 in Korea. Salmonellosis caused by non-typhoid *Salmonella* (NTS) gives rise to diarrhea, vomiting, abdominal pain, and enteric fever. Occasionally, systemic infection with bowel perforation, septicemia, and osteomyelitis are also caused by NTS (5-8).

At present, there are more than 2,500 *Salmonella* serovars in the world with new serovars emerging yearly. *Salmonella* serotyping is very important to the epidemiology study. Unquestionably, *Salmonella* serotyping is time-consuming and complex work for the serological identification of bacteria (9). To serotype *Salmonella*, lipopolysaccharide epitopes in bacterial membrane (O antigens) and flagella proteins (H antigens) should be identified with the respective antibodies. For the identification of *S. enterica* Typhi, additional antibody specific to capsular polysaccharides (Vi antigen) is essential. Until 1990, reference laboratories in Korea merely performed sero-grouping with only O antigen-specific and Vi–specific antibodies. Therefore, they were unable to complete serotyping of *Salmonella* due to expensive commercial antibodies and lack of interest in surveillance. Truly, at present, most poor or developing countries perform only sero-grouping of *Salmonella* because of the cost problem (10).

Korea National Institute of Health (KNIH) is the headquarters for the national surveillance of *Salmonella* in Korea. KNIH gathered *Salmonella* isolates and analyzed their epidemiological data from 17 Research Institutes of Health and Environment located in cities and provinces and 13 quarantine stations located in airports and harbors in Korea. The microbiologists in these 17 regional institutes covering all country and 13 quarantine stations isolated enteric bacteria from patients according to the standardized protocols distributed by KNIH. According to Korea's Infectious Diseases Prevention Act which has been enforced since 1954, certain infectious diseases must be reported to government authorities. These diseases are classified into four classes according to the grade of danger and threat to public health. Among the infectious diseases caused by *Salmonella* species, typhoid fever and paratyphoid fever are classified as Class 1 notifiable infectious diseases, and NTS-causing diseases are classified as Class 4.

As Korea has developed, the desire for an advanced social hygiene system for the well-being of the people has increased.

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Transportation, water supply and drainage, medical and food-processing systems were developed. Between the 1990s and 2000s, Korea had one of the highest economic growths in the world. This rapid growth resulted in dramatic changes in life-styles as well as in incidence of foodborne *Salmonella* (11).

In this review, three major *Salmonella* serovars, *S. enterica* Typhi, *S. enterica* Enteritidis, and *S. enterica* Typhimurium, and several remarkable outbreaks caused by rare *Salmonella* serovars in 1998-2007 are discussed. As complete serotyping of *Salmonella* was started and its security electronic database was constructed in national reference laboratories from 1998, this study is the first review of the status of the human *Salmonella* infections trend in Korea during 1998-2007. Finally, overseas-travel associated infection cases and the effects on serovar prevalence in Korea are also discussed.

THREE PREVALENT SEROVARS

From 1998 to 2007, *S. enterica* Typhi, *S. enterica* Enteritidis, and *S. enterica* Typhimurium were the most frequent *Salmonella* serovars in diarrhea patients and foodborne diseases in Korea (Fig. 1, Table 1) (12). From 422 to 2,252 culture-proven *Salmonella* infection cases and their isolates have been identified every year since 1998. The percentage of these 3 serovars among *Salmonella* isolates was over 70% almost every year from 1998 to 2007.

S. enterica Typhi is a causative pathogen of typhoid fever. Typhoid was an endemic enteric fever disease in Korea. It is not only systemic infection with high morbidity but also a common public health problem in Korea. The overall incidence of typhoid fever was 0.41 per 100,000 population from 1992 to 2000 (4). The number of *S. enterica* Typhi isolates was always among the three

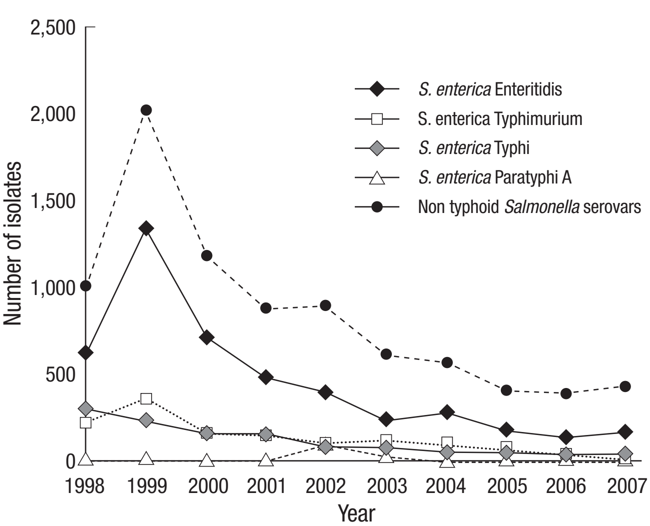

Fig. 1. Incidence of four serovars, *S. enterica* Typhi, *S. enterica* Paratyphi A, *S. enterica* Typhimurium and *S. enterica* Enteritidis and nontyphoidal *Salmonella* serovars excluding *S. enterica* Typhi, 1998-2007.
Source: Reference (12).

Table 1. Top 15 *Salmonella* serovars from foodborne and waterborne diseases and their number of isolates, 1998-2007. Bold characters represent 3 major serovars, *S. enterica* Typhi, *S. enterica* Typhimurium, and *S. enterica* Enteritidis. Underlined serovars show emerging serovars causing remarkable foodborne outbreaks which had been rare in Korea

Rank	1998		1999		2000		2001		2002		2003		2004		2005		2006		2007	
	Serovar	No.	Serovar	No.	Serovar	No.	Serovar	No.	Serovar	No.	Serovar	No.	Serovar	No.	Serovar	No.	Serovar	No.	Serovar	No.
1	**Enteritidis**	626	**Enteritidis**	1,344	**Enteritidis**	718	**Enteritidis**	477	**Enteritidis**	385	**Enteritidis**	232	**Enteritidis**	270	**Enteritidis**	167	**Enteritidis**	135	**Enteritidis**	163
2	**Typhi**	297	**Typhimurium**	351	**Typhi**	153	**Typhi**	147	**Typhimurium**	105	**Typhimurium**	116	**Typhimurium**	95	**Typhimurium**	68	**Typhi**	37	Infantis	62
3	**Typhimurium**	213	**Typhi**	227	**Typhimurium**	142	**Typhimurium**	141	Paratyphi A	91	**Typhi**	77	**Typhi**	40	**Typhi**	34	**Typhimurium**	37	**Typhi**	44
4	Infantis	29	Newport	93	Braenderup	101	Schwarzengrund	35	Braenderup	84	Paratyphi A	38	Braenderup	17	Montevideo	16	London	28	Othmarschen	41
5	Bareilly	13	Infantis	43	Derby	51	London	33	Typhi	66	Braenderup	31	London	17	Braenderup	11	Braenderup	19	**Typhimurium**	27
6	Hadar	12	Braenderup	35	London	43	Montevideo	32	Schwarzengrund	61	London	24	Weltevreden	13	Rissen	10	Schwarzengrund	18	Paratyphi A	10
7	Ohio	12	Montevideo	31	Montevideo	25	Rissen	16	London	28	Bardo	21	Rissen	12	London	9	Hillingdon	17	Braenderup	7
8	Schwarzengrund	10	Give	15	Hadar	13	Litchfield	16	Montevideo	22	Haardt	11	Infantis	10	Infantis	8	Paratyphi A	14	Hillingdon	6
9	Give	8	Hadar	11	Infantis	9	Mbandaka	16	Heidelberg	14	Rissen	11	Paratyphi A	8	Paratyphi A	8	Infantis	11	Weltevreden	6
10	Livingstone	8	Derby	11	Rissen	7	Blockley	10	Pakistan	12	Blockley	8	Hadar	7	Anatum	8	Montevideo	9	Corvallis	6
11	Braenderup	7	Thompson	11	Senftenberg	7	Infantis	9	Rissen	7	Aagona	8	Larochelle	7	Virchow	6	Rissen	8	Anatum	5
12	Mbandaka	7	Mbandaka	6	Mbandaka	6	Braenderup	9	Senftenbergrg	6	Paratyphi B	8	Kingston	6	Agona	4	Virchow	6	Derby	5
13	Kentucky	6	Blockley	6	Bareilly	5	Paratyphi A	7	Haardt	6	Senftenbergrg	8	Montevideo	6	Corvallis	4	Weltevreden	6	Haardt	5
14	Agona	5	Goldcoast	6	Give	4	Haardt	6	Virchow	6	Pakistan	7	Thompson	6	Schwarzengrund	4	Give	6	Paratyphi B	4
15	Derby	5	Paratyphi A	5	Paratyphi A	3	Haifa	6	Paratyphi B	5	Infantis	6	Agona	5	Thompson	4	Corvallis	5	Virchow	4
Total*		1,303		2,252		1,336		1,027		957		677		607		429		422		462

Source: Reference (12).
*Total number of isolates in each year includes the number of other serovar isolates in addition to the number of top 15 serovars.

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most prevalent *Salmonella* serovars in Korea from 1998 to 2007, excluding 2002 (Table 1). In 2002, *S. enterica* Paratyphi A and *S. enterica* Braenderup were the third and fourth most prevalent respectively followed by *S. enterica* Typhi. During 1961-1963, *S. enterica* Typhi was the most dominant serovar in Daegu, Korea, showing 92.6% frequency (13).

Unlike other serovars, *S. enterica* Typhi infects only humans. Therefore, with good hygiene and control of healthy carriers, the incidence of typhoid fever could be decreased (4). Korea Ministry of Health has controlled typhoid fever as a Class 1 notifiable disease. KNIH receives *S. enterica* Typhi isolates along with the epidemiological data from medical doctors according to the Infectious Diseases Prevention Act. Vi-passive hemagglutination for preliminary test and *S. enterica* Typhi isolation and identification are performed with the stool and the blood culture of the patients. In addition, quarantine stations obtain stool samples from overseas travelers who come from high-risk infectious diarrheal disease areas. Regional Health & Environment Institutes then isolate and identify the pathogens from the specimens (14).

The number of *S. enterica* Typhi isolates has decreased gradually since 1998. Busan and Gyeongsang-do, which face the East Sea, were higher incidence areas for typhoid fever than other Korea peninsulas (4). The integron-associated multidrug-resistant (MDR) *S. enterica* Typhi was first identified in Korea in 1999. The MDR isolates were resistant to six antimicrobial agents that were ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, streptomycin, tetracycline, and gentamicin. All the resistance determinants, *aacA4b*, *catB8*, *aadA1*, *dfrA1*, *aac(6')-IIa*, and *blaP2*, were clustered in about a 50 kb plasmid (15). Even though most *S. enterica* Typhi strains are susceptible to antimicrobial agents, drugs for the treatment of typhoid fever should be chosen carefully (16). From the early 2000s, nalidixic acid resistant *S. enterica* Typhi isolates were also identified in Korea. The genetic relation by PFGE revealed that the nalidixic acid resistant *S. enterica* Typhi in Korea was closely linked to those in India, Nepal, and Bangladesh. Increased overseas travelers were one of the main reasons. From the 1990s in Korea, ciprofloxacin was recommended as the drug of choice for typhoid fever (4). Since then, ciprofloxacin has been used widely in other developing and developed countries. According to recent phage-typing study of *S. enterica* Typhi, major phage type transition from M1 and E1 to A occurred in Korea from 1992 to 2006 (17).

S. enterica Typhimurium is a zoonotic pathogen infecting domestic animals and causing salmonellosis in humans (18). *S. enterica* Typhimurium was one of the major foodborne pathogens in Korea during 1998-2007 (Table 1). It was a more serious problem because the frequency of MDR *S. enterica* Typhimurium human isolates increased yearly. MDR *S. enterica* Typhimurium definitive type (DT) 104 first emerged in Korea in 1997 (19). *S. enterica* Typhimurium DT104, which harbors SGI1, has been identified as a worldwide threat to human and animal health, and showed a high degree of clonality between isolates obtained from different countries (20-22). Over 50% of MDR *S. enterica* Typhimurium DT104 isolates during 1997-2007 in Korea were resistant to five antimicrobial agents that were ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT) like the antibiogram of pandemic *S. enterica* Typhimurium DT104. During 1997-1998, there were five *S. enterica* Typhimurium DT104-associated foodborne-disease outbreaks nationwide (Table 2). Some *S. enterica* Typhimurium DT104 Korean isolates had an additional genetic arrangement of antibiotic resistance determinants with those of pandemic *S. enterica* Typhimurium DT104 (19). A PFGE experiment revealed that some *S. enterica* Typhimurium DT104 Korean isolates had an indistinguishable PFGE pattern with those of *S. enterica* Typhimurium DT104 isolates from American cattle (20). Even *S. enterica* Typhimurium swine isolates in Korea were resistant to at least 4 antimicrobial agents with class 1 integron. Among the isolates, DT104 was found by phage typing (23). *Salmonella* Genomic Island 1 (SGI1) in *S. enterica* Typhimurium DT104 chromosome harboring resistance determinants with two class 1 integrons was first identified only in *S. enterica* Typhimurium, but it has been discovered in other *Salmonella* serovars now (24), for example, *S. enterica* Derby (25), *S. enterica* Paratyphi B (26), and *S. enterica* Schleissheim (S. Kim, unpublished data). DT104 represented approximately 7% of phage types of all *S. enterica* Typhimurium human isolates. However, the major phage type was U302 (approximately 45%) (S. Kim, unpublished data).

S. enterica Enteritidis has been the most ubiquitous *Salmonella* serovar from diarrhea patients since 1998 in Korea. The percentage of *S. enterica* Enteritidis from all isolated *Salmonella* spp. from 1998 to 2007 was 47.5% (Table 1). This result indicated that half of the *Salmonella* isolates from humans were *S. enterica* Enteritidis. Similarly, *S. enterica* Enteritidis was the most prevalent serovar among the NTS serovars between 2000-2002 in the world (27). Main infection sources were poultry and eggs

Table 2. Selected *Salmonella* outbreaks in Korea mentioned in this review

Salmonella	Year	Source	Region	Number of patients	Reference
S. enterica Typhimurium DT104	1997-1998	Pork and beef	Nation-wide	41	Kim et al., 2009 (19)
S. enterica Enteritidis	1999	Boiled cockle and beef	Hamyang	>200	Kim et al., 1999 (30)
S. enterica London	2000-2001	Powdered milk	Nation-wide	>70	Kim et al., 2003 (33)
S. enterica Paratyphi A	2002	Water	Busan	>200	Kim et al., 2003 (40)
S. enterica Infantis	2007	Not found	Jeollanam-do	>49	S. Kim, unpublished data
S. enterica Othmarschen	2007	Eggs, squash, and seafood	Guri	72	Kim et al., 2007 (45)

which were contaminated easily with *S. enterica* Enteritidis. Molecular epidemiological and phage typing study with *S. enterica* Enteritidis isolated from patients and chickens revealed that common phage types and PFGE patterns were found in both isolates. The common phage types in isolates from both patients and chickens were PT1 and PT21. Moreover, they were also the most predominant types among the isolates (28). The PFGE patterns of *S. enterica* Enteritidis isolates from various sources by using *Xba*I, *Spe*I, or *Not*I restriction enzyme were highly clonal and related (29).

In 1999, there were huge foodborne-disease outbreaks caused by *S. enterica* Enteritidis in Korea (Table 2). The outbreaks which resulted in more than 200 inpatients and one death, occurred by consumption of *S. enterica* Enteritidis contaminated boiled cockle and beef (30). In addition to these outbreaks, there were many other outbreaks caused by *S. enterica* Enteritidis infections in 1999. Consequently, the number of *S. enterica* Enteritidis isolates showed the highest peak in 1999 (Fig. 1).

Most of *S. enterica* Enteritidis isolates from layers were not MDR (23). However, antimicrobial resistance rate of human isolates has been increasing yearly. Resistance to clinically important antimicrobial agents such as quinolone and cephalosporine has increased in *S. enterica* Enteritidis isolates. Resistance rate to nalidixic acid was 21.6% which was higher than that of *S. enterica* Typhimurium (12.1%) (31). PT1 was the most frequent phage type among nalidixic acid resistant isolates (31). Extended spectrum β-lactamase (ESBL)-producing *S. enterica* Enteritidis isolates were found. The ESBL type was TEM-52 which spread clonally and horizontally in Korea (32).

REMARKABLE OUTBREAKS BY RARE SEROVARS OF *SALMONELLA*

There were many *Salmonella* outbreaks in Korea during 1998-2007. Here, I would like to introduce four outbreaks caused by rare *Salmonella* serovars that are worthy of note in *Salmonella* human infection history in Korea (Table 2). The rare *Salmonella* serovars mentioned in this review have not been spotlighted or ranked among the most widespread *Salmonella* serovars in Korea before 1998.

S. enterica serovar London (antigenic formula: 3,{10}{15}: l,v: 1,6)

Salmonella enterica London had been a rare serovar in Korea as well as in the world until 1999. Until then, the outbreak patterns in Korea were traditional in that affected patients were restricted to certain areas (33). However, foodborne outbreaks occurred after 2000 and most of the affected patients were infants nationwide. Epidemiological and molecular study revealed that certain powdered milk for babies was contaminated with *S. enterica* London. Furthermore, the PFGE patterns of *S. enterica* London isolates from the powdered milk and the infant patients were indistinguishable (33). The powdered milk manufacturer received administrative penalties and recalled the powdered milk. Subsequently, infant patients decreased but human infections by *S. enterica* London continued to be reported until 2006. This has become a great concern. Three patients who suffered from gastroenteritis caused by *S. enterica* London were reported in 2004. The 3 isolates from the patients were CTX-M-14-type ESBL producing *S. enterica* London of which *Xba*I PFGE patterns were indistinguishable from those of the pathogens from powdered milk in 2000, indicating that the susceptible clone acquired the ESBL (34).

In brief, these outbreaks were triggered by massive transport of contaminated foods, a typical outbreak pattern in the present era. This *S. enterica* London case was the first early disease prevention by timely surveillance and the Korea PulseNet system that was started in 2000 and officially connected to PulseNet Asia-Pacific in 2006.

S. enterica serovar Paratyphi A (antigenic formula: 1,2,12: a: [1,5])

Salmonella enterica Paratyphi A was a rare serovar until 2001. Since 2002, *S. enterica* Paratyphi A has been ranked among the top 10 *Salmonella* serovars. *S. enterica* Paratyphi A is a causative pathogen for paratyphoid fever which is a Class 1 notifiable disease in Korea like typhoid fever (35). Outbreaks by this pathogen infection were not frequent in the world but were reported in India, Nepal, and Singapore (36-39). There was a big waterborne outbreak in Busan by *S. enterica* Paratyphi A infection in early 2002 (40). More than 200 people were hospitalized. Epidemiologists found that the water-supply system was contaminated with the bacteria. Most of the isolates were resistant to nalidixic acid. The resistance mechanism was due to the point mutation in the 83rd codon of *gyr*A gene as found by performing allele-specific PCR and restriction fragment length polymorphism (AS-PCR-RFLP). Recent studies showed that plasmid-mediated quinolone resistances were spreading to Enterobacteriacae, suggesting that the mechanisms of resistance to quinolone or fluoroquionolone are developing in bacteria (41).

Some patients were not cured after being treated with ciprofloxacin because of decreased susceptibility to the antimicrobial agent (42). Currently, nalidixic acid resistant *S. enterica* Paratyphi A is very common in Korea as well as India and Mid-East Asia (42, 43).

S. enterica serovar Infantis (antigenic formula: 6,7,14: r: 1,5)

In fact, *S. enterica* Infantis was not a rare serovar but steady during 1998-2007 (Table 1). It was the fifth common serovar in 2002 worldwide and had been commonly isolated from farm animals and their feed in Europe nations (27). In 2007, there was a huge outbreak caused by *S. enterica* Infantis infection in Jeollanam-

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do, a rural site in Korea (S Kim, unpublished data). Although 49 culture proven human cases were found from the outbreak, the source of contamination was not found. The antibiotic phenotype of the isolates was susceptible to 16 antimicrobial agents which were a standard set for antimicrobial test of enteric pathogens in Korean reference laboratories. Due to the outbreak, *S. enterica* Infantis ranked second most-frequent among serovars in 2007.

S. enterica serovar Othmarschen (antigenic formula: 6,7,14: g,m,[t]: -)

Many foodborne outbreaks arise in schools, parties, companies, and other gathering places today (44). Mass catering is rising because of increased provision of meals in public; so many people are exposed to possible foodborne diseases. An outbreak in 2007 caused by *Salmonella enterica* Othmarschen was such a case. In a funeral service, more than 300 mourners were exposed to contaminated foods with the pathogen and among them 72 persons became ill. The characteristics of this salmonellosis were severe diarrhea, abdominal pain, and fever. Yellow or white watery diarrhea for about 5 days with maximum 50 incidents was a typical symptom. *S. enterica* Othmarschen was isolated from those patients, food handlers, and foods containing eggs, squash, and seafood. The PFGE patterns of the outbreak isolates were all identical and indistinguishable from that of an American *S. enterica* Othmarschen isolate (45). It was very interesting that the identical clones were found in geographically distant nations even though *S. enterica* Othmarschen is such a rare *Salmonella* serovar in the world (46). All these molecular epidemiological evidences could be harvested because of the PulseNet International activities.

SALMONELLA SEROVARS FROM OVERSEAS TRAVELERS

For the quarantine activity and early detection of contagious diseases in airports and harbors, quarantine stations carry out rectal swabs or stool sampling from overseas travelers and crew who show fever, or notify symptoms of diarrhea, abdominal pain, vomit in questionnaire sheets and come from Thailand, the Philippines, Indonesia, Vietnam, Iran, Egypt, Cambodia, and North Korea. These nations are important for cholera epidemic according to a World Health Organization report (47).

During 2004-2005, the proportion of *Salmonella* isolates from overseas travelers who traveled to the above eight nations was approximately 7% in total *Salmonella* isolates. *S. enterica* Weltevreden was the most frequent *Salmonella* serovar from overseas travelers. The frequency was 19.7%. *S. enterica* Anatum and *S. enterica* Tallahassee followed at 8.5% and 7.0%, respectively (14). These three serovars had been rare in Korea, but after 2004, *S. enterica* Weltevreden and *S. enterica* Anatum ranked in top 10 prevalent serovars in Korea. Since the early 2000s, there have been many Korean wedding tourists and travelers to Malaysia, Thailand, and other East South-Asian nations. The 3 serovars were mostly isolated from such travelers. *S. enterica* Weltevreden has been isolated from seafood at relatively high frequency in those nations. In Thailand, *S. enterica* Weltevreden had been the most prevalent serovar among *Salmonella* isolates from 1993 to 2002 (48). Recently, there were outbreaks caused by *S. enterica* Weltevreden infections in France (49), Norway, Denmark, and Finland (50). These phenomena indicated that contaminated food trades and infected travelers between nations seriously affects the health of people in a distance and a nation's health defense (51).

CONCLUSIONS

In this study, I showed epidemiological evidence that the incidences of *S. enterica* Typhi, *S. enterica* Enteritidis, and *S. enterica* Typhimurium decreased significantly in Korea during the last 10 yr since 1998. It was a very encouraging epidemiologic trend because these serovars have been health-threatening pathogens in Korea as well as the world. However, the rates of imported cases and outbreaks caused by rare serovars increased during that time. As reviewed in this study, I would like to stress that newly emerging rare *Salmonella* serovars should be traced and investigated to control new type pathogens in the developed world.

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