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β-Glucuronidase-Negative Enterohemorrhagic *Escherichia coli* O26 Infections Associated with a Calf

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Enterohemorrhagic *Escherichia coli* (EHEC) infection is associated with severe disease in humans with symptoms such as bloody diarrhea and hemolytic-uremic syndrome. Among the EHEC serogroups, O26 is the second most frequently detected in Japan (1). Food-producing animals, particularly cattle, are known as potential reservoirs of EHEC. In this report, we identified EHEC O26 zoonotic infections originating from a calf in two members of one family in Yamaguchi Prefecture, Japan.

In February 2012, a 4-year-old girl residing in Yamaguchi Prefecture, Japan, presented with abdominal pain and watery diarrhea caused by EHEC O26, and her grandmother was identified as an asymptomatic carrier. Epidemiological surveillance established that for at least 2 weeks prior to disease onset, the girl consumed no beef or pork, which could have been contaminated with EHEC. However, both the girl and her grandmother had direct contact with three Japanese Black cattle (ages, 8 years, 300 days, and 90 days, respectively), which were raised in a cattle barn in the grandmother's house. To determine whether any of the cattle were a source of the EHEC O26 infection, stool and saliva samples from the animals were collected and examined.

For detection of EHEC O26, 1% rhamnose MacConkey agar (Becton Dickinson and Company, Sparks, Md., USA) supplemented with CT (CT-RMAC; Oxoid, Basingstoke, UK) was used as a selective media after culture enrichment in mEC broth with novobiocin (Eiken Chemical, Tochigi, Japan) for 24 h at 42°C. One stool sample from the 90-day-old calf yielded colorless colonies on the CT-RMAC plate, which were identified as EHEC O26.

All isolates were serotyped as O26:H11, which carries stx_1 and *eae* genes, using PCR with the O157-VT1, VT2 PCR typing kit plus (Takara Bio, Shiga, Japan), which contains primers for the *stx* gene and primers mSK1 and eaekas_1 for the *eae* gene (2). The biochemical phenotype of the isolates was examined using several analytical growth media, including a triple-sugar iron (TSI) slant (Kyokuto Pharmaceutical Industries, Tokyo, Japan), lysine indole motility (LIM) medium (Kyokuto Pharmaceutical Industries), and a cellobiose lactose indole β-D-glucuronidase (CLIG) slant (Kyokuto Pharmaceutical Industries). The isolates fermented glucose and lactose or sucrose in the TSI slant and weakly produced lysine decarboxylase in the LIM medium. No fluorescence of the isolates was observed in the CLIG slant under UV light, suggesting that the isolates could not produce β-glucuronidase, which cleaves the fluorogenic substrate, 4-methylumbelliferyl-β-D-glucuronide (MUG). The ability of β-glucuronidase production was further tested using the API ZYM system (Sysmex Biomériux Japan, Tokyo, Japan). The result was consistent with that from the CLIG slant, indicating that the isolates were β-glucuronidase-negative (MUG-negative). When incubated on CHROMagar O157 TAM (CHROMagar, Paris, France), the isolates produced mauve-colored colonies, which were similar to MUG-negative EHEC O157 colonies. In contrast to EHEC O157, EHEC O26 is generally MUG-positive as are other non-O157 EHEC (3). MUG-negative non-O157 EHEC is rare and infections with these serotypes are only occasionally reported (4,5).

The isolates (two from each clinical case and the calf) digested with the *Xba*I and *Bln*I enzymes were analyzed by pulsed-field gel electrophoresis (PFGE) according to the standard PulseNet protocol (6). The isolates showed indistinguishable (with *Xba*I digestion, Fig. 1A) or highly similar (with *Bln*I digestion, Fig. 1B) PFGE patterns, which indicated that it was highly likely that the isolates shared the same origin. Because cattle and calves are well recognized as potential EHEC reservoirs,

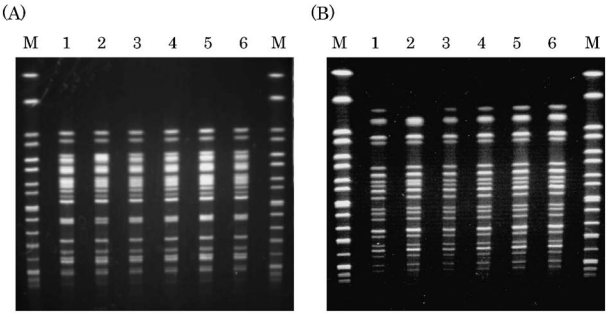

Fig. 1. Pulsed-field gel electrophoresis profiles of the EHEC O26 isolates digested with *Xba*I (A) and *Bln*I (B). Lane M, *Salmonella enterica* serovar Braenderup H9812 strain used as a standard size marker; lanes 1 and 2, EHEC O26 isolates from the calf; lanes 3 and 4, isolates from the 4-year-old girl; lanes 5 and 6, isolates from the girl's grandmother.

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it is suggested that the present cases had been infected with EHEC O26 from the 90-day-old calf.

In conclusion, the family infection that we observed was believed to have originated from a calf as a reservoir with an atypical phenotypic strain of EHEC O26. We recommend that atypical phenotypes should be considered when isolating and identifying EHEC. Moreover, in an EHEC outbreak, sufficient epidemiological surveillance should be performed to identify direct or indirect contact with reservoir animals, in addition to the ingestion of contaminated food, as a possible source of infection.

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Conflict of interest None to declare.

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