稀な腸管病原菌による集団感染事例の病因に関する研究

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Studies of the pathogenesis of outbreaks caused by rare enteric pathogens

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腸管出血性大腸菌 (Enterohemorrhagic *Escherichia coli* : EHEC) は, 1982 年に出血性腸炎の原因菌として最初に報告された比較的新しい病原菌である。本菌に関しては, ①出血性腸炎のほか, 溶血性尿毒症症候群(hemolytic-uremic syndrome: HUS)や脳症等を起こし，死亡する例もある，②牛が本菌のリザーバーである，③血清群としては O157, O26, O111 など多種あるが，重症化するのは O157 による場合が多いこと，などが明らかにされている。

2011 年 4〜5 月にかけて富山県をはじめとした北陸 3 県と横浜市で, 牛肉ユッケを原因食品とした EHEC による大規模な食中毒が発生した。患者数 181 名，HUS 発症者 34 名(19%)，HUS 34 名中 21 名が急性脳症を発症，5 名が死亡という非常に重症例の多い事件であった。181 名の患者糞便を対象とした細菌学的検査では，55 名から EHEC O111:H8 (stx_2)または EHEC O157:H7 (stx_1 & stx_2 / stx_1 / stx_2)，あるいはその両方が検出されるという非常に複雑な結果であった。さらに，*stx* を保有しない大腸菌 O111:H8 が 52 名の患者から分離された。この O111 は，Multilocus variable tandem-repeat analysis (MLVA) や pulsed-field gel electrophoresis (PFGE) により, stx_2 保有 O111:H8 と同様の遺伝的背景を持っていることが確認された。これまで報告された EHEC 感染症の病態では, 血便や HUS を呈する割合や死亡などの重症例は，EHEC O111 より，O157 による方が多いことが知られている。本事例では重症例が多い上，EHEC O111 と O157 の 2 種類の EHEC が検出された。そこで，この重症者の多い食中毒事例の病因をさらに詳細に検討するために，患者の免疫応答の一つである血清抗体価の上昇に着目し解析を行った。

一方，*Yersinia enterocolitica* も腸管感染症起因菌の一つであるが，感染事例の報告はそれ程多くはなく，わが国で 2018 年現在報告されている集団事例は 25 事例程度である。*Y. enterocolitica* のリザーバーとしては豚が知られているが，食中毒の原因食品・感染源についてはその多くが不明である。

2012 年に富山県で患者 4 名の *Y. enterocolitica* による集団感染事例が発生した。本事例の病因について明らかにする目的で，発生状況及び原因菌について疫学的解析を行うとともに，原因と推定された簡易水道水から原因菌を検出する方法について検討を行い，感染源の解明を図った。

これら稀な病原菌による病因について検討したところ，以下の結果を得た。

Ⅰ．腸管出血性大腸菌による食中毒事例の病因について

1. 181 名の患者糞便を対象とした細菌学的検査の結果, 55 名から EHEC O111:H8 (*stx*$_2$) または EHEC O157:H7 (*stx*$_1$ & *stx*$_2$ / *stx*$_1$ / *stx*$_2$)，あるいはその両方が検出された。内訳は，EHEC O111 のみ検出 25 名，O111 & O157 検出 12 名，O157 のみ検出 18 名，両菌陰性 126 名であった。

中でも，HUS 発症者 34 名では，EHEC O111 のみ検出 9 名，O111 & O157 検出 8 名，O157 のみ検出 1 名，両菌陰性 16 名であった。また，急性脳症発症者 21 名では，EHEC O111 のみ検出 7 名，O111 & O157 検出 6 名，両菌陰性 8 名であった。死亡者 5 名では，3 名から EHEC O111 のみ検出，他の 2 名は陰性であった。

2. 患者 181 名のうち，60 名から収集された血清について，凝集反応（Microagglutination: MA）試験により，*E.coli* O111 及び O157 の O 抗原に対する血清抗体価を測定した結果は以下の通りであった。

1) 患者 60 名の血清抗体価では，O111 に対する抗体陽性者は 45 名 (75.0%)であったが，O157 に対しては 10 名 (16.7%) であり，O111 に対する抗体陽性率が O157 陽性者に比べて有意に高かった ($p<0.001$)。

2) *E. coli* O111, O157 の両方あるいはどちらか一方が分離された患者 41 名の抗体陽性者は，O111 に対して 33 名 (80.0%) であったのに対し，O157 に対しては 8 名 (19.5%) であり，O111 に対する抗体陽性率は O157 に対する陽性者に比べて明らかに高かった。これらの患者の *E. coli* O111 に対する血清抗体価は，最も高い値は 1:10,240 であったのに対し, O157 に対する最も高い血清抗体価は 1:320 であった。

3) *E. coli* O111 に対する血清抗体価の中央値は，HUS 患者および血便患者ではいずれも 1:1,280 であったのに対し, 下痢便患者では 1:40 と低かった。また, HUS 患者と下痢患者，血便患者と下痢患者を比較すると，いずれも HUS 患者，血便患者の血清抗体価の方が有意に高かった ($p<0.01$)。

4) *E. coli* O111, O157 の両方が分離されなかった患者 19 名の抗体陽性者は, O111 に対して 12 名 (63.2%) ，O157 に対して 2 名 (10.5%) であり，O111 に対する抗体陽性率は O157 に対する抗体陽性者より高かった。これらの患者の血清抗体価の中央値は，O111 に対しては 1:640 であるのに対し，O157 に対しては 1:20 であった。これらを比較すると，O111 に対する血清抗体価が有意に高かった ($p<0.01$)。

3. 上述した血清診断の結果から，本事例の病因において EHEC O111:H8 (*stx*$_2$) が主要な役割を果たしたことが示唆された。

4. さらに，EHEC O157 のみが分離された患者の血清で，O111 に対する抗体価が陽性であった事例も確認されたことから，複数の EHEC に感染している患者の場合，患者便から分離される EHEC が主要な起因菌であるとは限らないことが示唆された。また, *E. coli* に対する血清抗体価を測定することは, 複数の EHEC による感染症において，それが O157 以外の血清群においても，きわめて有用であることが明らかとなった。

Ⅱ. *Y. enterocolitica* による集団感染事例の病因について

1. 2012 年 7～8 月，富山県において *Y. enterocolitica* O8 による患者 4 名の集団感染事例が発生した。感染源追及の過程で，患者らが共通に飲用していた簡易水道水から大腸菌は検出されなかったが，一般細菌数が最大 700 CFU /mL 検出され，本飲用水は水質基準である一般細菌数 100 CFU /mL を超えていることが判明した。

2. この簡易水道水を感染源と疑い，起因菌である *Y. enterocolitica* O8 を分離する方法について検討した。すなわち，水から *Y. enterocolitica* を分離することは非常に困難であると考え，効果的に集菌する方法として，本菌の O 抗原に対す

る抗体を磁気ビーズに感作して自家免疫磁気ビーズを作製し，これを用いて簡易水道水から *Y. enterocolitica* O8 を分離することに成功した。とりわけ，水道水中の夾雑菌により *Y. enterocolitica* のビーズへの吸着
が抑制されることを考慮し，増菌培養液を 10 倍希釈して培養することで *Y. enterocolitica* O8 をより高率に分離することができた。

3. 分離株について，制限酵素 *Not 1* による分子疫学的解析法の一つである PFGE により解析した結果，水道水から分離された *Y. enterocolitica* O8 株と 4 名の患者便から分離された株は同一の PFGE 型を示し，同一起源の由来株であることが明らかとなった。この結果により，患者 4 名は簡易水道水を飲用したことによる集団食中毒であると判断するに至った。

4. 簡易水道水が本菌に汚染された原因は，貯水タンクのパイプに注入されることになっている塩素タンクが空であったことと推定された。このことから，飲用水については，塩素濃度を適切に維持し，衛生的に管理する体制が重要であることが再認識された。

5. 塩素消毒による衛生管理が不十分な簡易水道水を原因とする *Y. enterocolitica* O8 集団感染事例は，日本では初めての報告である。

このように，腸管出血性大腸菌による非常に重症例の多い大規模食中毒事例において，原因菌として EHEC O111:H8 と O157:H7 の 2 種類の菌が検出された。一般的に EHEC の血清群の中では，O157 の方が，O111 より病原性が強いと考えられているが，本集団事例の病因においては，EHEC O111:H8(*stx*$_2$)が O157 より主要な役割を果たしたことが，菌の分離状況に加え，血清学的診断によっても裏付けることができた。また，希な病原菌の一つである *Y. enterocolitica* O8 による集団感染事例において，その感染源が塩素管理の不十分な簡易水道水であったことを解明した。簡易水道水を原因とする *Y. enterocolitica* による集団感染事例は，日本では初めての報告である。

以上のように，2 つの集団感染症事例について，詳細な細菌学的検討を行うことにより，稀な腸管病原菌による感染症の病因を明らかにすることができた。

Studies on the etiology of outbreaks caused by rare enteric pathogens

Enterohemorrhagic *Escherichia coli* (EHEC), which were first reported as a causative agent of hemorrhagic enteritis in 1982, are newly emerging as bacterial pathogens. Regarding the major attributes of EHEC infection, three features are generally considered: i) EHEC can cause hemorrhagic colitis and occasionally death due to characteristic complications, such as hemolytic uremic syndrome (HUS) and acute encephalopathy (AE); ii) cows are a natural reservoir of EHEC, and iii) although the most common EHEC serogroups are O157 followed by O26, O91, O103, O111, O121, and O145, severe cases, such as those with bloody diarrhea, HUS, AE, and death, are often related to serogroup O157.

A large outbreak of EHEC serogroups O157 and O111 occurred in the Hokuriku region and Kanagawa Prefecture from April to May 2011. The causative food was a raw beef dish called *yukhoe*. A total of 181 cases were reported in this outbreak, which included 34 cases with HUS, 21 with AE, and 5 deaths. EHEC O111:H8 (stx_2) and/or O157:H7 (stx_1stx_2, stx_1, or stx_2) were isolated from the stool samples of 55 among the 181 patients by bacteriological examinations. In addition, *stx*-negative *E. coli* O111 were also isolated from the stool samples of 52 patients. The *stx*-negative *E. coli* O111

strain had a genetic background identical to the EHEC O111:H8 (*stx*$_2$) strain, as determined with pulsed-field gel electrophoresis (PFGE) and a multiple-locus variable-number of tandem repeat analysis (MLVA). In this outbreak, many severely affected patients were reported, and two serotypes of EHEC O111 and/or O157 were detected in stool samples of the affected patients. To further understand the etiology of this foodborne outbreak, we performed serological studies on antibodies to O-antigen of *E. coli* O157 and O111 in the sera of patients.

On the other hand, *Yersinia enterocolitica* is also a causative agent of enteric infection. However, only 25 outbreaks of *Y. enterocolitica* have been reported in Japan as of 2018. Swine are suspected of being a major reservoir for *Y. enterocolitica*, but the causes of foodborne infection or sources of infection are not clear. In 2012, an outbreak of illness among four patients caused by *Y. enterocolitica* occurred in Toyama Prefecture. In order to evaluate the etiology of the outbreak, we tried to isolate *Y. enterocolitica* from the tap water which was suspected to be the cause of the infection, using an immunomagnetic concentration method which was an unique isolation method, and the molecular epidemiological analysis was performed. Etiological studies on the outbreaks caused by these rare enteropathogenic bacteria were conducted, and the following results were obtained:

1. Etiological studies on the large outbreak caused by yukhoe *contaminated with EHEC O157 and O111.*

1) In the outbreak, EHEC were isolated from stool samples of 55 patients among a total of 181. The isolates consisted of EHEC O111 and/or O157 (with stx_1, stx_2, or stx_1 and stx_2); EHEC O111 was isolated from 25 cases, both EHEC O111 and O157 from 12, EHEC O157 from 18, and no EHEC from 126. Regarding the EHEC isolation among the 34 HUS cases, 9 cases were positive for EHEC O111, 8 for both EHEC O111 and O157, 1 for EHEC O157, and 16 for no EHEC. Among the 21 AE cases, 7 were positive for EHEC O111:H8, 6 for both EHEC O111:H8 and O157:H7, and 8 for no EHEC. Among the five cases that died, three were found to be positive for EHEC O111:H8, and two were negative for EHEC.
2) A microagglutination (MA) assay was conducted to measure antibodies to *E. coli* O111 and O157 in sera collected from 60 patients during the foodborne outbreak affecting 181 patients. The results were as follows: the detection rates for antibodies to O111 (45/60, 75.0%) were significantly higher than those for antibodies to O157 (10/60, 16.7%) ($p<0.001$). Among 41 patients from whom O111 and/or O157 were isolated, the detection rates for antibodies to O-antigens

of *E. coli* were 33 (80.0%) for O111 and 8 (19.5%) for O157. These results showed that the positive rate for antibodies to O111 was markedly higher than that for O157. The maximum MA titer of antibodies to O111 was 1:10,240, while that to O157 was 1:320. The median value of the titers for antibodies to O111 was 1:280 in the HUS and bloody diarrhea cases and 1:40 in the diarrhea cases. Comparing the MA titer in the HUS and bloody diarrhea cases with that in the diarrhea cases showed that the MA titer of antibodies to O111 in the former was significantly higher than in the latter ($p<0.01$). In the 19 patients without EHEC, the detection rates for O-antigens of *E. coli* were 12/19 (63.2%) for O111 and 2/19 (10.5%) for O157. Therefore, the positive detection rate of antibodies to O111 was markedly higher than the positive rate of antibodies to O157, as was noted in the EHEC-positive cases. The median titer for antibodies to O111 was 1:640, while that for antibodies to O157 was 1:20. On a comparison, the serum antibody titers were higher in those with O111 than in those with O157 ($p<0.01$).

3) Based on the results of the serological diagnosis, EHEC O111:H8 stx_2 was identified as a key player in the etiology of the outbreak.

4) In addition, the serum of five patients with EHEC O157 isolates showed

additional positivity for antibodies to O111. Our results suggest that the isolates obtained from the stool specimens of patients were not always a causative pathogen, especially in EHEC infections caused by several serogroups of EHEC. Finally, the serological diagnosis using patients' sera seems to be very useful for determining the cause of foodborne illness outbreaks by multiple serogroups of EHEC infections.

2. Etiological studies on waterborne outbreak caused by Y. enterocolitica

1) A water-borne outbreak affecting four patients caused by *Y. enterocolitica* O8 occurred in 2012 in Toyama Prefecture. Four patients had experienced a gastrointestinal illness from July to August 2012. No common source of infection, except possibly water, was identified. Water samples were obtained from the small water-supply system of this area. In the samples, coliforms were not detected, but the standard plate count of bacteria yielded a maximum of 700 CFU/mL, which exceeded the standard value for the purity of tap water (100 CFU/mL).

2) Because the tap water was suspected of being the source of infection, we explored ways to isolate *Y. enterocolitica* O8, which was detected from the patients' stool samples. We used immunomagnetic separation (IMS) to

efficiently isolate *Y. enterocolitica* O8 from water samples. We attempted to prepare immunomagnetic beads coated with an antibody specific to the O8 antigen of *Y. enterocolitica.* As a result, *Y. enterocolitica* O8 was efficiently isolated from the tap water samples. We also improved the efficiency of isolating *Y. enterocolitica* O8 by combining dilution of enrichment culture and artful plating.

3) The PFGE patterns of three isolates from water samples and five clinical isolates from the four patients were identical; therefore, these strains were concluded to be the outbreak strains. Finally, we concluded a waterborne outbreak with *Y. enterocolitica* O8 to be the cause of infection using tap water from the affected area supplied via a small water-supply system.

4) Contamination of tap water with *Y. enterocolitica* was presumed to have occurred when the tank of chlorine that was used as disinfectant for the water-supply system ran empty. These findings underscore the importance of appropriately maintaining the water-supply system.

5) This is the first case report of an outbreak of *Y. enterocolitica* due to tap water from a small water-supply system as a causative facility.

In large-scale foodborne outbreaks with severe symptoms caused by EHEC, two kinds of EHEC serogroups (O111:H8 and O157:H7) were detected as causative bacteria. Generally, EHEC O157 is thought to be a more severe pathogen than O111; however, in this outbreak, EHEC O111:H8 (*stx*$_2$) played a more major role than O157 in the pathogenesis, as evidenced by the isolation of EHEC from the patients' stool samples and serological diagnosis of the patients' serum samples. Regarding the outbreak caused by *Y. enterocolitica*, which is a rare pathogenic bacteria, we identified the source of infection and showed that the causative infectious substance was tap water supplied by the water-supply facility due to a system malfunction. This is the first case report of an outbreak of *Y. enterocolitica* due to tap water from a small water-supply system in Japan. In conclusion, for these two outbreaks, we clarified the etiology of infections caused by two rare enteric pathogens.

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Serodiagnosis Using Microagglutination Assay during the Food-Poisoning Outbreak in Japan Caused by Consumption of Raw Beef Contaminated with Enterohemorrhagic *Escherichia coli* O111 and O157

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A microagglutination (MA) assay to identify antibodies to *Escherichia coli* O111 and O157 was conducted in sera collected from 60 patients during a food-poisoning outbreak affecting 181 patients in Japan which was caused by the consumption of contaminated raw beef. Enterohemorrhagic *E. coli* (EHEC) O111:H8 and/or O157:H7 was isolated from the stools of some of the patients, but the total rate of positivity for antibodies to O111 (45/60, 75.0%) was significantly higher than that for antibodies to O157 (10/60, 16.7%). The MA titers of antibodies to O111 measured in patients with hemolytic-uremic syndrome and bloody diarrhea were higher than those measured in patients with only diarrhea. In patients from whose stool no isolates of *E. coli* O111 and O157 were obtained, the positive antibody detection rates were 12/19 (63.2%) for O111 and 2/19 (10.5%) for O157, and the MA titers of antibodies to O111 measured were higher than those to O157. Similarly, the MA titers of antibodies to O111 were significantly higher than those to O157, regardless of the other groups, including groups O111, O111 and O157, and O157. These serodiagnosis results suggest that EHEC O111:H8 stx_2 played a primary role in the pathogenesis of this outbreak. Furthermore, our findings suggest that the isolates from the patients' stool specimens were not always the major causative pathogen in patients with multiple EHEC infections, because the sera from patients from whose stools only O157 was isolated were positive for antibodies to O111. Measuring antibodies to *E. coli* O antigen is helpful especially in cases with multiple EHEC infections, even with a non-O157 serotype.

Enterohemorrhagic *Escherichia coli* (EHEC) strains cause a variety of human illnesses, such as uncomplicated diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome (HUS), and related acute encephalopathy. In 1983, Karmali et al. first presented data revealing a possible etiologic role of EHEC in HUS (1). The correlation between HUS and EHEC O157 infection has been widely reported and has supported the conclusion that EHEC is a causative pathogen (2–5). Conversely, HUS has less frequently been reported in outbreaks caused by non-O157 EHEC (i.e., EHEC O104) (6–8). On the other hand, HUS patients without EHEC isolates have frequently been described (3, 4, 9, 10). Detecting EHEC in the stools of patients administered antibiotics long after the onset of diarrhea can be challenging because the organisms are typically isolated only from the diarrhea stool tested soon after onset (9, 11–14). Therefore, alternative laboratory diagnostic methods have been used, such as the detection of Shiga toxin (Stx) protein in the patients' stools or the detection of antibodies against Stx (15) or lipopolysaccharides (LPSs) of *E. coli* O antigens in the sera of the patients (16–18). The presence of antibodies to the LPSs of *E. coli* O antigen in the sera of patients with HUS was first reported by Chart et al. (3) in 1991. Thereafter, antibodies to the LPSs of *E. coli* O antigen have been measured using methods such as enzyme-linked immunosorbent assay (ELISA) (2, 3, 9, 10), indirect hemagglutination assay (IHA) (4), and passive hemagglutination assay (PHA) (19). In Japan, a direct agglutination test has been used to detect antibodies to *E. coli* O antigens (20, 21) after mixing a patient's serum with heat-inactivated whole bacterial cells that were used as the antigen. Therefore, this assay has been called the bacterial agglutination test (21) or the microagglutination (MA) assay using microplates (22). In Japan, this serodiagnostic test is typically performed in research laboratories (23) but not in clinical laboratories because the assay is time-consuming. Nevertheless, the assay is listed under the Infectious Diseases Control Law in Japan as one of the methods for the diagnosis of EHEC infection. In the case of patients with only HUS, their sera are used for detecting antibodies to O antigens of EHEC.

Japan had 2,900 to 4,600 cases of EHEC infection per year from 2001 to 2010 and approximately 100 HUS cases per year from 2006 to 2010 (23). Moreover, outbreaks of food poisoning caused by the consumption of raw beef and the liver of cows contaminated with EHEC are increasing (24). The serogroups of EHEC that have been detected are, from the most to the least frequent, O157, O26, O103, and O111 (23, 24).

In April and May 2011, an outbreak of food poisoning caused by EHEC O111:H8 and O157:H7 occurred in Toyama, Fukui, Ishikawa, and Kanagawa Prefectures in Japan. The Toyama pre-

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TABLE 1 Number of patients positive for antibodies to *Escherichia coli* O111 and O157 among patients with HUS, no HUS with bloody diarrhea, and no HUS with diarrhea determined by using the MA assay

Group[a]	*E. coli* strain isolated from patient stools	No. of patients positive[b] for antibody to the indicated strain/total no. of patients tested (%)							
		HUS patients		Non-HUS patients					
				Bloody diarrhea		Diarrhea		Total	
		O111	O157	O111	O157	O111	O157	O111	O157
A (n = 19)	None	10/10	2/10	2/3	0/3	0/6	0/6	12/19 (63.2)	2/19 (10.5)
B (n = 21)	O111:H8	9/10	2/10	4/5	1/5	5/6	0/6	18/21 (85.7)	3/21 (9.5)
C (n = 15)	O111:H8, O157:H7	8/8	4/8	4/5	1/5	1/2	0/2	13/15 (86.7)	5/15 (33.3)
D (n = 5)	O157:H7	0/0	0/0	0/0	0/0	2/5	0/5	2/5 (40.0)	0/5 (0.0)
Total (n = 60)		27/28 (96.4)	8/28 (28.6)	10/13 (76.9)	2/13 (15.4)	8/19 (42.1)	0/19 (0.0)	45/60[c] (75.0)	10/60[c] (16.7)

[a] Groups were categorized on the basis of the *E. coli* isolates obtained from stool specimens from the patients. See the text.
[b] An MA antibody titer of ≥1:160 was defined as positive.
[c] $P < 0.001$, Fisher's exact test.

fectural government finally reported that 181 patients, including 21 patients with acute encephalopathy and 5 deaths among 34 patients with HUS, were affected by the outbreak that occurred at 6 out of 20 restaurants of a barbecue restaurant chain. The causative food was epidemiologically determined to be a raw beef dish called *yukhoe*. In this outbreak, EHEC O111:H8 strains with stx_2 (O111 stx_2) and O157:H7 strains with stx_1, stx_2, or stx_1 and stx_2 (O157 stx_1 and stx_2) were isolated from the stool specimens of the patients. Furthermore, *E. coli* O111:H8 strains without *stx* (O111 *stx*-negative strains) were also isolated from 52 patients. This O111 *stx*-negative strain is a non-Stx-producing *E. coli* strain that has a genetic background identical to that of the O111 stx_2 strain, as determined by using pulsed-field gel electrophoresis (PFGE) analyses and multilocus variable-number tandem-repeat analysis (MLVA) (M. Watahiki, J. Isobe, K. Kimata, T. Shima, J. Kanatani, A. Nagata, K. Kawakami, M. Yamada, H. Izumiya, S. Iyoda, T. Morita-Ishihara, J. Mitobe, J. Terajima, M. Ohnishi, and T. Sata, unpublished data). Various combinations of strains exhibiting serotypes or toxin types of the EHEC and O111 *stx*-negative strains were isolated from stool specimens from the patients. However, none of the *E. coli* strains was isolated from 102 patients, including 14 HUS patients, and, consequently, the local public health center delayed starting a precise survey and declaring that a food-poisoning outbreak had occurred in order to prevent the infection from spreading further.

In this study, we measured the antibodies to the O antigens of *E. coli* O111 and O157 in the sera of patients with and without any isolates of EHEC or *E. coli* by using the MA assay, and we observed the antibody response to O111 and/or O157 in the patients. We studied the relationships of these results to the symptoms and isolates of the patients, and we also evaluated the usefulness of serodiagnosis in examining the antibody response of the host and in determining the major pathogen in cases with multiple EHEC infections in this outbreak.

MATERIALS AND METHODS

Food-poisoning outbreak in Toyama Prefecture. In the food-poisoning outbreak in 2011, 175 of 181 (96.7%) patients were from Toyama Prefecture, including 31 of 34 HUS patients (91.2%) and 4 of the 5 deaths that occurred in this prefecture. The 175 patients in Toyama corresponded to the following case definition for outbreak-related illness: the patients had consumed *yukhoe* at the implicated restaurants from 17 April through 25 April 2011 and developed one or more gastrointestinal tract symptoms and signs or HUS or had culture-confirmed infection with isolates of EHEC O111 stx_2 and/or O157 stx_1 and stx_2. The age distribution of the 175 patients was 1 to 70 years (mean, 25.5 years; 90 men and 85 women), and 156 patients (89.1%) had consumed *yukhoe*. HUS and acute encephalopathy accounted for the 4 deaths. The 31 HUS patients were aged 1 to 63 years (mean, 20.5 years; 11 men and 20 women). The EHEC strains isolated from the stools of the patients included 89 O111 strains consisting of 37 O111 stx_2 and 52 O111 *stx*-negative strains and 57 O157 strains consisting of 9 O157 stx_1, 24 O157 stx_2, and 24 O157 stx_1 and stx_2 strains.

Serum specimens. The 280 serum specimens used in this study were collected from 60 of the 175 patients and were obtained from 13 hospitals. These 60 patients were aged 0 to 15 years (13 patients), 16 to 60 years (45 patients), and >61 years (2 patients). Among the samples from these patients, 17 serum specimens were collected as single specimens from 17 patients, whereas the other serum specimens were collected as multiple specimens from 43 patients (2 to 20 serum specimens per patient); specifically, 2 to 19 serum samples were obtained from HUS patients. Among the 60 patients, 28 developed HUS (HUS patients), 13 did not develop HUS but had bloody diarrhea (bloody diarrhea patients), and 19 had only diarrhea (diarrhea patients). Furthermore, the 60 patients were divided into the following groups according to the serogroup profiles of the *E. coli* strains isolated from their stool specimens (Table 1): 19 patients with neither EHEC nor *stx*-negative *E. coli* O111 (group A), 21 patients with only O111 stx_2 and O111 *stx*-negative strains (group B), 15 patients with O111 stx_2, O111 *stx*-negative, and O157 stx_1 and stx_2 strains (group C), and 5 patients with only O157 strains (group D). The patients with O111 stx_2 and/or O111 *stx*-negative strains were included in the same group because, as mentioned in the introduction, the O111 *stx*-negative strains had a genetic background identical to that of the O111 stx_2 strains according to PFGE and MLVA analysis (data not shown). We examined 49 serum samples stocked in our laboratory as negative controls; these serum samples were comparable to the serum samples from the patients involved in this outbreak. As positive controls, we used immune rabbit serum with antibodies specific to each of the O antigens of the *E. coli* strains (O1, O18, O26, O111, and O157; Denka Seiken, Tokyo, Japan).

Bacterial strains used for antigen preparation. Antigens were prepared from the original *E. coli* isolates obtained during this outbreak: O111:H8 stx_2 (isolate E165) and O157:H7 stx_1 and stx_2 (isolate E045-1). In addition, we used other isolates stocked in our laboratory: *E. coli* O26:H11 (isolate EC3273), *E. coli* O1:H6 (isolate EC3061), and *E. coli* O18:H7 (isolate EC2922). *E. coli* O1, O18, and O26 strains were also used as controls because *E. coli* O1 and O18 strains are frequently isolated from healthy Japanese people and *E. coli* O26 is the second major serogroup of EHEC identified in Japan (23).

MA assays were performed as described by Vuddhakul et al. (20) with minor modifications. The bacterial strains were grown on Trypticase soy

agar (Becton, Dickinson and Company, Le Pont de Claix, France) at 35°C for 18 h. The cells were suspended in saline solution and autoclaved at 121°C for 1 h. The cells were then centrifuged at 2,000 × *g* for 15 min, the supernatants were discarded, and the cell pellets were resuspended in 5 ml of saline. After washing twice with saline, the cells were centrifuged at 200 × *g* for 5 min, and the supernatants were mixed with equal volumes of 2% formalin-saline and then incubated at 35°C for 1 h. The suspensions were again washed using saline and finally adjusted with saline to a 3 to 4 McFarland standard by using a turbidity meter (Densimat; Sysmex-bio-Mérieux, Marcy l'Etoile, France). The suspensions were subsequently used as *E. coli* O antigens.

Determination of titers of antibodies to O antigen of *E. coli*. The test sera were diluted 1:10 using sterile saline, inactivated by heating at 56°C for 30 min, and then centrifuged at 10,000 × *g* for 1 min. Next, 25-μl aliquots of the test sera were diluted 2-fold by mixing with 25 μl of sterile saline in 96-well V-shaped microtiter trays (BM Equipment, Tokyo, Japan). The highest dilution giving a clear agglutination pattern was considered the endpoint. The MA antibody titers were recorded as the reciprocal of the endpoint dilution of the test sera, yielding final serum dilutions ranging from 1:20 to 1:40,960. Lastly, to confirm the immunoglobulin class of the antibodies identified using the MA assay, the positive sera from the patients were treated with 2-mercaptoethanol (2ME; Wako Pure Chemical Industries, Tokyo, Japan) as described previously (19, 25).

Statistical analysis. We investigated the relationship between the antibodies to *E. coli* O antigens in the sera and the serogroup types of the *E. coli* strains isolated from patients' stools by using the HALBAU program (version 7.0). The level of significance was set at 0.05. The rates of positivity for serum antibodies to O111 and O157 strains were compared by using Fisher's exact test. The serum antibody titers were common log transformed, and titers of less than 10 were defined as 2, the minimal value. Statistical significance was determined by using U tests (for 2 groups) or Kruskal-Wallis tests (among 3 groups or more) and then applying the *post hoc* Bonferroni method.

Ethical considerations. This study was approved by the Ethical Review Board at the Toyama Institute of Health (approval no. 2 in 2012).

RESULTS

Specificity of MA assay. The rabbit immune sera with antibodies to the *E. coli* serogroups (O1, O18, O26, O111, or O157) reacted specifically with each of the *E. coli* O antigens prepared from original and laboratory isolates. No cross-reaction was observed (data not shown).

The MA titers of antibodies to *E. coli* serogroups O111, O157, and O26 for the 49 negative-control serum specimens are shown in Fig. 1a (Controls). The highest MA antibody titers in the negative-control sera were ≤1:80 for *E. coli* O111 and O157 and ≤1:40 for *E. coli* O26. The sera with MA antibody titers of <1:20 included 45 serum specimens (91.8%) with *E. coli* O111, 27 serum specimens (55.1%) with *E. coli* O157, and 40 serum specimens (81.6%) with *E. coli* O26. The MA titers of sera with antibodies to *E. coli* O1 and O18 were under 1:20 (data not shown). The sera with MA antibody titers of 1:20 to 1:40 were suggested to reflect potential EHEC infections in Japan (21). Collectively, MA antibody titers below 1:80 were considered negative results (Fig. 1a).

In this study, we also observed that the MA antibody titers of the patients' sera became negative after the sera were treated with 2ME, which strongly suggested that the antibodies detected using the MA assay belonged to the IgM class of immunoglobulins, which is the same finding reported previously (19, 25).

Distribution of MA titers measured for antibodies to *E. coli* O antigen. The MA titers measured for antibodies to the *E. coli* O antigen in the sera of the 60 patients are shown in Fig. 1b (Patients). The MA titers determined for antibodies to *E. coli* O111, O157, and O26 were in the range of ≤1:20 to 1:10,240, and the titers for antibodies to O1 and O18 were under 1:20 (data not shown). For *E. coli* O111, the number of patients with MA antibody titers of ≥1:160 was 45 (75.9%). The MA antibody titers of the sera collected from HUS patients and bloody diarrhea patients were high: the median values of the MA antibody titers were 1:640 in the HUS patients and 1:320 in the bloody diarrhea patients. However, the MA antibody titers of 9 serum specimens from one patient were under 1:20; this patient was a 70-year-old woman, one of the deceased patients who had the O111 stx_2 and O111 *stx*-negative isolates (group B in Table 1). This patient suffered from rheumatoid arthritis and had been receiving immunosuppressive drugs, and therefore, she might have been in an immunocompromised state. In contrast, for *E. coli* O157, the number of patients with MA antibody titers of ≥1:160 was 10 (16.7%). The highest MA antibody titer in sera obtained from these 10 patients with O157 infection was 1:320. In this study, we defined sera with MA antibody titers of ≥1:160 as being positive for *E. coli* O111 and O157 antibodies (Table 1). The mean interval of sampling of the sera from the date of onset was 6.9 days (range, 2 to 14 days) in 13 of 15 patients negative for antibodies to O111 and O157; data for 2 patients with ambiguous dates of onset were excluded.

Patients positive for antibodies to *E. coli* O111 and O157. We determined that the rate of positivity for antibodies to O111 (45/60, 75.0%) was significantly higher than that for antibodies to O157 (10/60, 16.7%) (Table 1; $P < 0.001$). The rates of positivity were significantly different among the patients belonging to groups A to C ($P < 0.001$) but not among those belonging to group D. In group A patients, from whose stool specimens no EHEC or *E. coli* O111:H8 *stx*-negative isolates were obtained, the rates of antibody positivity were 12/19 (63.2%) for antibodies to O111 and 2/19 (10.5%) for antibodies to O157. Among HUS patients in the same group, the rates of antibody positivity were 10/10 (100.0%) for antibodies to O111 and 2/10 (20.0%) for antibodies to O157. The rates of antibody positivity in the HUS and bloody diarrhea patients were higher than those in the diarrhea patients in group A. In group B patients, who had only *E. coli* O111:H8 isolates, the rates of antibody positivity were 18/21 (85.7%) for antibodies to O111 and 3/21 (9.5%) for antibodies to O157. In group C patients, with both *E. coli* O111:H8 and O157:H7 isolates, the rates of antibody positivity were 13/15 (86.7%) for antibodies to O111 and 5/15 (33.3%) for antibodies to O157. Among HUS patients in this group, the rates of antibody positivity were 8/8 (100%) for antibodies to O111 and 4/8 (50.0%) for antibodies to O157. Group D contained only 5 patients, with only *E. coli* O157:H7 isolates, but the rates of antibody positivity were 2/5 (40.0%) for antibodies to O111 and 0/5 for antibodies to O157. Furthermore, the rate of positivity for antibodies to O111 increased with severe symptoms, from diarrhea to bloody diarrhea or HUS.

Relationship between MA antibody titers and the symptoms of patients. The relationship between MA antibody titers and patients' symptoms is presented in Fig. 2. The median values of the MA titers measured for antibodies to O111 were 1:1,280 in 28 HUS patients, 1:1,280 in 13 bloody diarrhea patients, and 1:40 in 19 diarrhea patients. The MA titers for antibodies to O111 among the 3 symptom categories of the patients were determined to be significantly different by using the Kruskal-Wallis test ($P < 0.001$). Moreover, applying the Bonferroni method revealed that the MA antibody titers were significantly different between the

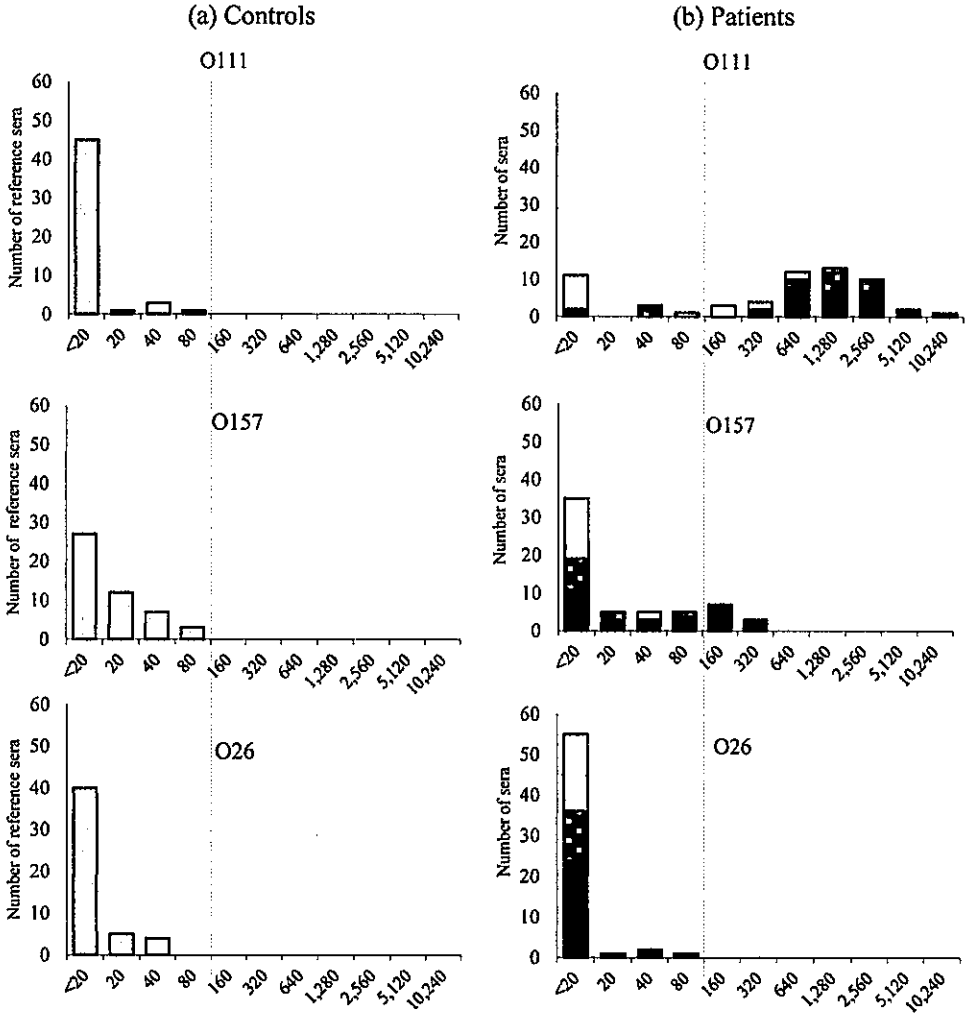

FIG 1 Histogram showing the MA titers measured for antibodies to whole bacteria: *E. coli* O111, O157, and O26. Sera were collected from 49 controls (a) and 60 patients involved in this outbreak (b). Specimens from 28 HUS patients (■), 13 bloody diarrhea patients (▩), and 19 diarrhea patients (□) were included. The cutoff value (dashed line) is 1:80.

HUS and diarrhea patients ($P < 0.01$) and between the bloody diarrhea and diarrhea patients ($P < 0.01$). However, no significant difference in the MA titers for antibodies to O111 was detected between the HUS and bloody diarrhea patients. Conversely, the MA titers for antibodies to O157 among the 3 symptom categories of the patients were determined to be significantly different by using the Kruskal-Wallis test ($P < 0.01$), and applying the Bonferroni method revealed that the MA titers for antibodies to O157 were significantly different between the HUS and diarrhea patients ($P < 0.05$).

Relationship between MA antibody titers and isolates of the patients. The relationship between MA antibody titers and the isolates obtained from the patients' stool specimens is presented in Fig. 3. The MA titers measured for antibodies to O111 and O157 were significantly different in groups A, B, and C ($P < 0.01$) but not in group D, although the MA titers for antibodies to O111 were higher than those for antibodies to O157 in group D. In group A patients, from whose stool specimens no *E. coli* O111 or O157 isolates were obtained, the median value of the MA titers for antibodies to O111 was slightly higher than that in patients in groups B and C (with *E. coli* O111:H8 and/or O157:H7 isolates). Furthermore, the MA titer for antibodies to O157 in group A was the highest among the 4 groups.

DISCUSSION

The remarkable features of the food-poisoning outbreak that occurred in Japan in 2011 are that 2 serotypes of EHEC O111:H8 and O157:H7 were isolated from certain patients, whereas no isolates of either EHEC O111:H8 or O157:H7 were obtained from samples of some patients with HUS, bloody diarrhea, or diarrhea. Only a few reports are available that have described outbreaks in which isolates of multiple serotypes of EHEC have been obtained from the stools of patients and that have also measured antibodies to the *E. coli* non-O157 serotype (26). To the best of our knowledge, this study is the first to describe the measurement and evaluation of antibodies to the O antigen of *E. coli* O111 and O157 by using the MA assay.

In our serological study, the rate of positivity for antibodies to O111 (75.0%) was higher than that for antibodies to O157 (16.7%). The MA titers measured for antibodies to O111 were

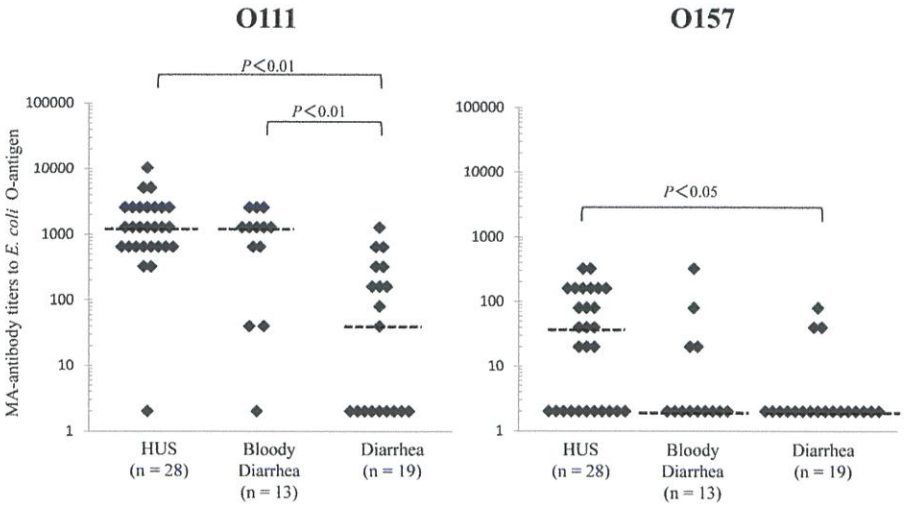

FIG 2 Relationship between antibody titers determined using the MA assay and the symptoms of patients. The MA titers measured for antibodies to *E. coli* O111 and O157 in sera in terms of the grouping of the patients' symptoms are shown. The categories of the positive antibodies in HUS, bloody diarrhea, and diarrhea patients are the same as those shown in Table 1. Data were analyzed using Kruskal-Wallis tests (followed by *post hoc* Bonferroni tests). Dashed line, median MA antibody titers in each group of symptoms.

significantly higher than those for antibodies to O157, regardless of the serogroups of *E. coli* isolated from the patients' stool specimens. One key finding was that positivity for antibodies to O111 (40.0%) but not O157 was present in group D, which was composed of patients with the *E. coli* O157:H7 isolate only. Moreover, this characteristic of elevated antibody levels was also recognized, and the titers for antibodies to O111 were higher than those for antibodies to O157 in the patients from whose stool no *E. coli* isolates were obtained (group A). These results suggest that EHEC O111:H8 stx_2 was the major pathogen in this outbreak. However, our findings do not exclude the possibility of a role of the O157 stx_1 and stx_2 isolates in the outbreak, because the MA antibody titers measured for O157 were significant in 10 patients, consisting of 8 HUS patients and 2 bloody diarrhea patients (Table 1 and Fig. 2). In this study, we also determined that the antibodies measured using the MA assay were IgM, because these antibodies lost their binding activity after treatment with 2ME. The antibodies detected are considered to have been elevated during this outbreak and were not from a past infection.

Previously, Paton et al. reported an outbreak caused by dry fermented sausages containing multiple serotypes of EHEC (26), and they detected antibodies to the LPSs (O antigens) of *E. coli* O111 and O157 by performing Western blotting. Paton and colleagues reported that the antibodies to *E. coli* O antigen detected in the patients' sera were of the same serotype as those to the O antigen of *E. coli* isolated from the patients' stool specimens (26).

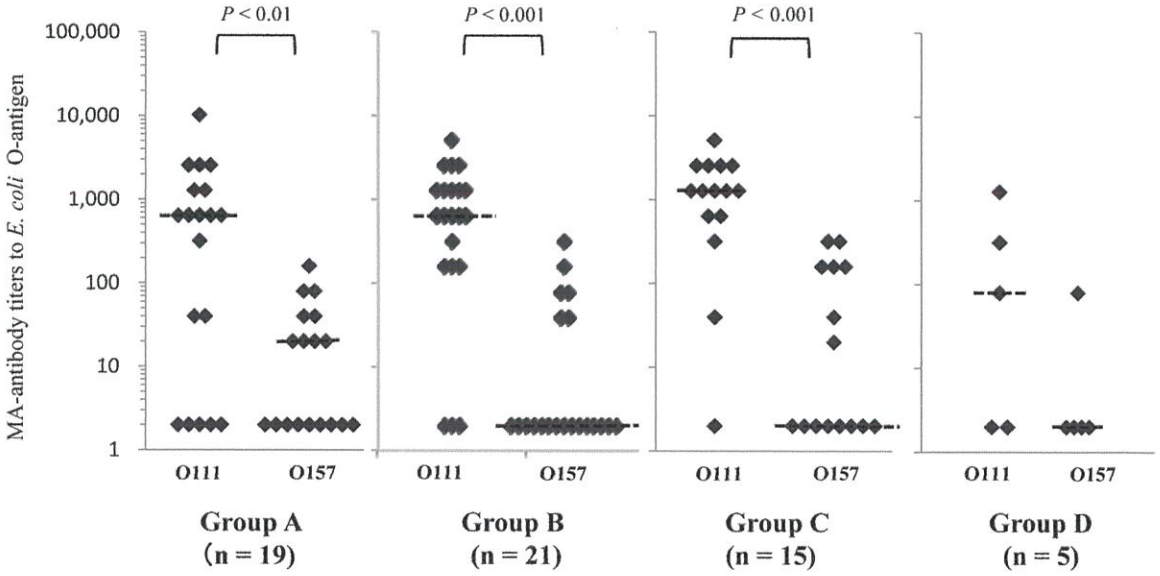

FIG 3 Relationship between MA antibody titers of sera and isolates from stools of patients. The MA titers measured for antibodies to *E. coli* O111 and O157 in sera were plotted, and statistical analyses were performed. Groups A to D are the same as those in Table 1. Data were analyzed using U tests. Dashed line, median MA titers.

In contrast, others have reported detecting antibodies to another type of *E. coli* O antigen which was distinct from the *E. coli* O antigen isolated from the patients' stools (4, 10, 21). These studies reported that the antibodies may cross-react with pathogens of other serotypes (4) or that the serological test used might have been unsuitable for specifically detecting EHEC without O157 (10) and that the patients might have been concurrently infected with *E. coli* O157 and non-O157 strains (21). However, these studies investigated only a single patient in sporadic cases; they failed to evaluate the results for reaction of antibodies to the O antigen of *E. coli*. Our results showed that the antibodies to the *E. coli* O antigens in patients' sera were not always consistent with those to the O antigens of *E. coli* isolated from the patients' stools in the outbreak described here; this has not been reported in previous cases of EHEC infection.

In this outbreak, we found that no *E. coli* O111:H8 and O157:H7 isolates were obtained from the stool specimens of certain HUS patients and that certain patients had only isolates of O157, but their serum antibodies were determined to be positive only for *E. coli* O111. In the gastrointestinal tracts of these patients, both EHEC O111 and O157 were considered to contribute to the production of stx_2. Although EHEC O157 has previously been implicated in HUS, reports indicate that no pathogen was frequently detected in patients' stools at the time of acute illness (4, 9, 10). In Japan, no causative pathogen has been detected in the stool specimens of approximately 30% of HUS patients (24, 25). Therefore, measuring antibodies by using the MA assay can facilitate the diagnosis of EHEC infection in patients from whose stool no *E. coli* isolates are obtained.

In conclusion, by using the MA assay to perform serodiagnosis, we have provided evidence that O111 stx_2 played a primary role in the pathogenesis of the food-poisoning outbreak in Japan described in this study. Furthermore, our results reveal that the isolates obtained from the stool specimens of patients were not always the major causative pathogen in multiple EHEC infections. Thus, antibodies to *E. coli* O antigens should be measured, especially in cases with EHEC infection, even with a non-O157 serotype.

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原　著

簡易水道水を原因と特定できた *Yersinia enterocolitica* O8 による集団感染事例

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要　旨

2012年7～8月，富山県において塩素管理が不十分な簡易水道水が原因で *Yersinia enterocolitica* O8による患者4名の集団感染事例が発生した．患者らが飲用していた簡易水道水から，大腸菌は検出されなかったが，一般細菌数が最大700CFU/mL検出され，水質基準である100CFU/mLを超えていた．また，この簡易水道水から免疫磁気ビーズを用いた培養法で *Y. enterocolitica* O8が分離された．*Y. enterocolitica* O8のpulsed-field gel electrophoresis解析の結果，水道水から分離された株と4名の患者便から分離された株は同一由来株であることが明らかとなった．簡易水道の貯水タンクのパイプに注入されることになっている塩素タンクは空であったことが原因であると推定された．予防策として，塩素濃度を適切に維持し，管理する体制が重要であると考えられた．簡易水道水を原因とする *Y. enterocolitica* O8集団感染事例は，日本では初めての報告である．

〔感染症誌　88：827～832，2014〕

序　文

Yersinia enterocolitica はヒトに発熱，下痢，腹痛を主訴とする胃腸炎を引き起こす．しかしながら，幼児から青年期へ年齢が高くなると，回腸末端炎，腸管膜リンパ節炎，虫垂炎様症状を呈し，また，上気道に感染すると咳や咽頭痛などの症状が現れる．ときには肝膿瘍や髄膜炎などの原因になることも知られ[1]，その病像は多彩である．そして，発熱，右下腹部痛，および白血球増多が観察されるため，しばしば虫垂炎と誤診されることもある[2)3)]．

Y. enterocolitica は0～44℃で発育し，28～29℃を至適温度とするが，4℃以下でも増殖できる好冷菌で[4)5)]，水中で増殖することや5℃の水中での生残性についても報告されている[6]．その特徴として，培養温度により生化学性状が異なること，高いアルカリ環境での抵抗性を示すことが挙げられる．血清学的性状は76種のO抗原，44種のH抗原，6種のK抗原が確認されており，1991年にはそれらの組み合わせにより81の血清型が報告された[7]．H抗原やK抗原は決定方法が複雑であるため，通常はO抗原のみによる血清群別が行われている．

日本では1972年に静岡県で *Y. enterocolitica* による集団食中毒が発生しているが[8]，この事例では，原因食品は給食であると推定されたが，菌は検出されず，特定には至っていない．本菌による食中毒は2002年に8件発生しているが，2000～2012年の13年間には17件と少なく，また，これらの事例のうち原因食品が特定されたのは，平成16年に長野県の学校給食施設で発生したリンゴサラダを原因とする事例のみである[9]．

2012年7～8月に富山県で発生した *Y. enterocolitica* O8による患者4名の集団感染事例では，塩素管理が不十分な簡易水道水が原因であると特定された．本報告では，簡易水道水から菌株を分離し，臨床分離株との分子疫学的解析から，集団感染が発生した背景について考察した．

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材料と方法

1. 患者便由来 *Y. enterocolitica*

患者便由来 *Y. enterocolitica* は，患者らが診察を受けたA病院の細菌検査室で分離され，当所に搬入された．この病院では，エルシニア腸炎を疑う患者便について，エルシニア選択分離培地とSS寒天培地（極東製薬工業）を併用して培養している．エルシニア選択分離培地は121℃15分で滅菌したエルシニア選択基礎培地（Oxoid）にYersinia Selective Supplement (Oxoid, SR0109) を加え平板とした．本事例においても，5名の患者便についてエルシニア選択分離培地（25℃培養）とSS寒天培地（37℃培養）で24～48時間培養した結果，いずれの便からも *Y. enterocolitica* O8が分離された．*Y. enterocolitica* はエルシニア選択分離培地から分離されたが，SS寒天培地からは分離されなかった．

2. 水質基準検査

患者宅の簡易水道水およびこの水道水の水源地周辺を流れる河川水について，水道水の水質基準である一般細菌数測定および大腸菌定量試験を行った．一般細菌数測定は水道水を 10^{0} から 10^{-3} まで段階希釈した検水を標準寒天で混釈し，35℃24 hr培養後，生育したコロニーを数えた．大腸菌定量試験はColilert(IDEXX Laboratories)とQuanti-Tray/2000(IDEXX Laboratories)を用いて，36℃で24 hr培養後に判定した(MPN/100mL)．患者が摂取した冷蔵庫内製氷室の氷は，3つにわけて（40mL，160mL，500mL）融解し，一般細菌数測定を行った．

3. *Y. enterocolitica* の分離

患者宅の簡易水道水および河川水について，既報に従い *Y. enterocolitica* の分離を試みた[10]．また，上述した氷の融解水と冷蔵庫の製氷室のふき取り検体についても，同様の試験を行った．

簡易水道水と河川水については3Lを，氷の融解水については一般細菌数検査の残りすべてを孔径0.45 μmのセルロースアセテートメンブランフィルター（ADVANTEC）でろ過濃縮し，そのフィルターを0.25%ペプトン・0.25%マンニトール加リン酸バッファー緩衝液（PMP）15mLに入れ，5 minボルテックスで振り出し，7.5mLずつをそれぞれ5℃で5日間，30℃で48 hr培養した．ふき取り検体については，生理食塩水2mLによく振り出したあと，その1mLずつを10mLのPMPに接種し，5℃で5日間，30℃で48 hrそれぞれ培養した．

培養液1mLに0.5% KOH加生理食塩水2mLを加えて15sec振盪後，その1白金耳をCefsulodin-Irgasan-Novobiocin (CIN) 培地（Merck Milipore）およびマッコンキー寒天培地(栄研化学)に塗抹し，30℃で24 hr培養した．水質基準検査の結果で簡易水道水中の一般細菌数が多かったことから，寒天平板培地上に生育した雑菌により典型的集落を釣菌できず偽陰性となることを考慮し，培養液を10倍希釈したものについても同様の処理を行い，平板培地に塗抹した．定型的集落を釣菌し，TSI（日水製薬），LIM（日水製薬），胆汁エスクリン寒天培地により生化学性状を確認した．

4. 免疫磁気ビーズを用いた *Y. enterocolitica* O8の分離

上述した通常の培養法に加え，市販抗血清エルシニアエンテロコリチカO群別用免疫血清の血清群O8（デンカ生研）で感作した免疫磁気ビーズを作製し，これを用いて検水から *Y. enterocolitica* O8を選択的に濃縮した．培養液1mLに20μLの感作磁気ビーズを接種し，20 min回転混和した．その後，1% Tween80加PBSにて2回洗浄し，磁石で集められたペレットに50μLのBrain Heart Infusion (Becton, Dickinson and Company) を接種し懸濁した．懸濁液を上述の方法と同様にKOH処理後，CIN培地とMackonky培地に塗抹し，30℃で24 hr培養した．

5. *Y. enterocolitica O8* の生化学性状

医療機関から搬入された患者便由来および当所で分離した水道水由来の *Y. enterocolitica* O8について，TSIおよびLIMに接種し，35℃および28℃培養した．生物型を決めるため，硝酸塩還元，オルニチン脱炭酸，β-ガラクトシダーゼ，インドール産生性，炭水化物（D-キシロース，乳糖，トレハロース）からの酸の産生性について，API20EおよびAPI50CH（シスメックス・ビオメリュー）を用いて調べた．レシチナーゼ活性は卵黄を5%に加えた普通寒天で，また，ピラジナミダーゼ活性[11]は文献に従い，それぞれ28℃48時間培養で調べた．

6. pulsed-field gel electrophoresis (PFGE) による分子疫学的解析

PFGE解析は既報[12]を参考に，下記の条件で行った．制限酵素反応は，*Y. enterocolitica* O8は30Uの *Not*I（ニッポンジーン），分子量マーカーとして用いた *Salmonella* enterica serovar Braenderup H9812 strainは30Uの *Xba*I（ニッポンジーン）を用いて1晩静置した．その後，パルスタイム5～8sec，電圧6 V/cm，14℃で19.5 hr泳動した．

成績

1. 集団感染の概要

2012年7月，A病院の小児科でエルシニア腸炎と診断された患者4名は，右下腹部に痛み，発熱，下痢，嘔吐，リンパ節炎，回盲部炎，回腸末端炎を呈した(Table 1，P1～P4)．患者3名（P2～P4）はA町に在住

Table 1 Patients infected with *Yersinia enterocolitica* O8

No.	Date of onset	Age	Gender	Resident area	Tap water	Symptoms
P1	2013/7/8	1	Male	B-city	small scale water system	diarrhea, fever, ileocecitis
P2	2013/7/14	8	Male	A-town	small scale water system	abdominal pain, diarrhea, fever, terminal ileitis
P3	2013/7/19	9	Female	A-town	small scale water system	abdominal pain, diarrhea, fever, ileocecitis, lymphadenosis, vomiting
P4	2013/7/24	7	Female	A-town	small scale water system	abdominal pain, fever, terminal ileitis, vomiting
P5	2013/8/14	4	Male	A-town	small scale water system	abdominal pain, fever, lymphadenosis, terminal ileitis

Table 2 Results of microbiological examinations of enviromental samples in this study

No.	Sample	Source*	Viable bacteria count (CFU/mL)	MPN of *E. coli* (/100 mL)	Isolation of *Y. enterocolitica* by using PMP medium							
					without IMB**				with IMB			
					5℃, 5 days		30℃, 48 hr		5℃, 24 hr		30℃, 24 hr	
					×1	×10	×1	×10	×1	×10	×1	×10
S1	Tap water	House of P2 and P4	700	<3	−	+ (O:8)	−	−	+ (O:8)	+ (O:8)	−	+ (O:8)
S2	Tap water	House of P3	482	<3	−	+ (O:8)	−	−	−	+ (O:8)	−	+ (O:8)
S3	River water	River A near the source of tap	62	3.1	−	−	−	−	−	−	−	−
S4	River water	River B near the source of tap	37	5.2	−	−	−	−	−	−	−	−
S5	Ice (40 mL)	Refrigerator ice supplying apparatus of house of P5	47	NT	−	−	−	−	−	−	−	−
S6	Ice (160 mL)	Refrigerator ice supplying apparatus of house of P5	30.5	NT	−	−	−	−	−	−	−	−
S7	Ice (500 mL)	Refrigerator ice supplying apparatus of house of P5	9	NT	−	−	−	−	−	−	−	−
S8	Swab	Refrigerator ice supplying apparatus of house of P5	NT***	NT	−	−	−	−	−	−	−	−
S9	Swab	Refrigerator ice supplying apparatus of house of P5	NT	NT	−	−	−	−	−	−	−	−

*Patient no. is described in Table 1.
**IMB; immunomagnetic beads.
***NT; Not tested.

し，同じ簡易水道水を飲用していた．患者（P1）はB市に在住し，上記3名とは別の簡易水道水を飲用していた．他に共通した喫食歴などは認められなかった．一方，8月に同じくA病院の小児科で1人の保育園児が腹痛，発熱，リンパ節炎，回腸末端炎を呈し，エルシニア腸炎患者（P5）と診断された．この患者の居住地では上記の患者らとは別の共同井戸が設置されているが，日ごろ，上述した患者3名（P2〜P4）と同じA町の簡易水道水を利用している親戚宅で過ごしていた．なお，患者は全員10歳未満であった．

2. 簡易水道水の水質基準検査

A町の簡易水道水由来である，患者P2およびP4（兄弟）の自宅の水道水（S1）と患者P3の自宅の水道水（S2）の2検体からは，大腸菌は検出されなかった（Table 2）．一般細菌数については，S1は700CFU/mL，S2は482CFU/mLと，どちらの検体も基準の100CFU/mLを超えていた．また，A町の簡易水道水の水源地周辺を流れる河川水2検体（S3，S4）からは，大腸菌3.1および5.2/100mL，一般細菌数62および37CFU/mLがそれぞれ検出された．

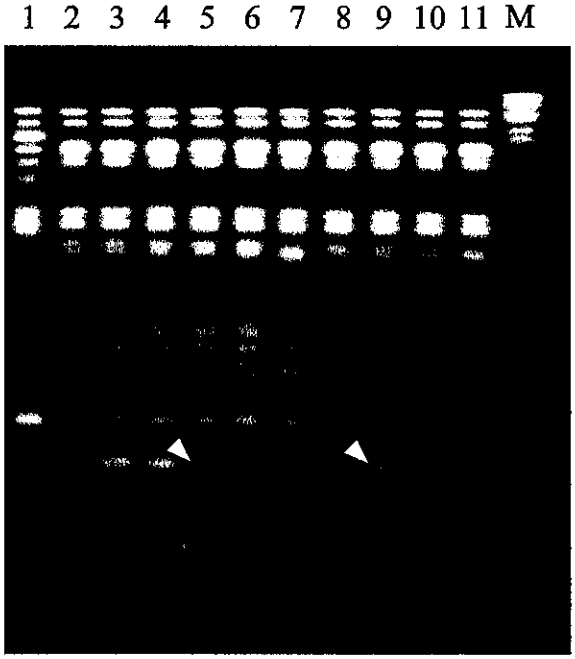
患者P1は1歳で，家族からの聞き取りによると，患児には日ごろ簡易水道水を飲用させていなかったため，自宅の簡易水道水の検査は実施しなかった．

患者P5の自宅の井戸水は，厚生センターで一般細菌数および大腸菌の検査が行われ，どちらも水質基準内であった．また，患者P5がよく利用していた親戚宅の簡易水道水は，先の患者らの事件を受けてすでに塩素消毒がなされていたため，検査しなかった．ただし，この親戚宅で塩素消毒前の簡易水道水で作られたと思われる冷蔵庫の製氷室の氷（S5〜S7）については，一般細菌数測定および大腸菌定量試験を行った．その結果，一般細菌数は9〜30.5CFU/mL，大腸菌は不検出であり，水質基準内であった．

3. 患者らが飲用していた簡易水道水からの *Y. enterocolitica* の分離

結果をTable 2に示した．免疫磁気ビーズを用いない培養法では，5℃および30℃培養の増菌液を直接塗抹した方法どちらにおいても，簡易水道水2検体，

Fig. Pulsed-field gel electrophoresis patterns with *Not*I digestion of *Y. enterocolitica* isolates. Differences in the band brightness isolated from the same patient (P3; Table 1) were indicated by arrowheads (lanes 5 and 9). Lane 1, an isolate stocked in our laboratory; lane 2 and 3, an isolate from tap water from the house of P2 and P4; lane 4, an isolate from tap water from the house of P3; lane 5 and 9, a clinical isolate from P3; lane 6, a clinical isolate from P2; lane 7, a clinical isolate from P1; lane 8, a clinical isolate from P4; lane 10, a clinical isolate from P5; lane 11, a clinical isolate from a patient in 2010; and lane M, size marker (*Salmonella* enterica serovar Braenderup H9812 strain digested with *Xba*I).



河川水2検体いずれからも *Y. enterocolitica* は検出されなかった．しかしながら，5℃ 培養液を10倍に希釈した増菌液を塗抹した方法では，簡易水道水2検体（S1，S2）から *Y. enterocolitica* O8が検出された．

免疫磁気ビーズを用いた分離法では，5℃ 培養の増菌液を直接塗抹した方法で患者P2とP4の自宅の水道水（S1）から *Y. enterocolitica* O8が分離された．また，5℃ 培養，30℃ 培養液を10倍に希釈した液を塗抹した方法では，簡易水道水2検体（S1，S2）から *Y. enterocolitica* O8が検出された．

河川水2検体からは *Y. enterocolitica* は分離されなかった．また，P5がよく訪れていた親戚宅の冷蔵庫の氷と冷蔵庫のふき取り検体からも *Y. enterocolitica* は分離されなかった．

4. *Y. enterocolitica* O8の性状

患者便および簡易水道水から分離された *Y. enterocolitica* O8の生化学性状試験を行った結果，TSI培地はA/Aであり，リジン脱炭酸，エスクリン加水分解は陰性であった．分離菌の運動性は，35℃ 培養ではほとんど認められなかったが，28℃ 培養では明らかに認められた．一方，硝酸塩還元，オルニチン脱炭酸，β-ガラクトシダーゼ，インドール産生性陽性，レシチナーゼ活性陽性，ピラジナミダーゼ活性陰性であった．また，炭水化物（D-キシロース，乳糖，トレハロース）からの酸の産生はすべて陽性であり，すべての菌株の生物型は1Bであった．

5. PFGEによる分子疫学的解析

水道水（S1，S2）由来3株，患者P3由来2株のうち1株，患者P4由来株，および患者P5由来株の計6株の *Y. enterocolitica* O8は同一パターンを示した（Fig.）．また，患者P3の残りの1株，患者P2由来株は上記6株のバンドパターンと比較して，1本のバンドの輝度のみが異なった．また，A町の簡易水道水を飲用していなかった患者P1由来株と2010年の臨床分離株のバンドパターンは，これら集団株とは異なったが，Tenoverらの基準[13]によると，バンドパターンの相違は5バンド以内であった．一方，県外で分離された当所保存株のバンドパターンは，今回の集団株のバンドパターンと比べ10バンド以上異なっていた．

6. 県内10定点医療機関の *Y. enterocolitica* 分離数

2004～2011年にかけて県内10カ所の定点医療機関から報告された糞便由来の *Y. enterocolitica* は，計33株であった．そのうち23株（69.7%）は，今回集団感染事例が発生した地域にあるA病院からの報告であった．

考　察

水道水を原因とする *Y. enterocolitica* O8集団感染事例は日本ではこれまで報告はない．患者らが居住する地域は北アルプスの雪解け水が地下に流れ込み，豊富な地下水が湧き出ることで有名である．したがって，この地域では湧き水をタンクに溜めて塩素を注入して利用する「簡易水道」を利用している住民が多い．本簡易水道については，管理者は自治体（町長）であるが，実際の管理は住民らで組織する管理組合が行っていた．本事例はそのような背景の地域で発生した．

今回の結果では，患者便および簡易水道水から分離された *Y. enterocolitica* O8の生化学性状および生物型は一致した．PFGEでは，A町の簡易水道水由来3株と患者由来3株（P3，P4，P5）のバンドパターンが一致した．また，患者から分離された他の2株（P2，P3）は上記6株と比較して1本のバンドの輝度のみが異なっていたが，P3からこれら2種類のバンドパターンを示す株が分離されていることと，Tenoverらの基準をもとに考慮した結果，8株は同一由来であ

ると考え，集団感染株由来であると判定した．厚生センターの調査によると，本事例の原因となった簡易水道の貯水タンクのパイプに注入されることになっている塩素タンクは空であった．住民らは消毒されないままの簡易水道水を飲用していたものと推定された．事実，飲用された水道水の一般細菌数は水道法の基準値[14]の約5～7倍であった．これまでの報告から，水源を汚染する可能性として野生動物の糞などが混入する場合が考えられるが[15]，周辺に生息する野生動物の調査は実施できなかったため，関連性は明らかではない．また，患者1名（P5）は他の3名（P2～P4）と比べ約1カ月遅く発症しており，水道水の塩素消毒が行われた後であった．この患者は塩素消毒前の水道水で作られた氷を喫食していたため，氷が感染源であると考えられた．しかしながら，製氷室に残っていた氷から *Y. enterocolitica* は検出されず，一般細菌および大腸菌は水質基準内であった．製氷室の氷がいつ作られたかは不明であったので，長期間の保存により *Y. enterocolitica* が死菌あるいは培養できない状態となっていたかもしれない．

一方，患者P1は他の4名と同時期にエルシニア腸炎と診断されたが，異なる市町村に在住し，利用する水道水の系統も異なった．また，家族は患児に水道水を飲用させていなかったため，この患児の感染源は不明である．しかしながら，この患者から分離された株と，2010年に同じB市に居住する患者から分離された株は，県外で分離された当所保存株より，今回の集団株とのバンド数の違いが少なく，集団株に類似していた．上記2名の患者は集団感染事例の4名の患者らと隣接する市町村に居住していることから，遺伝的に近い *Y. enterocolitica* O8の株がこの地域に土着している，あるいは野生動物が保有し，汚染を広げているなどの可能性が考えられ，その関連性に興味がもたれる．実際，この地域にあるA病院の患者便由来 *Y. enterocolitica* 分離数は，県内の他の地域の病院と比較して多い．A病院から分離された *Y. enterocolitica* の血清群や生化学性状は不明であるが，この地域でエルシニア腸炎の患者が多く診断されるのは，この地域の住民らが簡易水道水を飲用していることと関連するかもしれない．あるいは，この病院では細菌検査に *Yersinia* の選択分離培地であるエルシニア選択分離培地を常用していることにより，担当者による *Y. enterocolitica* の分離を容易にしていることも影響しているのかもしれない．

今回われわれは感染源を特定するため，免疫磁気ビーズを用いて，目的とする *Y. enterocolitica* を選択的に濃縮する方法を試した．これまで，集団事例の感染源特定のためにこの方法を用いて *Y. enterocolitica* を分離した報告はなく，今回が最初である．その結果，5℃ 24 hr培養液（原液と10倍希釈液）と30℃24 hr培養の10倍希釈液から，*Y. enterocolitica* O8を検出することが可能であった．これに対し，免疫磁気ビーズを用いない培養法においては，5℃5日間培養の10倍希釈液のみ，*Y. enterocolitica* O8が分離された．この結果は，免疫磁気ビーズを用いて選択的に濃縮する方法が *Y. enterocolitica* の分離，すなわち，早期の感染源特定に有用であることを示している．同時に，一度増菌した培養液を10倍希釈することが，雑菌の生育による偽陰性を回避するのに有用であったことも示した．

本研究によって，簡易水道水が *Y. enterocolitica* による集団感染の原因となりうることが明らかとなった．本事例では管理者に対し，水道施設等改善勧告書が交付され，水道法に規定する衛生上必要な措置を講じることや，水質検査及びその記録の保存などが指示された．予防策として，簡易水道水の塩素濃度を適切に維持し，管理する体制が重要である．

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利益相反自己申告：申告すべきものなし

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Water-Borne Outbreak of *Yersinia enterocolitica* O8 Due to a Small Scale Water System

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A water-borne outbreak of *Yersinia enterocolitica* O : 8 associated with a small-scale water system occurred during July-August 2011 in Toyama Prefecture, Japan. *Escherichia coli* was not detected in tap water from the small-scale water system. However, the maximum concentration of viable bacteria in the tap water was 700CFU/mL, which exceeds the legal standard for purity of tap water (100CFU/mL). Furthermore, *Y. enterocolitica* O8 was isolated from the tap water with the use of immunomagnetic beads prepared with anti-*Y. enterocolitica* O8 antibodies. Pulsed-field gel electrophoresis analysis identified 3 isolates from tap water and 5 isolates from 4 patient stool specimens as belonging to the outbreak strain. An epidemiological investigation revealed improper management of the residual chlorine concentration in the tap water. This is the first report of an outbreak of *Y. enterocolitica* due to tap water from a small-scale water system in Japan.

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