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Laboratory Investigation of Enteroaggregative *Escherichia coli* O Untypeable:H10 Associated with a Massive Outbreak of Gastrointestinal Illness

YOSHIHIRO ITOH,[1]* ISAO NAGANO,[2] MIYUKI KUNISHIMA,[3] AND TAKAYUKI EZAKI[1]

Department of Microbiology[1] and Department of Parasitology,[2] Gifu University School of Medicine, Tsukasa-machi 40, and Gifu Prefectural Health and Environment Research Center, Noishiki 4-6-33,[3] Gifu 500, Japan

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A massive outbreak of gastrointestinal illness occurred in Tajimi city, Gifu prefecture, in June of 1993 in which 2,697 children in elementary and junior high schools developed severe diarrhea. Stool specimens from 30 children with severe protracted diarrhea were studied. Twenty-seven strains of enteroaggregative *Escherichia coli* (EAggEC) isolated from 12 of 30 patients all belonged to the same serotype, O untypeable (OUT):H10, and showed the same biochemical characteristics and antibiotic susceptibility pattern. These strains were negative for the virulence factors of the four standard categories of diarrheagenic *E. coli* (enterotoxigenic, enteropathogenic, enteroinvasive, and enterohemorrhagic). However, the isolates showed an aggregative pattern of adherence to HEp-2 cells and had a 60-MDa plasmid and an *astA* gene, which encodes heat-stable enterotoxin-1 production. These data suggested that the EAggEC serotype OUT:H10 was associated with this massive outbreak of gastrointestinal illness.

Four major categories of *Escherichia coli* that cause diarrhea have been identified: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), and enterohemorrhagic (EHEC) (13). Recently, *E. coli* strains that do not belong to any of the above-mentioned categories have been isolated from patients with diarrhea (21, 22). Accumulated evidence indicates the existence of a fifth distinct diarrheagenic category, enteroaggregative *E. coli* (EAggEC). EAggEC has been epidemiologically implicated as an etiological agent of diarrhea in some developing countries (2, 4, 9, 12). EAggEC strains are characterized by an aggregative pattern of adherence to HEp-2 cells (14, 22, 23). Virtually all EAggEC strains carry a 60-MDa plasmid that contains a gene that confers aggregative adherence (AA) and also a gene that encodes EAggEC heat-stable enterotoxin-1 (EAST-1) (16, 18), but the importance of EAggEC in diarrheal disease is not yet clear. A massive outbreak of gastrointestinal illness occurred at 16 schools in Tajimi city in the Gifu prefecture on 21 June 1993. Though bacteriological investigation was performed by the public health center in charge of this case, the source and the pathogen were not identified (7). We isolated EAggEC, for which the serotype has not yet been reported, from stool specimens taken after an outbreak of gastrointestinal illness in Japan and characterized the isolates with respect to their O:H serotype, plasmid profile, drug susceptibility pattern, pattern of adhesion to HEp-2 cells, production of various toxins, and possession of some virulence genes.

MATERIALS AND METHODS

Specimens and stool cultures. From 21 to 24 June 1993, 2,697 children at two kindergartens, eight elementary schools, and six junior high schools in Tajimi city, Gifu, were stricken with gastroenteritis. Among them, we studied 30 children with severe protracted diarrhea. Diarrheal fecal specimens were taken from naturally excreted feces and cultured by standard microbiologic techniques for *E. coli*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., *Aeromonas* spp., *Vibrio* spp., *Plesiomonas* spp., and *Clostridium perfringens*. Five lactose-positive colonies of typical *E. coli* growing on deoxycholate-hydrogen sulfide-lactose or *Salmonella-Shigella* agar plates were selected and identified on the basis of biochemical properties with commercial media (Nissui Seiyaku Co., Ltd., Tokyo, Japan).

Agglutination test. Isolates suspected of being *E. coli*, as a result of the biochemical tests, were examined for O antigen with polyvalent and monovalent antisera to O1, O6, O8, O15, O18, O20, O25, O27, O26, O28ac, O29, O44, O55, O63, O78, O86a, O111, O112ac, O114, O115, O119, O124, O125, O126, O127a, O128, O136, O142, O143, O144, O146, O148, O151, O152, O153, O157, O158, O159, O164, O166, O167, O168, and O169 (Denka Seiken Co., Ltd., Tokyo, Japan) by slide and tube agglutination of heated (100°C, 1 h) bacterial suspensions. The H antigens were subsequently determined as follows: isolates with intensified mobility were grown at 37°C for 5 h with Trypticase soy broth (Difco Laboratories, Detroit, Mich.), the resulting bacteria were treated with equal volumes of 1% (vol/vol) formalin–physiological saline, and tube agglutination tests were carried out with monovalent antisera (Denka Seiken Co., Ltd.).

Drug susceptibility test. The MICs for the isolates were determined by the agar plate dilution method according to the standards of the Japan Society of Chemotherapy (10). The antibiotics ampicillin, cephalothin, chloramphenicol, kanamycin, nalidixic acid, streptomycin, and tetracycline were supplied from Wako Pure Chemical Industries, Ltd., Osaka, Japan. The MIC was defined as the lowest concentration of a drug needed to achieve complete inhibition of growth.

Plasmid isolation and gel electrophoresis. Plasmid DNA was isolated by the method of Kado and Liu (11) and electrophoresed in 0.7% agarose gels. The agarose gels were then stained with ethidium bromide and photographed under UV light.

HEp-2 cell adherence assay. HEp-2 cell adherence of bacterial isolates was assayed as described by Vial et al. (22, 23). Bacteria were incubated with HEp-2 cells for 3 h in the presence of 1% methyl-α-D-mannoside at 37°C in 5% CO_2. Wells were then washed three times with Hanks balanced salt solution, and cells were fixed with 70% ethanol and stained with 10% Giemsa stain. Coverslips were mounted on glass slides and examined under an oil immersion lens of a light microscope. The isolates were then observed for the presence of diffuse adherence, localized adherence, or AA to HEp-2 cells. A diffuse-adherence strain (*E. coli* 251), a localized-adherence strain (*E. coli* 886L), and an AA strain (*E. coli* JM221), all kindly provided by the Gifu Prefectural Health and Environment Research Center, were used as controls.

Determination of enterotoxins in *E. coli*. The conditions used to culture isolates for production of verotoxin (VT), heat-labile toxin (LT), and heat-stable enterotoxin (ST) were as follows: briefly, isolates were grown in the Casamino Acids-yeast extract medium described by Evans et al. (6) at 37°C for 18 h by shaking and culture supernatant fluids obtained by centrifuging the cultures at 1,000 × *g* for 30 min were used for a reversed passive latex agglutination test and enzyme-linked immunosorbent assay. The levels of production of VT and LT from isolates were determined by a reversed passive latex agglutination test (Denka Seiken Co., Ltd.). The production of ST was determined by enzyme-linked immunosorbent assay (Colist enzyme immunoassay kit; Denka Seiken Co., Ltd.).

* Corresponding author. Mailing address: Department of Microbiology, Gifu University School of Medicine, Tsukasa-machi 40, Gifu 500, Japan. Phone: 058-267-2239. Fax: 058-267-0155. E-mail: tezaki@cc.gifu-u.ac.jp.

PCR. PCR analysis was performed with a thermal cycler from Perkin-Elmer Cetus (Norwalk, Conn.). The multiplex PCR for the detection of the VT, ST, LT, and *invE* genes was described previously by Fumiaki et al. (8). Savarino et al. designed oligonucleotide primers to amplify the *astA* gene region, which encodes EAST-1 production (18). These primers were used to detect the *astA* gene. Recently Schmidt et al. developed a PCR with primers complementary to the EAggEC probe (by cloning an *Eco*RI-*Pst*I fragment of plasmid pCVD432) (19). This PCR assay designed by Schmidt et al. was performed to identify EAggEC. Briefly, the isolates examined by PCR were grown on tryptic soy agar overnight at 37°C. DNA was extracted from the bacteria by resuspending the cells in a small volume (1 ml) of sterile deionized water and boiling the suspension for 10 min. The PCR mixture (total volume, 50 μl) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.25 mM each deoxynucleoside triphosphate, a 5-μl aliquot of the boiled bacterial suspension, 1 U of *Taq* polymerase (TaKaRa Shuzo Co., Ltd., Kyoto, Japan), and 0.2 μM each primer. The VT, ST, LT, and *invE* genes were amplified for 25 cycles (the PCR cycle included denaturation for 30 s at 94°C, primer annealing for 60 s at 47°C, and extension for 90 s at 72°C). The *astA* gene was amplified for 30 cycles (the PCR cycle included denaturation for 30 s at 94°C, primer annealing for 30 s at 55°C, and extension for 30 s at 72°C). The EAggEC gene was amplified for 30 cycles (the PCR cycle included denaturation for 40 s at 94°C, primer annealing for 60 s at 53°C, and extension for 60 s at 72°C). The amplified DNA product was resolved by agarose gel electrophoresis and visualized under UV transillumination after the gel was stained with ethidium bromide.

RESULTS

Outline of outbreak. An outbreak of gastrointestinal illness occurred in Tajimi city in the Gifu prefecture from 21 to 24 June 1993. Of 6,636 children who ate the school lunch at 16 schools (two kindergartens, eight elementary schools, and six junior high schools), 2,697 (rate of attack, 40.6%) were stricken with gastroenteritis. Major symptoms were stomachache (73.6%), nausea (51.1%), and diarrhea (39.9%). The incubation period for the onset of gastrointestinal illness was 40 to 50 h on average. There were no common foods other than the school lunches that were cooked in the same kitchen of the school lunch center. This school lunch center provided lunches for 22 schools (the above-described 16 schools and another 6 schools), and the 22 schools were dispersed geographically over a broad section of Tajimi city. There were no patients from the above-mentioned other six schools in this outbreak. As these six schools were closed June 21, the school lunches of this day were assumed to contain the causative food by the public health center in charge of this outbreak. The contents of the school lunch of June 21 were bread, noodles, macaroni salad, milk pudding, fried vegetables, and milk. The public health center performed a bacteriological investigation for *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, *Staphylococcus aureus*, *Yersinia*, *Aeromonas*, *Vibrio*, *Plesiomonas*, and *Clostridium perfringens* on about 20 school lunches, on naturally excreted feces of 109 patients, one sample of vomitus from a patient, five drinking water samples, and 34 cotton gauze pads used for cleaning at the cooking institution (cooking appliances, cutting board, kitchen sink, etc.). However, the specific common serotype of *E. coli* mentioned below was not isolated from these samples. The Gifu prefecture reported to the Ministry of Health and Welfare that the source and the pathogen were not identified in this outbreak (7).

Stool examination. The 30 stool specimens collected from diarrheal patients were examined bacteriologically; *E. coli* strains were the dominant isolates in all cases, and no other pathogenic bacteria were isolated. Twenty-seven strains of *E. coli* isolated from 12 of 30 patients had the same biochemical characteristics (Table 1).

Serotyping. All *E. coli* isolates that had the same biochemical characteristics were serotyped as H10, but the serotype of the O antigen could not be determined with the antisera available.

Drug susceptibility testing. The MICs of each antimicrobial agent for *E. coli* O untypeable (OUT):H10 are summarized in Table 2. All isolates were susceptible to nalidixic acid, chloramphenicol, streptomycin, kanamycin, and cephalothin. All strains were resistant to ampicillin. The susceptibility patterns of all *E. coli* OUT:H10 strains were similar.

TABLE 1. Biochemical characteristics of 27 isolates of EAggEC

Test	Reaction
Voges-Proskauer	−
Motility	+
Simmons citrate utilization	−
H_2S (TSI)	−
Phenylalanine deaminase	−
Lysine decarboxylase	+
Ornithine decarboxylase	+
Arginine dihydrolase	−
Malonate utilization	−
D-Tartrate (Jordan) utilization	+
Esculin	+
Christensen urea	−
β-Galactosidase (ONPG[a])	+
β-D-Glucuronidase	+
$NO_3^- \rightarrow NO_2^-$	+
Gas from glucose	+
Acid from:	
Glucose	+
Sucrose	−
Raffinose	−
Arabinose	+
Inositol	−
Xylose	+
Mannitol	+
Lactose	+
Salicin	+

[a] ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

Plasmid profiles. OUT:H10 isolates carried a 60-MDa plasmid, and it was the sole plasmid in all the strains (Fig. 1).

HEp-2 cell adherence assay. All 27 *E. coli* OUT:H10 isolates were examined for adhesion. The diffuse-adherence strain (*E. coli* 251) adhered uniformly to the entire cell surface (Fig. 2A). The localized-adherence strain (*E. coli* 886L) attached to cells and formed microcolonies in distinct regions of the cell surfaces (Fig. 2B). The AA strain (*E. coli* JM221) showed AA to cells. All 27 isolates showed equivalent levels of AA to cells in a stacked-brick arrangement. The pattern of adhesion of *E. coli* JM221 to HEp-2 cells is shown in Fig. 2C, and one strain of the 27 isolates that adhered to HEp-2 cells is shown in Fig. 2D.

Production of various toxin from isolates. OUT:H10 isolates did not produce toxins (ST, LT, or VT).

Detection of various virulence genes in isolates by PCR amplification. Twenty-seven OUT:H10 strains gave negative

TABLE 2. MICs of antibiotics for 27 isolates of EAggEC

Antibiotic[a]	No. of strains for which the MIC (μg/ml) was:				
	1.56	3.13	6.25	50	≧200
NA	6	21			
CP	2	25			
SM		14	13		
TC				27	
ABPC					27
KM		27			
CET			27		

[a] NA, nalidixic acid; CP, chloramphenicol; SM, streptomycin; TC, tetracycline; ABPC, ampicillin; KM, kanamycin; CET, cephalothin.

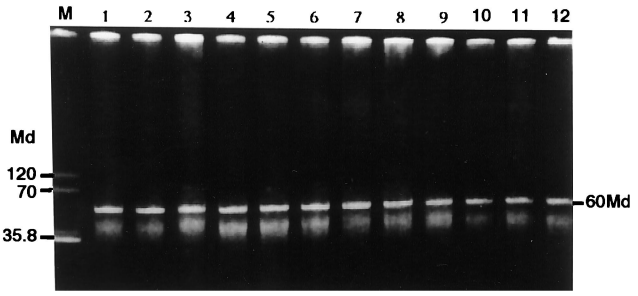

FIG. 1. Agarose gel electrophoresis of plasmid DNA from *E. coli* OUT:H10 strains (lanes 1 to 12) isolated from 12 patients with diarrhea. Lane M, *E. coli* V517 (plasmid 35.8-Md), *E. coli* K12 W3350/R222 (plasmid 70-Md), and *E. coli* Rts1 (plasmid 120-Md) as plasmid DNA size markers; lanes 1 to 12, strains carrying the same 60-MDa plasmid.

PCR results for VT, ST, LT, *invE*, and EAggEC genes, while amplification of the *astA* gene isolates showed positive PCR results (Fig. 3). Amplification of the OUT:H10 strains resulted in similar 124-bp DNA fragments in all strains.

DISCUSSION

Several categories of *E. coli* have been firmly established as important enteropathogens, including EPEC, ETEC, EIEC, and EHEC. The propensity for certain strains of *E. coli* isolated from patients with diarrhea to adhere to HEp-2 cells was first reported by Cravioto et al. (5). They found that 80% of tested strains of classical EPEC infantile diarrhea serotypes adhered to HEp-2 cells but that this was an uncommon property among ETEC, EIEC, and EHEC. In a recent study, evidence to implicate a fifth distinct new category, EAggEC, was presented. EAggEC strains are characterized by their unique patterns of adherence to HEp-2 cells as well as to the glass between the cells in a characteristic stacked-brick appearance (15, 17).

In this study, all 27 strains of *E. coli* isolated from 12 of the 30 patients with severe diarrhea showed serotype OUT:H10 and had the same biochemical characteristics. Of these 27 strains, which possess a 60-MDa plasmid and the *astA* gene, which encodes production of EAST-1, all showed the same aggregative pattern of adherence to HEp-2 cells and the same pattern of drug susceptibility. On the basis of these points, as

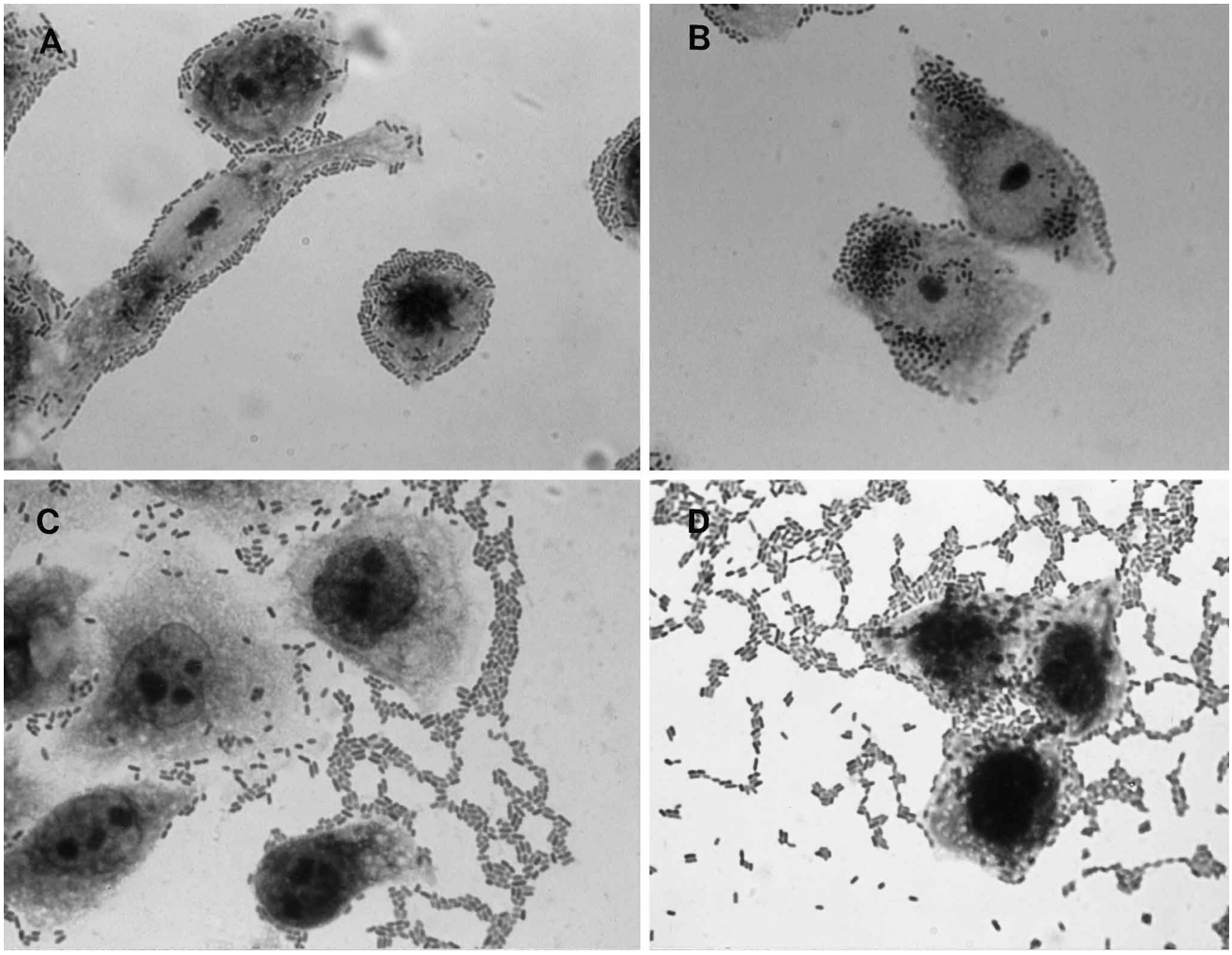

FIG. 2. Results of a HEp-2 cell adherence assay in which cells were stained with Giemsa and observed with an oil immersion light microscope. The Giemsa stain shows a diffuse-adherence pattern for *E. coli* 251 (A), a localized-adherence pattern for *E. coli* 886L (B), an AA pattern for *E. coli* JM221 (C), and an AA pattern in a stacked-brick arrangement for one *E. coli* OUT:H10 strain from 27 isolates (D) on HEp-2 cells.

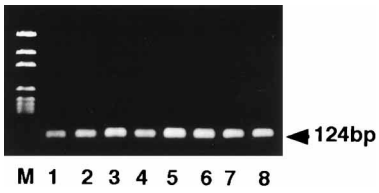

FIG. 3. PCR amplification of the *astA* genes from eight *E. coli* OUT:H10 strains. Lanes 1 to 8 show isolates from eight patients with diarrhea that were positive for *astA* gene amplification (124 bp). Lane M contains pGEM DNA markers (Promega Corp., Madison, Wis.).

no other pathogenic bacteria were isolated from the stool specimens of the patients, we concluded that EAggEC OUT:H10 was associated with this outbreak.

In the HEp-2 cell adherence assay, three distinct patterns of adherence can be discerned: localized, diffuse, and aggregative (3, 5). EAggEC strains are characterized by cells adhering to HEp-2 cells in a stacked-brick-like lattice, called AA (15, 17). AA is associated with the presence of a 60-MDa plasmid (22). The 27 isolated strains showed AA in the HEp-2 cell adherence assay (Fig. 2D) and carried a sole 60-MDa plasmid (Fig. 1). On the basis of observations from the above-mentioned two assays, these isolates were determined to be EAggEC. Furthermore, Savarino et al. reported that an EAggEC strain, 17-2, isolated from a Chilean child with diarrhea contains a single 60-MDa plasmid and that the 60-MDa plasmid is associated with EAST-1 production (16, 18). EAST-1 is genetically and immunologically distinct from ETEC STa (16). Vial et al. showed that EAggEC causes characteristic lesions in rabbit and rat ileal loops and possesses a 55- to 65-MDa plasmid carrying genes coding for the production of virulence factors (22). The pathogenic mechanism by which EAST-1 causes acute and persistent diarrhea is not yet clear, and the role of EAST-1 activity in human small intestines has not been fully elucidated. But several epidemiological studies have implicated EAggEC as a causative organism of diarrhea in infants and young children (2, 3, 12).

Baudry et al. studied the fragments from 60 MDa of the EAggEC strain that were used as DNA probes to diagnose EAggEC infections (1). The PCR assay designed by Schmidt et al. (19) to identify EAggEC was performed, but all isolates were negative by this PCR assay. Schmidt et al. reported 50 *E. coli* strains that demonstrated AA to HEp-2 cells; 7 (14%) were negative by the EAggEC PCR assay. The reason why not all EAggEC strains gave positive results in the EAggEC PCR assay is unknown (19), but some argue that different categories of EAggEC strains may exist (1, 20). It is possible that the 27 isolates exhibit sequence variations in their primer binding sites. These 27 isolated strains might be of a type different from that of EAggEC strains found in the past. A comprehensive study of gastrointestinal illness caused by the EAggEC serotype OUT:H10 has not been done. This serotype has been described only in a report by Zepeda-Lopez and Gonzalez-Lugo (24), who used this serotype strain as an AA standard strain for investigation of HEp-2 cells with prefixed cells.

After serotyping, we generally perform a PCR assay to identify *E. coli*. However, when O serotyping is not possible, it is difficult to decide in which category an isolated strain belongs. When large-scale gastrointestinal illness occurs, a serotype OUT strain is easily overlooked as a pathogen. In this outbreak, we thought these isolates belonged to a conventional category (EPEC, ETEC, EIEC, and EHEC); in particular, EAggEC, which belongs to a new category, was not considered to be a pathogenic bacterium. EAggEC appears to be particularly important as a cause of persistent diarrhea in children in developing countries (1). EAggEC is found widely in South America, Southeast Asia, and India (3, 4, 12). Though the source and the pathogen were not officially identified (7), our data provide suggestive evidence that EAggEC serotype OUT:H10 was associated with this massive outbreak of gastrointestinal illness. The epidemiologic significance and relative importance of EAggEC as a diarrheal pathogen and public health burden has still not been established. As EAggEC is a new category of diarrheagenic *E. coli*, reports of gastrointestinal illness caused by EAggEC have been few in Japan. Further studies should be conducted in order to elucidate the significance of the virulence factor of EAggEC for humans.

REFERENCES

1. **Baudry, B., S. J. Savarino, P. Vial, J. B. Kaper, and M. M. Levine.** 1990. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J. Infect. Dis. **161:**1249–1251.
2. **Bhan, M. K., P. Raj, M. M. Levine, J. B. Kaper, N. Bhandari, R. Surivastava, R. Kumar, and S. Sazawal.** 1989. Aggregative *Escherichia coli* associated with diarrhea in a cohort of rural children in India. J. Infect. Dis. **159:**1061–1064.
3. **Bhan, M. K., V. Khoshoo, H. Sommerfelt, R. Pushker, S. Sazawal, and R. Srivastava.** 1989. Enteroaggregative *Escherichia coli* and *Salmonella* associated with nondysenteric persistent diarrhea. Pediatr. Infect. Dis. J. **8:**499–502.
4. **Carvioto, A., A. Tello, A. A. Navarro, J. Ruiz, H. Villafan, F. Uribe, and C. Eslava.** 1991. Association of *Escherichia coli* HEp-2 adherence patterns with type and duration of diarrhoea. Lancet **337:**262–264.
5. **Cravioto, A., R. J. Gross, S. Scotland, and B. Rowe.** 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional enteropathogenic serotypes. Curr. Microbiol. **3:**95–99.
6. **Evans, D. J., Jr., D. G. Evans, and S. L. Gorbach.** 1973. Production of vascular permeability factor by enterotoxigenic *Escherichia coli* isolated from man. Infect. Immun. **8:**725–730.
7. **Food Sanitation Division, Environmental Health Bureau, Ministry of Health and Welfare.** 1993. Case reports of food poisoning in Japan 1993, p. 120. Ministry of Health and Welfare, Tokyo, Japan. (In Japanese.)
8. **Fumiaki, I., T. Ogino, K. Itoh, and H. Watanabe.** 1992. Differentiation and detection of pathogenic determinants among diarrheagenic *Escherichia coli* by polymerase chain reaction using mixed primers. Nippon-rinshyoh **50:**343–347.
9. **Gomes, T. A. T., P. A. Blake, and L. R. Trabulsi.** 1989. Prevalence of *Escherichia coli* strains with localized, diffuse, and aggregative adherence to HeLa cells in infants with diarrhea and matched controls. J. Clin. Microbiol. **27:**266–269.
10. **Japan Society of Chemotherapy.** 1981. Method for minimal inhibitory concentration (MIC) determination of antimicrobial agents by the agar dilution technique. Committee report. Chemotherapy (Tokyo) **29:**76–79.
11. **Kado, C. I., and S.-T. Liu.** 1981. Rapid procedure for the detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
12. **Levine, M. M., V. Prado, R. Robins-Browne, H. Lior, J. B. Kaper, K. Gicquelais, J. P. Nataro, P. Vial, and B. Tall.** 1988. Use of DNA probe and HEp-2 cell adherence assay to detect diarrheagenic *Escherichia coli*. J. Infect. Dis. **158:**224–228.
13. **Levine, M. M.** 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis. **155:**377–389.
14. **Nataro, J. P., J. B. Kaper, R. Robins Browne, V. Prado, P. Vial, and M. M. Levine.** 1987. Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr. Infect. Dis. J. **6:**829–831.
15. **Nataro, J. P., Y. Deng, D. R. Maneval, A. L. German, W. C. Martin, and M. M. Levine.** 1992. Aggregative adherence fimbriae I of enteroaggregative *Escherichia coli* mediate adherence to HEp-2 cells and hemagglutination of human erythrocytes. Infect. Immun. **60:**2297–2304.
16. **Savarino, S. J., A. Fasano, D. C. Robertson, and M. M. Levine.** 1991. Enteroaggregative *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. J. Clin. Invest. **87:**1450–1455.
17. **Savarino, S. J., P. Fox, D. Yikang, and J. P. Nataro.** 1994. Identification and characterization of a gene cluster mediating enteroaggregative *Escherichia coli* aggregative adherence fimbria I biogenesis. J. Bacteriol. **176:**4949–4957.
18. **Savarino, S. J., A. Fasano, J. Watson, B. M. Martin, M. M. Levine, S. Guandalini, and P. Guerry.** 1993. Enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 represents another subfamily of *E. coli* heat-stable toxin. Proc. Natl. Acad. Sci. USA **90:**3093–3097.
19. **Schmidt, H., C. Knop, S. Franke, S. Aleksic, J. Heesemann, and H. Karch.** 1995. Development of PCR for screening of enteroaggregative *Escherichia coli*. J. Clin. Microbiol. **33:**701–705.
20. **Scotland, S. M., H. R. Smith, B. Said, G. A. Willshaw, T. Cheasty, and B.**

Rowe. 1991. Identification of enteropathogenic *Escherichia coli* isolated in Britain as enteroaggregative or as members of a subclass of attaching and effacing *E. coli* not hybridising with the EPEC adherence factor probe. J. Med. Microbiol. **35:**278–283.
21. **Smith, H. R., S. M. Scotland, G. A. Willshaw, B. Rowe, A. Cravioto, and C. Eslava.** 1994. Isolates of *Escherichia coli* O44:H18 of diverse origin are enteroaggregative. J. Infect. Dis. **170:**1610–1613.
22. **Vial, P. A., R. Robins Browne, H. Lior, V. Prado, J. B. Kaper, J. P. Nataro, D. Maneval, A. Elsayed, and M. M. Levine.** 1988. Characterization of enteroadherent-aggregative *Escherichia coli*, a putative agent of diarrheal disease. J. Infect. Dis. **158:**70–79.
23. **Vial, P. A., J. J. Mathewson, H. L. Dupont, L. Guers, and M. M. Levine.** 1990. Comparison of two assay methods for patterns of adherence to HEp-2 cells of *Escherichia coli* from patients with diarrhea. J. Clin. Microbiol. **28:**882–885.
24. **Zepeda-Lopez, H. M., and G. M. Gonzalez-Lugo.** 1995. *Escherichia coli* adherence to HEp-2 cells with prefixed cells. J. Clin. Microbiol. **33:**1414–1417.