The Journal of Veterinary Medical Science

NOTE

Public Health

Emergence of *Salmonella enterica* subsp. *enterica* serovar Chester in a rural area of Japan

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J. Vet. Med. Sci.
82(5): 580–584, 2020
doi: 10.1292/jvms.20-0033

Received: 21 January 2020
Accepted: 9 March 2020
Advanced Epub:
19 March 2020

ABSTRACT. In Japan, only one outbreak of *Salmonella enterica* subsp. *enterica* serovar Chester (*S.* Chester) has been confirmed in 1999. We performed a single-center retrospective case review of *S.* Chester infections that occurred in a rural area of Japan in 2016 (n=8). Case 5 and 6 occurred in twin infants who had contact with a pet dog. The dog's stool culture was positive for *S.* Chester. Pulsed-field gel electrophoresis and cluster analysis of *S.* Chester strains revealed that all the isolates appeared to be derived from the same genetic clone. Emergence of *Salmonella* infection can be overlooked if cases are not reported to health authorities; therefore, core hospitals should play a role to alert the occurrence of public health issue.

KEY WORDS: bacteremia, dog, emerging infectious disease, molecular epidemiology, *Salmonella* infection

Nontyphoidal *Salmonella* (NTS) is a major cause of bacterial enterocolitis, and the consumption of contaminated food often causes foodborne outbreaks [2, 7, 9, 11]. In addition to contaminated food, NTS infections can also be acquired through contact with pets and other animals [28]. Furthermore, they can progress to invasive infections such as bacteremia, osteomyelitis, arthritis, and meningitis [8, 19, 24].

Salmonella enterica subsp. *enterica* serovar Chester (*S.* Chester) is an NTS that has been isolated from various animals as well as frozen food around the world [2, 10]. Although multinational outbreaks of *S.* Chester were reported in 2010 [10, 25], 2012 [11], and from 2014 to 2015 [9], in the United States *S.* Chester accounted for only 0.1% of all culture-confirmed *Salmonella* infections during the 11-year period from 2006 to 2016 [3]. Similarly, *S.* Chester has rarely been isolated in Japan [14–17, 20, 23]. To date, only one outbreak of *S.* Chester has been confirmed, and this involved the ingestion of cuttlefish chips in 1999 [27].

This report describes an emergence of *S.* Chester that occurred in a rural area of Japan, which was identified by molecular epidemiological investigation using pulsed-field gel electrophoresis (PFGE).

We retrospectively reviewed the medical charts of eight patients of *S.* Chester infection diagnosed at a regional core hospital from October to November 2016. Information on clinical features such as age, sex, diagnosis, underlying disease, bacterial culture, serotype, likely route of infection, symptoms, laboratory data, antibiotic use, and outcome were abstracted from the patients' medical records.

Bacteriological investigations were carried out as follows: trypticase soy agar (TSA II) with 5% sheep blood (Japan BD, Tokyo, Japan), bromothymol blue (BTB) lactose agar (Japan BD, Tokyo, Japan), chocolate agar (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), and *Salmonella Shigella* agar (Japan BD, Tokyo, Japan) were used to isolate the organisms from blood, urine, and stool (Supplementary Table 1). The isolates were identified as *Salmonella* species based on their biochemical properties, and they were serotyped using anti-*Salmonella* serum (Denka Seiken Co., Ltd., Tokyo, Japan) and confirmed as *S.* Chester. PFGE and cluster analysis were performed using XbaI and BlnI restriction enzymes (Roche Diagnostics, Rotkreuz, Switzerland) to determine whether isolated strains were derived from a same genetic clone of *S.* Chester according to the method described previously [29].

The study was approved by the Asahi General Hospital Ethics Committee (IRB no. 2018091820).

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(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

A summary of the demographic and clinical characteristics of eight cases of *S.* Chester infection is shown in Table 1. All patients lived within a 15-km radius of the border between Ibaraki and Chiba prefectures. These cases were reported to local public health centers because we strongly suspected these cases were derived from a foodborne outbreak. Further health, dietary, and contact surveys were conducted by the local health authorities; however, no clear evidence was obtained to identify the source of infection of these patients with the exception of case 5 and 6. The clinical features of the cases are shown in Table 2. Out of seven patients in whom blood cultures were performed, three (43%) were tested positive (Supplementary Table 1). Although the choice of antibiotics and the duration of antibiotic treatment varied among the cases, no deaths were reported.

The patients identified as case 5 and 6 had a history of close contact with their indoor pet dog. Case 6, the twin sister of case 5, was admitted to hospital three days after her twin's diagnosis. Her food intake included breastmilk, formula milk, and iron supplementation. *S.* Chester was isolated from a stool sample collected on admission. She was not treated with antibiotics because she was afebrile on admission. Though *S.* Chester was not isolated from the stools of either of her parents, it was found in the feces of the family's pet dog. Furthermore, case 6 had persistently positive results on periodic follow-up stool cultures over a period of >12 months.

The PFGE patterns of the strains isolated from all the human cases and the pet dog of the family of case 5 and 6 matched, and cluster analysis revealed that all the isolates appeared to be derived from the same genetic clone (Fig. 1A and 1B).

This is the first report of the emergence of a relatively rare serovar of *Salmonella* sp., *S.* Chester, in Japan since 1999. We speculate that all the *S.* Chester isolates in this study may have been derived from the same genetic clone, and although there is no direct evidence that this is so, our previous reports support this hypothesis. In our previous reports of *Salmonella* serovar Agona, *S.* Agona strains were divided into several genetic clones based on a single nucleotide polymorphism (SNP) analysis of whole genome sequencing (WGS) data, and the grouping of those clones was identical to that found on PFGE using two types of restriction enzymes [26, 31]. Moreover, *Salmonella* serovar Infantis strains were almost identically subdivided into several genetic clones based on SNP analysis of WGS data compared to those identified using PFGE [21, 30]. These results suggest that strains of *Salmonella* serovars can be accurately divided into genetic clones using PFGE with two types of restriction enzymes, as was performed in this study. A further study using SNP analysis of WGS data, is required to confirm that the *S.* Chester strains identified in this study were derived from the same genetic clone.

S. Chester has rarely been isolated from ordinary foods in various studies in Japan. Katoh *et al.* reported serovars of NTS isolated from samples of domestic chicken meat sampled from 1992 to 2012 [15], but *S.* Chester was not among them. On a national basis, the results of food contamination surveys by prefectural institutes of public health have found miscellaneous foods

Table 1. Cases of *Salmonella enterica* subsp. *enterica* serovar Chester infection around the prefectural border between Ibaraki and Chiba in Japan (n=8 cases)

Case no.	Date of admission	Age (years)	Sex	Address	Diagnosis	Underlying disease	Suspected source of infection
1	2-Oct-16	2	F	Ibaraki	Bacteremia, enteritis	—	Unknown
2	6-Oct-16	1	F	Ibaraki	Enteritis	—	Unknown
3	7-Oct-16	2	M	Chiba	Enteritis, UTI	Asthma	Unknown
4	3-Nov-16	4	M	Chiba	Bacteremia, enteritis	—	Unknown
5	11-Nov-16	0	F	Chiba	Enteritis	MD twin, LBW	A pet dog
6	14-Nov-16	0	F	Chiba	Enteritis	MD twin, LBW	A pet dog
7	16-Nov-16	65	F	Ibaraki	Bacteremia, UTI	Intellectual disability, cholangitis	Unknown
8	17-Nov-16	7	M	Chiba	Enteritis	—	Unknown

F, female; M, male; UTI, urinary tract infection; MD, monochorionic diamniotic; LBW, low birth weight.

Table 2. Clinical features of *Salmonella enterica* subsp. *enterica* serovar Chester infection (n=8 cases)

Case no.	Duration of diarrhea (days)	Duration of fever (days)	Abdominal pain	Vomiting	Bloody stool	WBC (/μl)	CRP (mg/d*l*)	Initial antibiotics	Duration of antibiotic therapy (days)	Outcome
1	3	3	−	−	−	10,800	7.26	CTRX	12	Alive
2	2	1	−	−	+	NA	NA	AZM	3	Alive
3	6	6	−	+	−	7,000	2.19	AMPC	7	Alive
4	4	5	+	−	−	9,400	1.68	CTX	14	Alive
5	2	2	−	+	−	9,100	5.33	CTX + ABPC	9	Alive
6	1	0	−	−	+	11,400	0.17	—	—	Alive
7	0	14	−	−	−	6,500	6.62	CTRX	21	Alive
8	8	8	−	−	+	9,500	1.08	FOM	9	Alive

WBC, white blood cell; CRP, C-reactive protein; CTRX, ceftriaxone; NA, not available; AZM, azithromycin; AMPC, amoxicillin; CTX, cefotaxime; ABPC, ampicillin; FOM, fosfomycin.

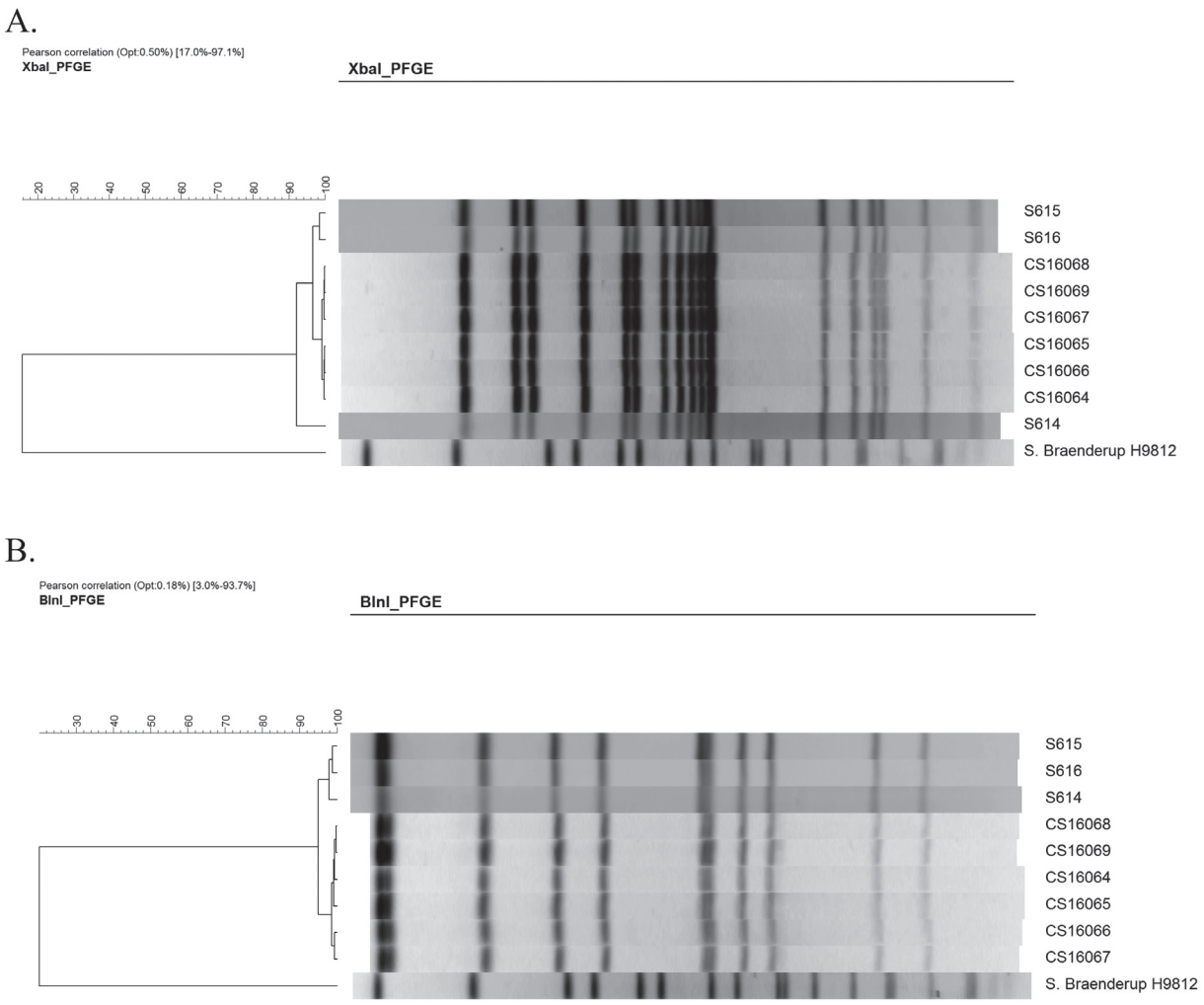

Fig. 1. *Salmonella enterica* subsp. *enterica* serovar Chester DNA fingerprints generated via pulsed-field gel electrophoresis (PFGE). (A) PFGE pattern digested with XbaI; (B) PFGE pattern digested with BlnI. Each fingerprint is shown as follows: S615, Case 1; S616, Case 2; CS16064, Case 3; CS16065, Case 4; CS16066, Case 5; CS16067, Case 6; S614, Case 7; CS16068, Case 8; CS16069, the dog owned by the family of case 5 and 6.

to be contaminated by NTS, but *S.* Chester has not been identified in these surveys [14, 16, 17].

According to the national surveillance, *S.* Chester has rarely been isolated from humans in Japan [20]. On the other hand, multinational outbreaks of *S.* Chester have been reported in several other countries in recent years [2, 9, 11, 25]. This suggests that different genetic features contribute to pathogenicity or adaptation to the environment in *S.* Chester strains isolated in Japan and those identified in other countries. Results from studies of other bacterial species support this hypothesis. For example, different clonal groups of enterohemorrhagic *Escherichia coli* serovar O157 (O157) have different pathogenicity, and different distribution of *E. coli* O157 across several countries, contributed the difference in the number of patients with severe symptoms due to O157 reported in different affected countries [18]. Therefore, investigating the differences in *S.* Chester strains from Japan and those isolated in other countries may provide useful information that could be used to control the spread of *S.* Chester. A multi-country genomic-level study, such as those performed for *S.* Infantis and *S.* Agona [12, 32], would be required.

The clinical characteristics of the *S.* Chester cases in this study suggest that the rare isolation of *S.* Chester from human in Japan may not be due to the low virulence of the organism for human. In this study, patients with *S.* Chester infection developed enteritis, bacteremia, and urinary tract infection. These symptoms are consistent with the features of other NTS serotypes such as *S.* Enteritidis, *S.* Typhimurium, and *S.* Newport, which are often isolated from humans [7]. Therefore, *S.* Chester could emerge once the organism contaminates ordinary foods such as meat, vegetables, and fish products.

There is a serious barrier to early detection of an emergence of NTS in Japan. NTS infections are not designated infectious diseases nor notifiable diseases by the national Infectious Disease Control Law; thus, public health authorities cannot detect emergence of NTS at an early stage, even if the number of patients with NTS increases in hospitals in an area. The early detection of an emerging pathogen is key to minimizing the hazard for humans due to the emergence.

The results of this report suggest that the accumulation of rare NTS serovar cases can be an indicator for clinicians in central

hospitals to report suspected outbreaks to local public health centers, even if there is no direct evidence of the cases being linked. Taylor *et al*. suggest that an outbreak should be suspected when a rare serotype of *Salmonella* spp. is detected in a circumscribed demographic group during a limited period [25]. We reported the present cases to local public health centers suspecting a foodborne outbreak because the cases were accumulated in a limited geographic area and occurred within a relatively short time period. This triggered molecular epidemiological analyses by a local governmental institute, which led to the demonstration of a *S.* Chester emergence that appeared to be derived from the same genetic group.

In general, when epidemiological analysis identifies the accumulation of monoclonal strains, there are two possibilities: outbreak or clonal dissemination [6]. Although additional epidemiological surveys were conducted by local public health centers, the source of infections could not be revealed in this study; in other words, this study could not demonstrate this accumulation of *S.* Chester strains was due to an outbreak of the organism. Apart from elderly persons, children, and immunocompromised individuals, patients with NTS infections do not always develop severe illness. This indicates that subclinical infections can be missed by clinicians [8]. This report is based on cases seen at a single center; therefore, we were unable to investigate all cases that occurred during this period. There may have been more cases in the area during this period, which may have provided important information to that could have enabled the source of the infection to be determined. To reveal whether this *S.* Chester accumulation was due to clonal dissemination of the organism, larger numbers of the organism, isolated in other areas of Japan should be investigated. Further studies are necessary.

Although there are reports in the literature of NTS being isolated from dogs [13, 22], to the best of our knowledge, there has been no previous reports of *S.* Chester in dogs. The rarity of *S.* Chester isolation in dogs parallels its rarity in humans according to the national surveillance data in Japan [20]. Notably, many other NTS serotypes isolated from dogs have also been frequently identified in human infections [22]. Therefore, as in human infections, a low level of contamination in food is likely to be major reason why the organism has rarely been isolated from dogs until now.

Although most of the patients in our study received antibiotic treatment during the acute stage of their illness, antibiotic therapy is generally not recommended for the treatment of NTS infections due to concerns about prolonging the gastrointestinal symptoms [1]. Therefore, we did not administer any antibiotics to case 6 because she did not develop fever, even though she had persistently positive results on periodic follow-up stool cultures. In general, prolonged carrier rates of NTS occurs in <2% of infected children [4]. As an exception to this general recommendation, one study suggested that antibiotic treatment should be used for NTS infections in infants aged <3 months [4]. Although routine follow-up stool culture is not currently recommended in the management of NTS infection [5], infants can be regarded as an exception considering their vulnerability to invasive infections.

In conclusion, the accumulation of *S.* Chester strains observed in this study revealed the emergence of an organism that appeared to be derived from the same genetic clone. Emergence of NTS infections can be overlooked unless appropriate reports are made and epidemiological investigations are performed. Regional core hospitals should make a caution when an accumulation of cases with a rare serotype of NTS is detected.

ACKNOWLEDGMENT. We thank the Division of Bacteriology, at the Ibaraki Prefectural Institute of Public Health for the initial analysis of the strain of *S.* Chester in case 1, 2, and 7.

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