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Molecular Epidemiology of Monophasic *Salmonella enterica* serovar O7: -: 1, 5 Isolates in Akita Prefecture, Japan

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Serotyping of *Salmonella* strains is epidemiologically important and useful for the surveillance and prevention of salmonellosis. Atypical monophasic *Salmonella* remains a public health concern; for example, the monophasic variant of *S. Typhimurium* (serovar O4: i: -) has often been identified since the 1990s and the serotype currently represents one of the most prevalent serotypes among human salmonellosis cases worldwide (1).

A total of 36 *Salmonella* isolates were collected from medical institutions at Akita prefecture, Japan, in 2010 (Fig. 1). The most frequently found serotype was *S. Infantis* (7 isolates), followed by *S. Braenderup* and *S. Thompson* (5 isolates, respectively). Of the *Salmonella* isolates collected, 4 isolates designated as Sa2495, Sa2508, Sa2513, and Sa2527 were serotyped as O7: -: 1, 5 according to standard serological typing procedures. Of these, 3 were isolated at Odate during the period from March 29 to October 21, 2010. Only Sa2508 was isolated from Akita city on August 2, 2010. Frequent isolation of such atypical monophasic variants raised the question of their phylogenetic origin.

Among the *Salmonella* O7 group, *S. Infantis* (serovar O7: r: 1, 5), *S. Thompson* (serovar O7: k: 1, 5), and *S. Bareilly* (serovar O7: y: 1, 5) have been often isolated from Akita prefecture. *S. Paratyphi C* and *S. Choleraesuis* (serovar O7: c: 1, 5) have been rarely isolated from diarrheal patients in Akita prefecture, but they have higher mortality rates in humans than other *Salmonella* serotypes (2). To determine the phase 1 flagellin (H1) type of the O7: -: 1, 5 isolates, 4 primers targeting the central variable regions of the H1 gene were designed; *fliC*, for type 'c' (5'-attctgtttcagtcggaat-3'), 'r' (5'-gatcaccagtaatagcagg-3'), 'k' (5'-gtcagtcgcatcagcaaagctt-3'), and 'y' (5'-gagcatctttaacgtggca-3'). These primers were coupled with a sense primer (5'-actcaggctccrtaacgc-3') described by Levy et al. (3) under the following polymerase chain reaction (PCR) conditions: 94°C for 2 min; 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; and 72°C for 2 min. The PCR-based analyses targeting *S. Choleraesuis*, designated Sa2201,

S. Infantis, designated Sa2514, *S. Thompson*, designated Sa2528, and *S. Bareilly*, designated Sa2550, generated amplicons of 405, 475, 529, and 720 bp, respectively (Fig. 2). The O7: -: 1, 5 isolates showed amplification of the fragment specific for type 'k', indicating that the O7: -: 1, 5 isolates were monophasic variants of *S. Thompson* (Fig. 2).

Using pulsed field gel electrophoresis (PFGE), we investigated the genetic relation between the O7: -: 1, 5 and *S. Thompson* isolates designated as Sa2497, which were isolated at Kita-akita on April 29, 2010, Sa2498, which was isolated at Odate on May 27, 2010, Sa2500, which was isolated at Odate on June 28, 2010, Sa2517, which was isolated at Yuzawa on September 10, 2010, and Sa2528, which was isolated at Akita city on December 6, 2010. The sample plug was prepared according to the United States CDC PulseNet protocol (4). Genomic DNA was digested with *NotI* (50 units/plug, Nippon gene, Toyama, Japan) or *XbaI* (50 units/plug, New England BioLabs, Tokyo, Japan) at 37°C for 4 hr. The

Fig. 1. Number of *Salmonella* clinical isolates in each area of Akita prefecture in 2010. Area divided into jurisdictions by the public health center.

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Fig. 2. PCR-based typing of *fliC* for O7: -1, 5. Lanes: M, 100 bp DNA size ladder (Takara, Shiga, Japan); Cs, *S. Choleraesuis* (Sa2201); In, *S. Infantis* (Sa2514); Th, *S. Thompson* (Sa2528); Ba, *S. Bareilly* (Sa2550); 1-4, O7: -1, 5 isolates (Sa2495, Sa2508, Sa2513, and Sa2527).

Fig. 3. PFGE patterns. Lanes: M, CHEF DNA size standard lambda ladder (Bio-Rad); 1-5, *S. Thompson* isolates (Sa2497, Sa2498, Sa2500, Sa2517, and Sa2528); 6-9, O7: -1, 5 isolates (Sa2495, Sa2508, Sa2513, and Sa2527).

sample plug was then subjected to PFGE with CHEF DRII (Bio-Rad, Tokyo, Japan) under the following conditions: (*NotI*) 6 V for 6.3 hr with pulse times of 1-9.4 sec and 12.2 hr with pulse time 9.4-12.4 sec; (*XbaI*) 6 V for 6.7 hr with pulse times of 5.3-33.8 sec and 13 hr with pulse time 33.8-44.5 sec. The PFGE patterns of the O7: -1, 5 were identical to those of the *S. Thompson* isolates, Sa2497, Sa2498, and Sa2500, which were isolated at Odate and the neighboring region of Kita-akita (Fig. 3). These results indicated that the O7: -1, 5 isolates were related to *S. Thompson* spreading to the north area of the Akita prefecture.

According to Garaizar et al. (5), monophasic

Salmonella can theoretically originate in 2 different ways. It may represent ancestral forms that did not acquire the second H antigen or the necessary switching mechanism during evolution. Alternatively, it may have originated as a variant of the biphasic strains, which have either lost the switching mechanism or the ability to express the second H antigen (6). Further studies will be required to warrant a mechanism by which the expression of H1 antigen has been lost in the O7: -1, 5 isolates.

In conclusion, our results indicate that most frequent serotype at Akita prefecture in 2010 was actually *S. Thompson*. The increase in such atypical monophasic

variants is a hindrance to salmonellosis surveillance. PCR-based serotyping is useful for the examination of atypical monophasic variants as a complementary tool to classical serological typing.

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Conflict of interest None to declare.

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