Applied and Environmental Microbiology

# Lineage-Specific Distribution of Insertion Sequence Excision Enhancer in Enterotoxigenic Escherichia coli Isolated from Swine

**Masahiro Kusumoto, Dai Fukamizu, Yoshitoshi Ogura, Eiji Yoshida, Fumiko Yamamoto, Taketoshi Iwata, Tadasuke Ooka, Masato Akiba and Tetsuya Hayashi**

***Appl. Environ. Microbiol.*** **2014, 80(4):1394. DOI: 10.1128/AEM.03696-13.**
**Published Ahead of Print 13 December 2013.**

AEM
Journals.ASM.org

# Lineage-Specific Distribution of Insertion Sequence Excision Enhancer in Enterotoxigenic *Escherichia coli* Isolated from Swine

**Masahiro Kusumoto,[a] Dai Fukamizu,[b] Yoshitoshi Ogura,[c,d] Eiji Yoshida,[e] Fumiko Yamamoto,[f] Taketoshi Iwata,[a] Tadasuke Ooka,[c] Masato Akiba,[a,g] Tetsuya Hayashi[c,d]**

Bacterial and Parasitic Disease Research Division, National Institute of Animal Health, Tsukuba, Ibaraki, Japan[a]; Fukuoka Chuo Livestock Hygiene Service Center, Fukuoka, Japan[b]; Division of Microbiology, Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Kiyotake, Miyazaki, Japan[c]; Division of Microbial Genomics, Department of Genomics and Bioenvironmental Science, Frontier Science Research Center, University of Miyazaki, Kiyotake, Miyazaki, Japan[d]; Animal Quarantine Service, Yokohama, Kanagawa, Japan[e]; Oita Livestock Hygiene Service Center, Oita, Japan[f]; Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Izumisano, Osaka, Japan[g]

**Insertion sequences (ISs) are the simplest transposable elements and are widely distributed in bacteria; however, they also play important roles in genome evolution. We recently identified a protein called IS excision enhancer (IEE) in enterohemorrhagic *Escherichia coli* (EHEC) O157. IEE promotes the excision of IS elements belonging to the IS*3* family, such as IS*629*, as well as several other families. IEE-mediated IS excision generates various genomic deletions that lead to the diversification of the bacterial genome. IEE has been found in a broad range of bacterial species; however, among sequenced *E. coli* strains, IEE is primarily found in EHEC isolates. In this study, we investigated non-EHEC pathogenic *E. coli* strains isolated from domestic animals and found that IEE is distributed in specific lineages of enterotoxigenic *E. coli* (ETEC) strains of serotypes O139 or O149 isolated from swine. The *iee* gene is located within integrative elements that are similar to SpLE1 of EHEC O157. All *iee*-positive ETEC lineages also contained multiple copies of IS*629*, a preferred substrate of IEE, and their genomic locations varied significantly between strains, as observed in O157. These data suggest that IEE may have been transferred among EHEC and ETEC in swine via SpLE1 or SpLE1-like integrative elements. In addition, IS*629* is actively moving in the ETEC O139 and O149 genomes and, as in EHEC O157, is promoting the diversification of these genomes in combination with IEE.**

Insertion sequence (IS) elements are the simplest transposable elements and are considered selfish (or parasitic) genetic elements. However, they also play important roles in genome evolution (1). The transposition and proliferation of IS elements induces not only insertional gene inactivation and the modification of gene expression (1) but also a variety of genomic rearrangements, such as deletions, inversions, and duplications (2, 3). In bacteria, several thousand types of IS elements have been identified from various species and strains (4) and classified into approximately 20 families (5).

IS-mediated bacterial genome diversification has been extensively studied in enterohemorrhagic *Escherichia coli* (EHEC) O157. EHEC O157 strains produce highly potent cytotoxins (Shiga toxins Stx1 and/or Stx2) and causes diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome; thus, it is one of the most serious food-borne infections worldwide (6). O157 strains contain many IS elements, and these elements play important roles in the diversification of the O157 genome. For example, the O157 strain RIMD0509952 (referred to as O157 Sakai) contains 25 types of IS elements (116 copies in total), and the most abundant is an IS*3* family member, IS*629* (23 copies) (7, 8). Our comparative genomic analysis of O157 clinical isolates revealed that many small structural polymorphisms associated with gene inactivation and/or deletion have been generated by IS*629* (9). More recently, we identified a novel protein called IS excision enhancer (IEE), which promotes IS*629* excision from the O157 genome in a transposase (TPase)-dependent manner. We demonstrated that various types of genomic deletions were generated upon IEE-mediated IS excision in IS-flanking regions (10). IEE also promotes the excision of other members of the IS*3*, IS*1*, and IS*30* families.

In the O157 genome, the gene encoding IEE (*iee*) is located in a large integrative element (IE) called SpLE1 (7). In non-O157 EHEC strains, *iee* is located on SpLE1-like IEs (11, 12). IEE homologs have been identified in a broad range of bacterial species and are encoded in genomic regions exhibiting low GC content and/or containing genes related to mobile genetic elements (MGEs) (10). These results suggest that IEE and its homologs have spread to a variety of bacterial strains by horizontal gene transfer. Although many *E. coli* strains have been sequenced, IEE is found primarily in EHEC isolates (10).

Pathogenic *E. coli* strains other than EHEC are also important etiological agents of zoonotic or food-borne disease in humans and of colibacillosis in domestic animals (13, 14). Enterotoxigenic *E. coli* (ETEC) is an important cause of diarrhea in children, which is associated with high morbidity and mortality in nonindustrialized countries. ETEC is the main cause of diarrhea in travelers to these countries (13). In swine, ETEC infections immediately after birth (neonatal diarrhea) and ETEC or Shiga toxin-producing *E. coli* (STEC) infections after weaning (postweaning diarrhea or edema disease) are responsible for significant economic losses due to diarrhea, growth retardation, and mortality (15, 16).

In this study, we examined the prevalence of *iee* in non-EHEC

Received 10 November 2013 Accepted 7 December 2013
Published ahead of print 13 December 2013
Address correspondence to Masahiro Kusumoto, kusu555@affrc.go.jp.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.03696-13.

doi:10.1128/AEM.03696-13

TABLE 1 Prevalence of the *iee* gene in *E. coli* isolated from domestic animals

| Serotype | No. of isolates (ETEC strains) | | | |
|---|---|---|---|---|
| | Carrier animal | | | *iee* gene positive |
| | Swine | Chicken[a] | Other(s) | |
| O2 | 11 (0) | 0 | 0 | 0 |
| O7 | 0 | 1 (0) | 0 | 0 |
| O8 | 5 (4) | 2 (0) | 0 | 0 |
| O10 | 0 | 4 (0) | 0 | 0 |
| O15 | 0 | 1 (0) | 0 | 0 |
| O16 | 1 (1) | 0 | 0 | 0 |
| O17 | 0 | 1 (0) | 0 | 0 |
| O18 | 0 | 1 (0) | 0 | 0 |
| O19 | 0 | 7 (0) | 0 | 0 |
| O25 | 0 | 1 (0) | 0 | 0 |
| O35 | 1 (1) | 0 | 0 | 0 |
| O39 | 0 | 3 (0) | 0 | 0 |
| O45 | 2 (1) | 2 (0) | 0 | 0 |
| O56 | 5 (4) | 0 | 0 | 0 |
| O68 | 0 | 1 (0) | 0 | 0 |
| O76 | 0 | 0 | 3[b] (0) | 0 |
| O84 | 0 | 1 (0) | 0 | 0 |
| O98 | 5 (5) | 0 | 0 | 0 |
| O103 | 1 (0) | 0 | 0 | 0 |
| O115 | 1 (0) | 0 | 0 | 0 |
| O116 | 15 (15) | 0 | 0 | 0 |
| O119 | 0 | 5 (0) | 0 | 0 |
| O121 | 2 (1) | 0 | 0 | 0 |
| O123 | 0 | 1 (0) | 0 | 0 |
| O132 | 1 (0) | 0 | 0 | 0 |
| O135 | 0 | 2 (0) | 0 | 0 |
| O138 | 6 (6) | 0 | 0 | 0 |
| O139 | 56 (23) | 0 | 0 | 23 (23) |
| O141 | 4 (2) | 0 | 0 | 0 |
| O149 | 17 (17) | 0 | 0 | 16 (16) |
| O157 | 2 (2) | 0 | 0 | 0 |
| O161 | 0 | 1 (0) | 0 | 0 |
| O164 | 3 (3) | 0 | 0 | 0 |
| O165 | 0 | 0 | 1[c] (0) | 0 |
| O167 | 0 | 1 (0) | 0 | 0 |
| O169 | 1 (0) | 0 | 0 | 0 |
| Untypeable or not tested | 19 (2) | 59 (0) | 0 | 0 |
| Total | 158 (87) | 94 (0) | 4 (0) | 39 (39) |

[a] These strains were all isolated from internal organs of chickens with extraintestinal diseases.
[b] These three strains were isolated from goats.
[c] This strain was isolated from cattle.

pathogenic *E. coli* strains isolated from domestic animals. Because the result indicated that the *iee* gene is distributed in specific lineages of ETEC isolated from swine, we further investigated the genomic structures of *iee*-containing IEs and the prevalence of IS elements that could be substrates for IEE in these ETEC strains.

## MATERIALS AND METHODS

**Bacterial strains and culture conditions.** We investigated 256 *E. coli* strains, all of which were isolated from diseased domestic animals in Japan between 1991 and 2010 (Table 1). The serotypes of the strains were determined using antisera obtained from Denka Seiken Co., Ltd. (Tokyo, Japan), or Statens Serum Institut (Copenhagen, Denmark). O157 Sakai and three sequenced non-O157 EHEC strains (O26:H11 strain 11368, O111:H− strain 11128, and O103:H2 strain 12009; all three were isolated from patients in Japan [11]) were also used. All strains were grown in Luria-Bertani (LB) broth (17) at 37°C.

**PCR-based genotyping of *E. coli* strains.** The presence of *iee* and a panel of major IS elements (IS*629*, IS*911*, IS*3*, IS*2*, IS*1*, IS*4*, IS*5*, IS*26*, IS*30*, and IS*621*) in the *E. coli* strains was examined by PCR using the primers listed in Table S1 in the supplemental material. The template DNA for PCR was prepared by the alkaline-boiling method, as previously described (18). PCR was performed in a 50-μl reaction mixture containing template DNA, 0.2 μM concentrations of each primer, 0.2 mM concentrations of each deoxynucleoside triphosphate (dNTP), PCR buffer, and 1.25 U of ExTaq DNA polymerase (TaKaRa Bio, Inc., Shiga, Japan) using 30 amplification cycles of 30 s at 95°C, 30 s at 60°C, and 1 min at 72°C. PCR amplification of genes encoding various virulence factors (VFs; LT, STa, STb, EAST1, Stx1, Stx2, F4, F5, and F18) was performed as described by Vu-Khac et al. (19).

**PFGE.** Pulsed-field gel electrophoresis (PFGE) was performed by clamped homogeneous electric field electrophoresis using a CHEF-DR III apparatus (Bio-Rad Laboratories, Inc., Hercules, CA). The genomic DNA of each strain was prepared as described by Akiba et al. (20). Genomic DNA in sliced plugs was digested at 37°C with 40 U of XbaI for 6 h or 30 U of SspI for 16 h (both enzymes were obtained from TaKaRa Bio, Inc.). Electrophoresis was performed in a 1% agarose gel in 0.5× Tris-borate-EDTA (TBE) buffer at 14°C at 6 V/cm for 22 h with a pulse time of 5 to 50 s (XbaI PFGE) or for 10 h with a pulse time of 4 s (SspI PFGE). For PFGE of XbaI-digested DNA, 100 μM thiourea was added to the TBE buffer to obtain clear banding patterns (21). Southern blot hybridization analysis of SspI-digested DNA was performed using an IS*629*-specific probe, as previously described (10).

The banding patterns obtained by PFGE of XbaI-digested DNA were analyzed using BioNumerics software version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium), followed by manual band assignment. Dendrograms were then generated using the unweighted pair group method with arithmetic mean (UPGMA) based on the Dice similarity index and with an optimization parameter of 1% band position tolerance.

**MLST and phylogenetic tree construction.** Multilocus sequence typing (MLST) was performed using the nucleotide sequences of the seven housekeeping genes *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* according to the protocols available in the *E. coli* MLST database (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli) (22). To determine the phylogenetic relationships of the *E. coli* strains, we concatenated the nucleotide sequences of the seven genes used for the MLST to generate pseudosequences and aligned them using CLUSTAL W in the software MEGA5 (23). A neighbor-joining tree was generated with a 1,000 bootstrap replicates.

**Analysis of the genomic structures of *iee*-containing elements.** The genomic structures of the SpLE1-like elements of the O139 and O149 strains were analyzed by PCR scanning, which is a long-range PCR-based genome comparison system (24). The primers and PCR conditions were identical to those previously described by Ohnishi et al. for whole-genome PCR scanning (WGPS) of O157 strains (24), except that the primer ieeIE-f was used instead of primer 113.3-f. To analyze the region of interest in strain E0231, we used the newly designed primer 113.9-r2 instead of 113.9-r because no amplicon was produced when the primer pair 113.8-f/113.9-r was used with this strain. In addition, to amplify both SpLE1-like IE/chromosome junctions in E0231, we designed primers IE0231-f and IE0231-r based on the results of random extension-based two-step PCR (RETS-PCR, a newly developed walking method described below). The sequences for the primers used in this analysis are shown in Table S2 in the supplemental material.

**RETS-PCR.** We developed a rapid walking method designated RETS-PCR to determine the sequences of the SpLE1-like IE/chromosome junctions in strain E0231. As outlined in Fig. 1, the RETS-PCR-based method comprises three steps: random extension, PCR using a single primer, and sequence determination (Fig. 1 presents the process used to analyze the left junction). In the first step, primer E0231J-R1, which comprises a

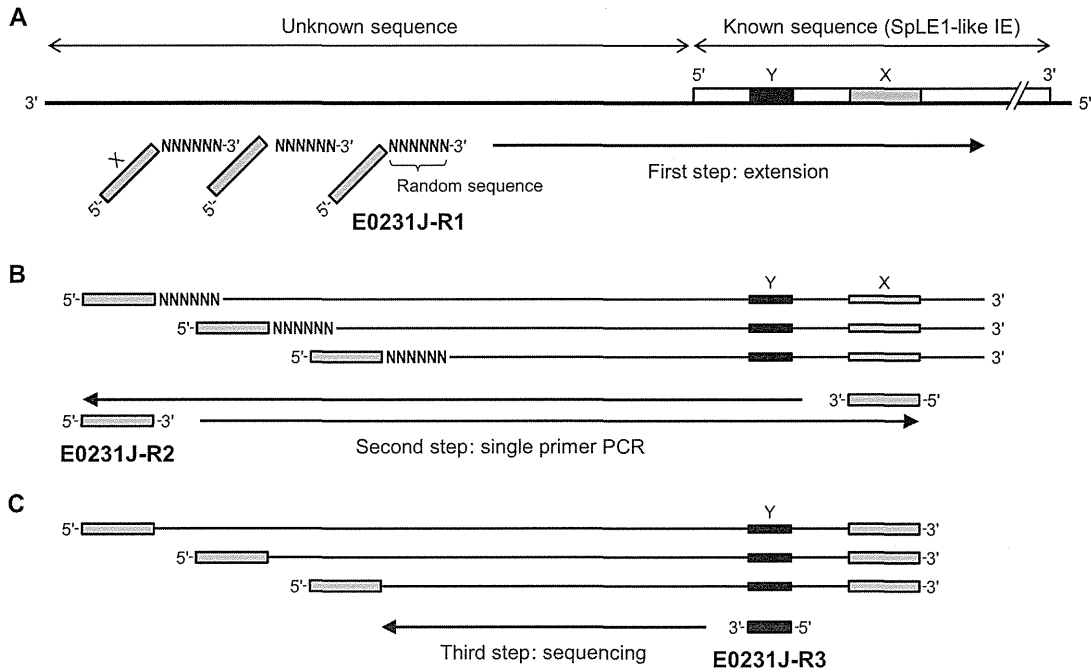


**FIG 1** RETS-PCR and the process used to determine unknown SpLE1-like IE/chromosome junctions. As an example, the process to determine the left SpLE1-like IE/chromosome junction in strain E0231 is presented. (A) In the first step, various ssDNA molecules are generated by random extension using primer E0231J-R1, which comprises a known sequence in the SpLE1-like IE (denoted as "X") and a random 9-base sequence. (B) In the second step, the ssDNA molecules act as templates for single-primer PCR (using primer E0231J-R2, which anneals only to sequence X) to amplify the DNA segments encompassing the junction between the known and unknown sequences. (C) In the third step, the sequences of the amplicons are determined using primer E0231J-R3, which aligns to a region located between the junction and X (denoted Y).

known sequence in the SpLE1-like IE (denoted as "X" in Fig. 1) and a random 9-base sequence in the 3′ region, was used to generate various single-stranded DNA (ssDNA) molecules by random extension using DNA polymerase (Fig. 1A). This step was performed in a 25-μl reaction mixture containing template DNA, 0.2 μM primer E0231J-R1, 0.2 mM concentrations of each dNTP, PCR buffer, and 0.625 U ExTaq DNA polymerase for 5 min at 95°C, 30 s at 30°C, and 1 min at 72°C. In the second step (Fig. 1B), the ssDNA molecules act as templates for single-primer PCR using primer E0231J-R2, and thus only molecules containing sequence X permit the amplification of DNA segments encompassing the junction. This step was performed in a 100-μl reaction mixture containing the total product of the first step, 0.4 μM primer E0231J-R2, 0.2 mM concentrations of each dNTP, PCR buffer, and 2.5 U of ExTaq DNA polymerase with 35 amplification cycles of 30 s at 95°C, 30 s at 60°C, and 1 min at 72°C. In the third step (Fig. 1C), the sequences of the amplicons were determined using the primer E0231J-R3, the sequence of which corresponds to an upstream region of X (denoted "Y" in Fig. 1C). The nucleotide sequences were identified by the dideoxy chain termination method (25) using a BigDye terminator cycle sequencing kit and an 3130xl sequencer (Applied Biosystems, Inc., Foster City, CA) according to the manufacturer's instructions. Similarly, the right junction was determined using the primers E0231J-F1, -F2, and -F3. The sequences for the primers used in this analysis are shown in Table S3 in the supplemental material.

**Sequence determination and genomic comparison of SpLE1-like IEs.** Amplicons covering the entire SpLE1-like IE of each strain, which were generated by PCR scanning as described above, were combined and sequenced using the Illumina MiSeq platform (Illumina, Inc., San Diego, CA). Libraries for each mixture were prepared using the Nextera XT DNA Sample Prep kit (Illumina, Inc.), and pooled libraries were subjected to multiplexed paired-end sequencing (251 cycles × 2) according to the manufacturer's protocol. The sequence reads were assembled using Velvet version 1.2.05 (26). The obtained sequences were annotated with the Microbial Genome Annotation Pipeline (http://www.migap.org/) (27) and were manually curated using IMC-GE software (In Silico Biology, Inc., Kanagawa, Japan). The sequence comparison of SpLE1-like IEs was performed using GenomeMatcher software (28).

**Nucleotide sequence accession numbers.** The sequences of the SpLE1-like IEs from strains E0046, E0092, E0124, E0217, E0223, and E0231 have been deposited in DDBJ/EMBL/GenBank under accession numbers AB786874 to AB786879.

## RESULTS AND DISCUSSION

**Screening, genotyping, and phylogenetic analysis of *iee*-positive *E. coli* strains.** We first determined the serotypes of 256 *E. coli* strains isolated from diseased domestic animals and identified *iee*-positive strains by PCR; *iee* was found only in strains of serotypes O139 (23 of 56 strains) and O149 (16 of 17 strains) isolated from swine (Table 1 and Fig. 2). O139 and O149 are major serotypes of ETEC that are associated with diarrhea in swine, and O139 is also one of the major serotypes of STEC that causes edema disease (15).

The 73 O139 and O149 strains were further examined by PCR for the presence of major IS elements of "pathogenic" *E. coli* and for genes encoding known VFs of ETEC and STEC (Fig. 2). PFGE analysis was also performed after digesting the genomic DNA with XbaI, and a dendrogram was generated to analyze the relatedness of these strains (Fig. 2). In addition, the sequence types (STs) of these strains were determined using the *E. coli* MLST database (22). The results of the clustering analysis of the 73 strains based on their XbaI digestion patterns correlated very well with the clustering based on the MLST analysis, and the strains were divided into three groups on the basis of their serotypes and VF profiles:

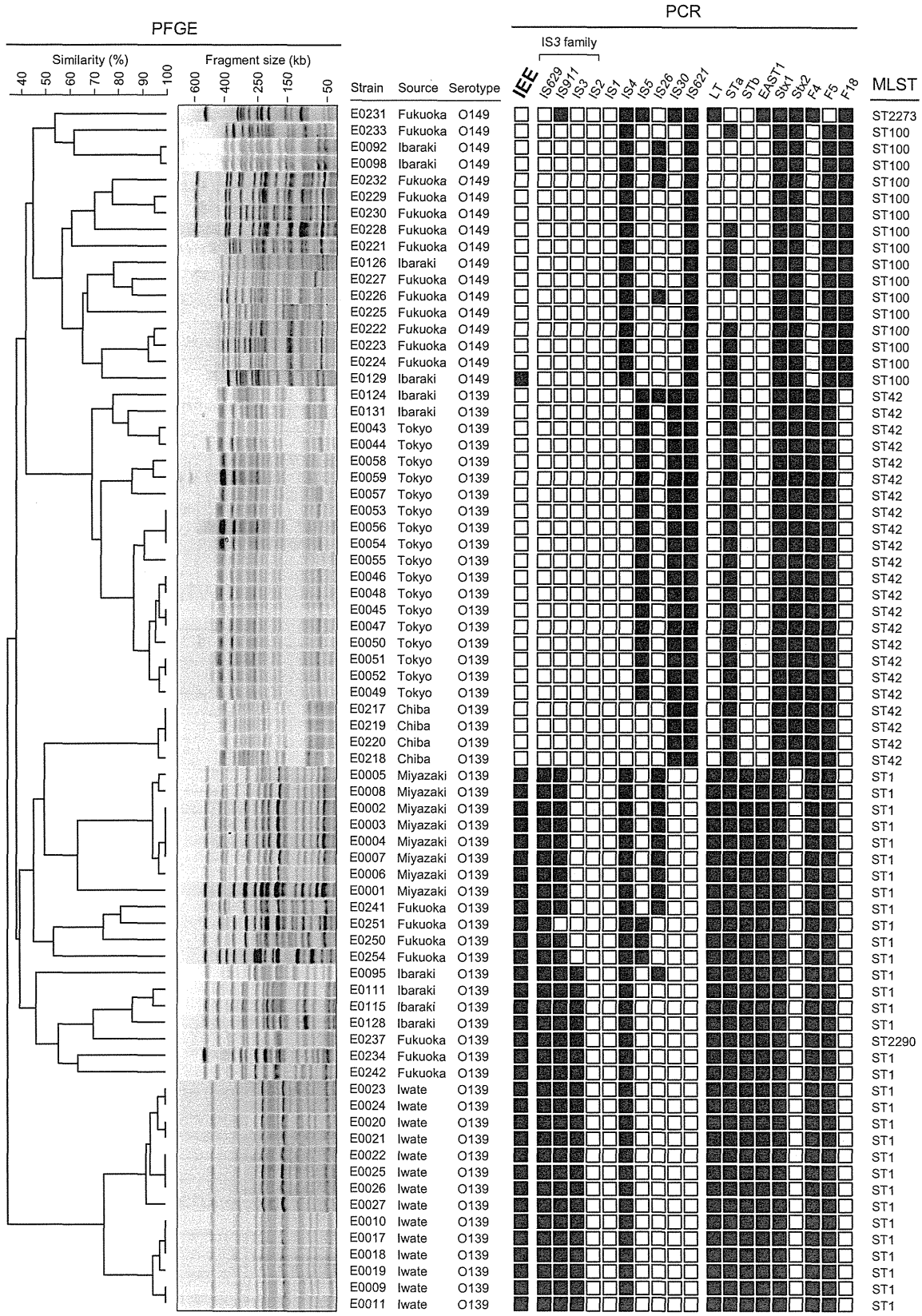


**FIG 2** Genotyping and phylogenetic analysis of *E. coli* strains. A dendrogram obtained by PFGE of XbaI-digested DNA from 73 *E. coli* O139 and O149 strains is shown on the left side. Information about each strain, the results of the PCR screening for genes encoding IEEs, IS elements, and VFs, and their STs as determined by MLST are aligned with the dendrogram. IS*629*, IS*911*, IS*3*, and IS*2* are all members of the IS*3* family. The presence or absence of each gene (or IS element) is indicated by an open or black square, respectively.

ETEC O149 (ST100 or ST2273), ETEC O139 (ST42), and STEC O139 (ST1 or ST2290). ST2290 is closely related to ST1 and contains only one single-nucleotide polymorphism.

Among the 17 ETEC O149 strains, only strain E0129 lacked *iee*. However, E0129 belongs to ST100 (a member of the ST165 clonal complex), like most of the *iee*-positive ETEC O149 strains, and its genotype was also very similar to those of the *iee*-positive strains (Fig. 2). All ETEC O139 strains possessed *iee*, but this gene was not found in any of the STEC O139 strains. Interestingly, the distribution of *iee* among the O149 and O139 strains correlated very well with the distribution of two IS*3* family members, IS*629* and IS*911* (Fig. 2). Although we identified ETEC strains with various serotypes isolated from swine (Table 1), the *iee*-positive strains were limited to strains of the O139 and O149 serotypes.

To determine the phylogenetic relationships of the ETEC O139 and O149 strains, we constructed a phylogenetic tree using the concatenated nucleotide sequences of the seven housekeeping genes used for MLST. In total, 50 genome-sequenced *E. coli* strains were included in this analysis (Fig. 3). Two of the 50 strains were the recently sequenced ETEC strains UMNK88 and UMNF18 (serotypes O149 and O147, respectively) (29). UMNK88 belongs to ST100, but UMNF18 belongs to ST10, which includes *E. coli* K-12. As shown in Fig. 3, the ETEC O139 (ST42) and STEC O139 (ST1 and ST2290) strains are relatively closely related to each other but distantly related to the two ETEC O149 lineages (ST100 or ST2273), which are located in distinct phylogenetic clusters. ST100 belongs to a cluster that also contains ST10. ST2273 belongs to a cluster that contains EHEC O26, O111, and O103 but is more closely related to O26 and O111 than to O103. These results indicate that IEE has spread to specific lineages of ETEC and EHEC strains by horizontal gene transfer.

**Analysis of *iee*-containing integrated elements.** The *iee* gene was found on the IE "UMNK88 island 8" in the O149 strain UMNK88 (29); this IE is similar to SpLE1 and the SpLE1-like IEs of EHEC, suggesting that *iee* may be encoded by SpLE1-like IEs in the *iee*-positive ETEC strains identified in the present study. To investigate the presence of SpLE1-like IEs and analyze their genomic structures in the O139 and O149 ETEC strains, we performed PCR scanning analysis as illustrated in Fig. S1A in the supplemental material. The results indicated that these strains contain SpLE1-like IEs, with the exception of strain E0231 (see Fig. S1B in the supplemental material; see also Fig. S2 in the supplemental material for the raw data). In E0231, no amplicons were obtained from the two segments containing the left and right SpLE1-like IE/chromosome junctions (ieeIE-f/113.4-r and 113.9-f/114-r). In addition, no amplicon was generated using the primers 113.8-f/113.9-r in E0231, but this region was amplified using a newly designed primer, 113.9-r2, as a substitute for 113.9-r (see Fig. S2B in the supplemental material), suggesting that some sequence polymorphism exists in the 113.9-r site of the E0231 genome. By sequencing the ieeIE-f/113.4-r amplicons, we confirmed that the amplified regions all contained the *iee* genes having an identical sequence. Importantly, no amplicon was obtained from any segments of any of the *iee*-negative O139 STEC strains examined. These results indicate that the O139 ETEC and O149 ETEC lineages acquired *iee* by SpLE1-like IEs.

The sequencing analysis of the ieeIE-f/113.4-r and 113.9-f/114-r amplicons revealed that SpLE1-like IEs are integrated in the *serX* tRNA gene in all O139 and O149 ETEC strains examined, with the exception of strain E0231 (see Fig. S2B in the supplemen-

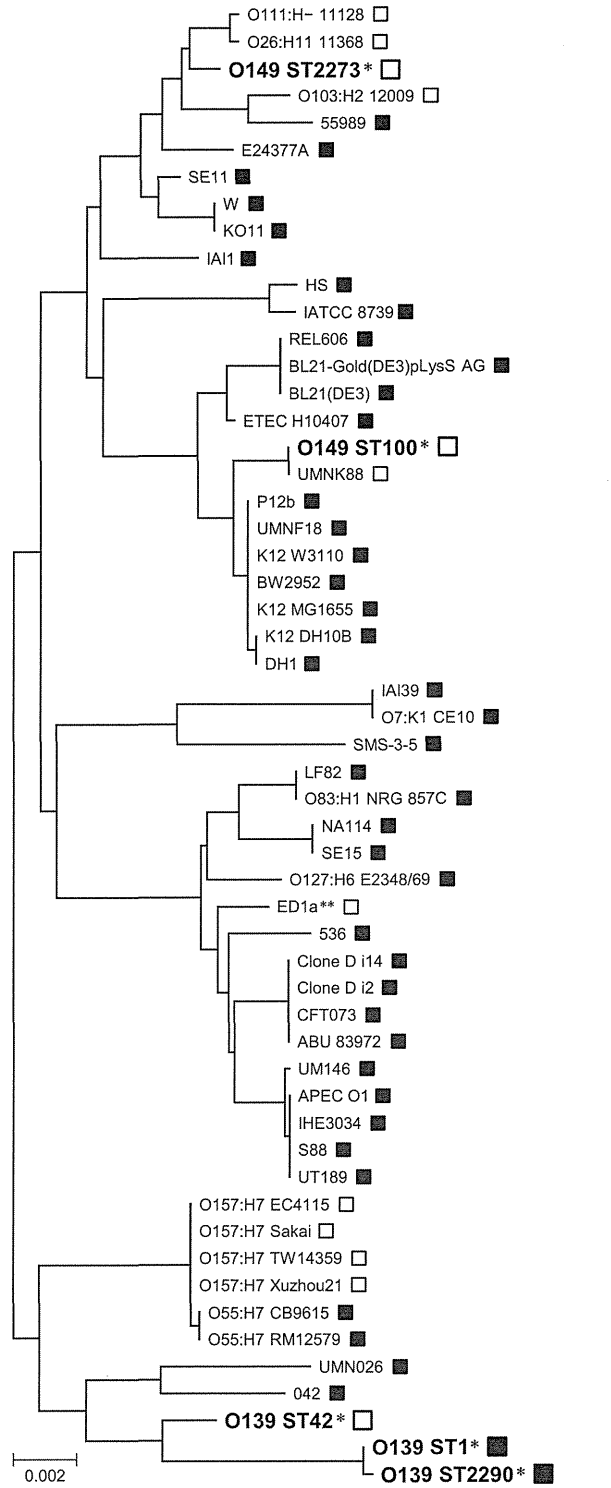


FIG 3 Phylogenetic tree of *E. coli* strains based on the sequences of seven housekeeping genes. The sequences of the seven housekeeping genes obtained in the MLST analysis were concatenated and aligned using CLUSTAL W in MEGA5 software (23), and a neighbor-joining tree was generated with 1,000 bootstrap replicates. All genome-sequenced *E. coli* strains are included in the phylogenetic representation, and the O139 and O149 lineages analyzed in the present study are indicated by a single asterisk (*). The scale bar represents the number of base substitutions. Open and black boxes indicate *iee*-positive and -negative strains, respectively. The ED1a strain marked with double asterisks (**) possesses *iee* on an IE that is not similar to SpLE1 (10).

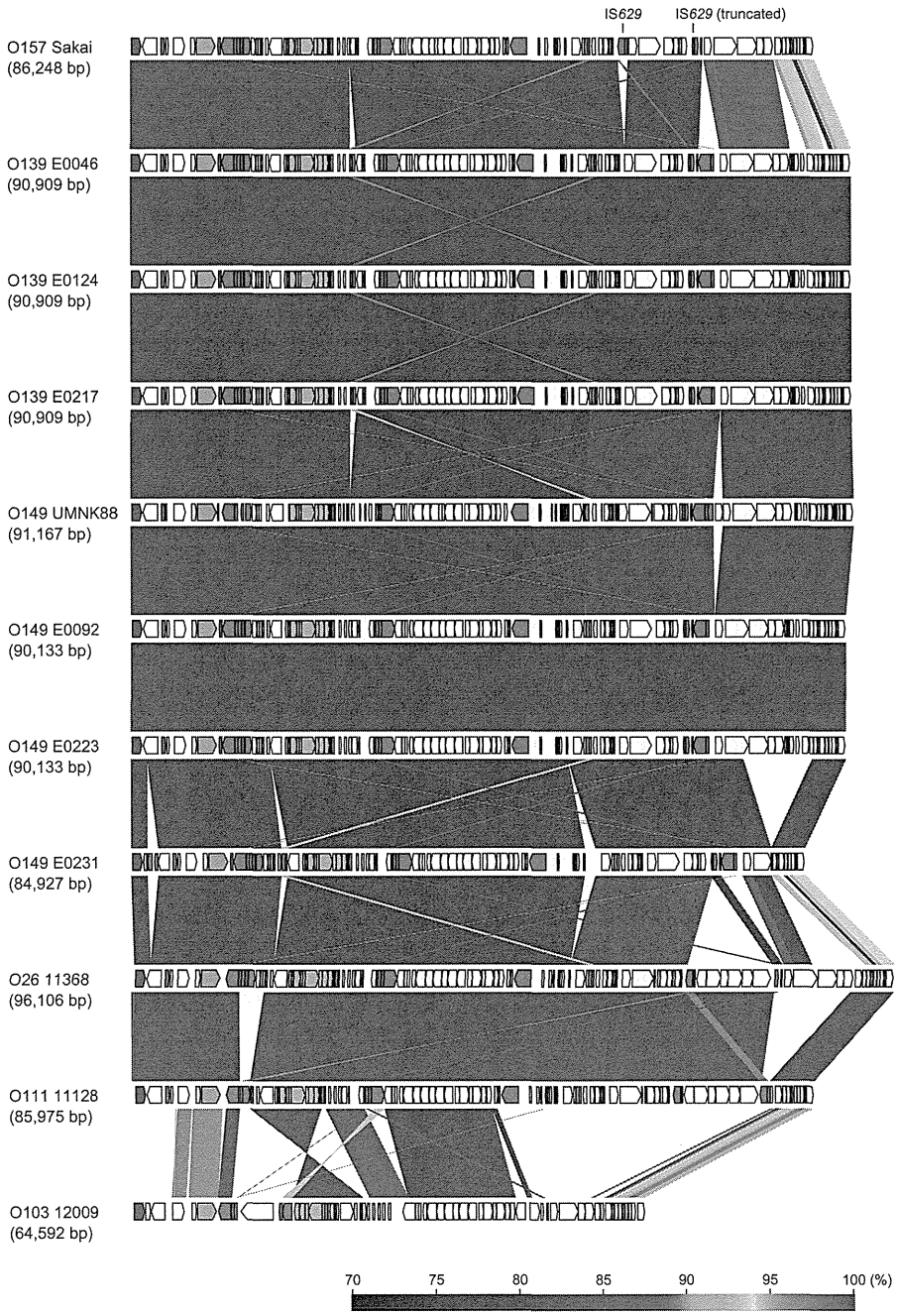


**FIG 4** Genomic comparison of the SpLE1-like IEs of the ETEC strains with the SpLE1 of EHEC O157 and the SpLE1-like IEs of non-O157 EHEC. The genes in each element are indicated by arrows; orange, blue, red, dark green, light green, and open arrows indicate the genes for IEE, integrase, IS TPase, Iha, the urease operon, and other functions, respectively. The locations of two copies of IS*629* in SpLE1 (one is intact and the other is truncated) are also indicated. The nucleotide sequence identities between the elements (cutoff ≥ 70% identity) are indicated by color shading according to the scale shown at the bottom of the figure.

tal material). The integration site of the SpLE1-like IE of E0231 (the *serW* tRNA gene) was identified using a newly developed rapid walking method designated RETS-PCR, as outlined in Fig. 1. Similar positional variations of SpLE1 were observed in EHEC O157; strain EDL933 contains two copies of SpLE1: one at *serX* and the other at *serW* (30). In the eight O157 strains that were analyzed by Ohnishi et al. using WGPS, SpLE1 was also found at the *serX* and/or *serW* loci (24). However, this variation in the integration site is not surprising because the *serX* and *serW* genes have identical nucleotide sequences and because a highly conserved phage-type integrase is shared by SpLE1 and SpLE1-like IEs.

In strain E0129 (the only *iee*-negative O149 ETEC strain), the *serW* and *serX* loci were both intact and showed no sign of the insertion of MGEs, as in the *iee*-negative O139 STEC strains. Because E0129 also belongs to ST100, as do most of the O149 ETEC strains, it is likely that this element has been deleted in this strain.

**Comparative analysis of SpLE1-like IEs.** The nucleotide sequences of SpLE1-like IEs from three ETEC O139 strains (E0046, E0124, and E0217) and three O149 strains (E0092, E0223, and E0231) were determined and compared to those of SpLE1 in O157 Sakai; SpLE1-like IEs in O26, O111, and O103 EHECs (strains 11368, 11128, and 12009, respectively); and island 8 in O149 UMNK88. Overall, the structures of the SpLE1-like IEs in the seven O139 and O149 ETEC strains were very similar to each other, whereas the deletion of an 8.7-kb segment was observed in the element of strain E0231 (Fig. 4). Although the elements of O139/O149 ETEC are also similar to SpLE1 and the SpLE1-like IEs of the O26 and O111 EHEC strains, their right end regions, particularly the far-right region have significantly diverged in sequence from those of the elements of O157, O26, and O111 (Fig. 4; see also Fig. S3 in the supplemental material). All other structural variations observed between the elements of EHEC strains and those of O139/O149 ETEC were small, and most appeared to have been generated by the insertion (or deletion) of IS elements. Considering that the three *iee*-positive ETEC O139 and O149 lineages are phylogenetically distant from each other and that the ST2273 lineage (O149 strain E0231) is closely related to O26 and O111 (Fig. 3), these findings suggest that the SpLE1-like IEs of these O139/O149 strains have been recently derived from an ancestor common to the EHEC elements and jumped into the three ETEC O139 and O149 lineages.

Yin et al. reported that the *iha* gene (which encodes a putative adhesin, Iha) and the *ure* operon (which encodes urease), both of which are encoded within SpLE1, are required for the efficient colonization of EHEC O157 strain EDL933 (the IE corresponding to SpLE1 is named "O island 48" in EDL933 [30]) in swine intestines (31). Because the *iha* gene and the *ure* operon are conserved in the SpLE1-like IEs of ETEC O139 and O149 (Fig. 4), the acquisition of SpLE1-like IEs may confer an advantage to these ETEC strains in colonizing the swine intestine. Although healthy cattle are considered the major reservoir for human infection with EHEC O157 (6), this microorganism has also been isolated from swine (32–35). Thus, although further studies are required, EHEC O157 and ETEC O139 and O149 may share an ecological niche (the swine intestine), thus allowing the transfer of SpLE1 or SpLE1-like IEs among these strains. Because genes required for conjugal transfer are not found in these IEs, a molecular mechanism underlying their transmission is another important issue to be elucidated.

**IS*629* in ETEC O139 and O149 strains and variation in its genomic copy number and insertion sites.** IEE promotes the excision of IS*629* and other IS*3* family members in a TPase-dependent manner. It also induces various genomic deletions upon IS excision and is thus implicated in the diversification of bacterial genomes (10). The genomic locations of IS*629* in the O157 genomes are highly variable and show complex patterns among the O157 strains (9). Because all *iee*-positive ETEC O139 and O149 strains also contain IS*629*, we investigated the genomic locations of IS*629* in these strains by PFGE analysis of SspI-digested genomic DNA, which was followed by Southern blot hybridization analysis using an IS*629*-specific probe (Fig. 5). This IS*629* fingerprinting analysis revealed that all *iee*-positive O139 and O149 strains possess multiple copies of IS*629*, a preferred substrate of IEE (10).

Intriguingly, SpLE1 of EHEC O157 carries two copies of IS*629* (one is intact, and the other is truncated), and all sequenced

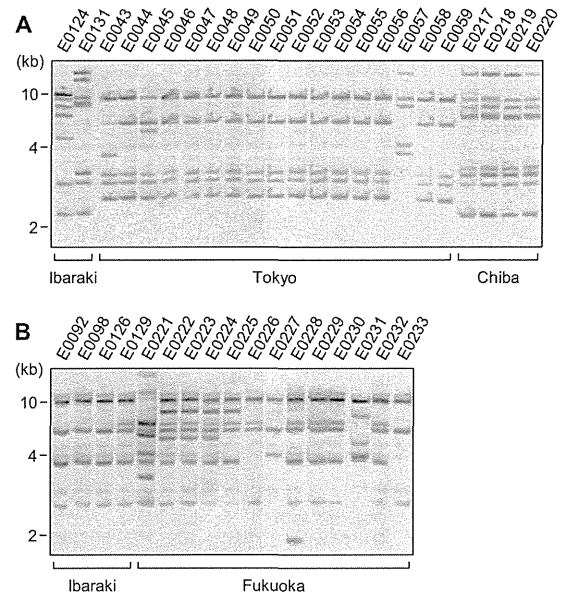


**FIG 5** Southern blot hybridization analysis of the ETEC O139 (A) and O149 (B) strains using an IS*629*-specific probe. SspI-digested genomic DNA was separated by PFGE and subjected to Southern blot hybridization analysis. The prefectures where the strains were isolated are indicated. The DNA probe was derived from the central part of the IS*629* sequence (nucleotide positions 308 to 607).

SpLE1-like IEs of the ETEC O139 and O149 strains also carry one truncated IS*629* copy. This result raises the possibility that these ETEC strains acquired at least one IS*629* copy, together with *iee*, via the transfer of an SpLE1-like IE, followed by the transposition and proliferation of IS*629* in these strains. We cannot exclude the possibility that these ETEC strains acquired IS*629* independently from SpLE1-like IEs, but the potential cotransfer of IEE and IS*629* merits further investigation.

The IS*629* fingerprinting patterns of the ETEC O139 and O149 strains also revealed considerable variation in copy numbers between these strains, as observed in EHEC O157. Although 17 O139 strains isolated in the Tokyo prefecture (E0043 to E0059) exhibited very similar XbaI digestion patterns in PFGE (Fig. 2), suggesting that they are closely related, some of the strains displayed IS*629* fingerprinting patterns that were distinct from the major pattern in this group (Fig. 5B). Similarly, O149 strains E0226 and E0227, which exhibited similar XbaI digestion patterns in PFGE, exhibited remarkable variation in their IS*629* fingerprinting patterns. Thus, it appears that IS*629* has actively transposed in these ETEC O139 and O149 strains, but it is also possible that some of the variation in the copy number of IS*629* is attributable to IEE-mediated IS excision, which could also have generated deletions in IS-flanking regions (10).

IEE promotes the excision of other IS*3* family members, such as IS*911*, IS*3*, and IS*2*, at the same efficiency as that for IS*629* (10). All of the IS elements are present in almost all *iee*-positive ETEC O139 and O149 lineages (Fig. 1). Although the excision frequencies of IS*1* and IS*30* is lower than that for the IS*3* family, these IS elements could also be substrates of IEE (10). IS*1* is distributed among all *iee*-positive ETEC O139 and O149 strains. IS*30* is found in one ETEC O149 lineage (ST100). Thus, although further research is required, the acquisition of these IS elements other than

IS*629* may have accelerated the genome diversification of ETEC O139 and O149, which in turn could have introduced important phenotypic variations in each lineage.

**Conclusions.** This study demonstrated that IEE is distributed specifically among three distinct ETEC lineages isolated from swine and is encoded by IEs similar to SpLE1 of EHEC O157. The SpLE1-like IEs are highly conserved in genomic structure among these ETEC lineages, and similar to SpLE1, they carry the *iha* gene and the *ure* operon, which are shown to be required for the efficient colonization of O157 in the swine intestine. These data suggest that IEE may have been transferred among EHEC and ETEC in swine via the acquisition of SpLE1-like IEs. Furthermore, because the IEE-positive ETEC lineages all contained multiple copies of IS*629*, a preferred substrate of IEE, and their genomic locations vary significantly between strains, IS*629* is likely actively moving on the ETEC genomes. As in O157, in combination with IEE, IS*629* is likely promoting the diversification of the ETEC genome.

## ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research (C), KAKENHI grant 24590543 to M.K., from the Japan Society for the Promotion of Science.

We thank Noriko Ido, Hiroko Matsukawa, Atsuko Matsumoto, Hiroto Nishino, Torata Ogawa, Horoshi Yoshizaki, and all of the prefectural Livestock Hygiene Service Centers for providing *E. coli* isolates and their strain information.

## REFERENCES

1. **Sinzelle L, Izsvak Z, Ivics Z.** 2009. Molecular domestication of transposable elements: from detrimental parasites to useful host genes. Cell. Mol. Life Sci. **66**:1073–1093. http://dx.doi.org/10.1007/s00018-009-8376-3.
2. **Kothapalli S, Nair S, Alokam S, Pang T, Khakhria R, Woodward D, Johnson W, Stocker BA, Sanderson KE, Liu SL.** 2005. Diversity of genome structure in *Salmonella enterica* serovar Typhi populations. J. Bacteriol. **187**:2638–2650. http://dx.doi.org/10.1128/JB.187.8.2638-2650.2005.
3. **Wei J, Goldberg MB, Burland V, Venkatesan MM, Deng W, Fournier G, Mayhew GF, Plunkett G, III, Rose DJ, Darling A, Mau B, Perna NT, Payne SM, Runyen-Janecky LJ, Zhou S, Schwartz DC, Blattner FR.** 2003. Complete genome sequence and comparative genomics of *Shigella flexneri* serotype 2a strain 2457T. Infect. Immun. **71**:2775–2786. http://dx.doi.org/10.1128/IAI.71.5.2775-2786.2003.
4. **Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M.** 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. **34**:D32–D36. http://dx.doi.org/10.1093/nar/gkj014.
5. **Chandler M, Mahillon J.** 2002. Insertion sequences revisited, p 305–366. *In* Craig NL, Craigie R, Gellert M, Lambowitz AM (ed), Mobile DNA II. ASM Press, Washington, DC.
6. **Mead PS, Griffin PM.** 1998. *Escherichia coli* O157:H7. Lancet **352**:1207–1212. http://dx.doi.org/10.1016/S0140-6736(98)01267-7.
7. **Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, Han CG, Ohtsubo E, Nakayama K, Murata T, Tanaka M, Tobe T, Iida T, Takami H, Honda T, Sasakawa C, Ogasawara N, Yasunaga T, Kuhara S, Shiba T, Hattori M, Shinagawa H.** 2001. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res. **8**:11–22. http://dx.doi.org/10.1093/dnares/8.1.11.
8. **Makino K, Ishii K, Yasunaga T, Hattori M, Yokoyama K, Yutsudo CH, Kubota Y, Yamaichi Y, Iida T, Yamamoto K, Honda T, Han CG, Ohtsubo E, Kasamatsu M, Hayashi T, Kuhara S, Shinagawa H.** 1998. Complete nucleotide sequences of 93-kb and 3.3-kb plasmids of an enterohemorrhagic *Escherichia coli* O157:H7 derived from Sakai outbreak. DNA Res. **5**:1–9. http://dx.doi.org/10.1093/dnares/5.1.1.
9. **Ooka T, Ogura Y, Asadulghani M, Ohnishi M, Nakayama K, Terajima J, Watanabe H, Hayashi T.** 2009. Inference of the impact of insertion sequence (IS) elements on bacterial genome diversification through analysis of small-size structural polymorphisms in *Escherichia coli* O157 genomes. Genome Res. **19**:1809–1816. http://dx.doi.org/10.1101/gr.089615.108.
10. **Kusumoto M, Ooka T, Nishiya Y, Ogura Y, Saito T, Sekine Y, Iwata T, Akiba M, Hayashi T.** 2011. Insertion sequence-excision enhancer removes transposable elements from bacterial genomes and induces various genomic deletions. Nat. Commun. **2**:152. http://dx.doi.org/10.1038/ncomms1152.
11. **Ogura Y, Ooka T, Iguchi A, Toh H, Asadulghani M, Oshima K, Kodama T, Abe H, Nakayama K, Kurokawa K, Tobe T, Hattori M, Hayashi T.** 2009. Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. **106**:17939–17944. http://dx.doi.org/10.1073/pnas.0903585106.
12. **Ogura Y, Ooka T, Asadulghani Terajima J, Nougayrede JP, Kurokawa K, Tashiro K, Tobe T, Nakayama K, Kuhara S, Oswald E, Watanabe H, Hayashi T.** 2007. Extensive genomic diversity and selective conservation of virulence-determinants in enterohemorrhagic *Escherichia coli* strains of O157 and non-O157 serotypes. Genome Biol. **8**:R138. http://dx.doi.org/10.1186/gb-2007-8-7-r138.
13. **Kaper JB, Nataro JP, Mobley HL.** 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. **2**:123–140. http://dx.doi.org/10.1038/nrmicro818.
14. **Nataro JP, Kaper JB.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11**:142–201.
15. **Frydendahl K.** 2002. Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Veterinary microbiology. **85**:169–182. http://dx.doi.org/10.1016/S0378-1135(01)00504-1.
16. **Holland RE.** 1990. Some infectious causes of diarrhea in young farm animals. Clin. Microbiol. Rev. **3**:345–375.
17. **Sambrook J, Russel DW.** 2001. Molecular cloning, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. **Ooka T, Terajima J, Kusumoto M, Iguchi A, Kurokawa K, Ogura Y, Asadulghani M, Nakayama K, Murase K, Ohnishi M, Iyoda S, Watanabe H, Hayashi T.** 2009. Development of a multiplex PCR-based rapid typing method for enterohemorrhagic *Escherichia coli* O157 strains. J. Clin. Microbiol. **47**:2888–2894. http://dx.doi.org/10.1128/JCM.00792-09.
19. **Vu-Khac H, Holoda E, Pilipcinec E, Blanco M, Blanco JE, Dahbi G, Mora A, Lopez C, Gonzalez EA, Blanco J.** 2007. Serotypes, virulence genes, intimin types and PFGE profiles of *Escherichia coli* isolated from piglets with diarrhoea in Slovakia. Vet. J. **174**:176–187. http://dx.doi.org/10.1016/j.tvjl.2006.05.019.
20. **Akiba M, Uchida I, Nishimori K, Tanaka K, Anzai T, Kuwamoto Y, Wada R, Ohya T, Ito H.** 2003. Comparison of *Salmonella enterica* serovar Abortusequi isolates of equine origin by pulsed-field gel electrophoresis and fluorescent amplified-fragment length polymorphism fingerprinting. Veterinary microbiology. **92**:379–388. http://dx.doi.org/10.1016/S0378-1135(02)00422-4.
21. **Liesegang A, Tschape H.** 2002. Modified pulsed-field gel electrophoresis method for DNA degradation-sensitive *Salmonella enterica* and *Escherichia coli* strains. Int. J. Med. Microbiol: **291**:645–648. http://dx.doi.org/10.1078/1438-4221-00180.
22. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. **60**:1136–1151. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x.
23. **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.** 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum-parsimony methods. Mol. Biol. Evol. **28**:2731–2739. http://dx.doi.org/10.1093/molbev/msr121.
24. **Ohnishi M, Terajima J, Kurokawa K, Nakayama K, Murata T, Tamura K, Ogura Y, Watanabe H, Hayashi T.** 2002. Genomic diversity of enterohemorrhagic *Escherichia coli* O157 revealed by whole genome PCR scanning. Proc. Natl. Acad. Sci. U. S. A. **99**:17043–17048. http://dx.doi.org/10.1073/pnas.262441699.
25. **Sanger F, Nicklen S, Coulson AR.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U. S. A. **74**:5463–5467. http://dx.doi.org/10.1073/pnas.74.12.5463.
26. **Zerbino DR, Birney E.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. **18**:821–829. http://dx.doi.org/10.1101/gr.074492.107.
27. **Sugawara H, Ohyama A, Mori H, Kurokawa K.** 2009. Microbial genome annotation pipeline (MiGAP) for diverse users. Poster and software dem-

onstrations, S001-1-2. 20th International Conference on Genome Informatics (GIW2009), Yokohama, Japan.
28. **Ohtsubo Y, Ikeda-Ohtsubo W, Nagata Y, Tsuda M.** 2008. GenomeMatcher: a graphical user interface for DNA sequence comparison. BMC Bioinformatics **9**:376. http://dx.doi.org/10.1186/1471-2105-9-376.
29. **Shepard SM, Danzeisen JL, Isaacson RE, Seemann T, Achtman M, Johnson TJ.** 2012. Genome sequences and phylogenetic analysis of K88- and F18-positive porcine enterotoxigenic *Escherichia coli*. J. Bacteriol. **194**: 395–405. http://dx.doi.org/10.1128/JB.06225-11.
30. **Perna NT, Plunkett G, III, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Posfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA, Blattner FR.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **409**:529–533. http://dx.doi.org/10.1038/35054089.
31. **Yin X, Wheatcroft R, Chambers JR, Liu B, Zhu J, Gyles CL.** 2009. Contributions of O island 48 to adherence of enterohemorrhagic *Escherichia coli* O157:H7 to epithelial cells in vitro and in ligated pig ileal loops. Appl. Environ. Microbiol. **75**:5779–5786. http://dx.doi.org/10.1128/AEM.00507-09.
32. **Nakazawa M, Akiba M, Sameshima T.** 1999. Swine as a potential reservoir of Shiga toxin-producing *Escherichia coli* O157:H7 in Japan. Emerg. Infect. Dis. **5**:833–834. http://dx.doi.org/10.3201/eid0506.990618.
33. **Keen JE, Wittum TE, Dunn JR, Bono JL, Durso LM.** 2006. Shigatoxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. Emerg. Infect. Dis. **12**:780–786. http://dx.doi.org/10.3201/eid1205.050984.
34. **Milnes AS, Stewart I, Clifton-Hadley FA, Davies RH, Newell DG, Sayers AR, Cheasty T, Cassar C, Ridley A, Cook AJ, Evans SJ, Teale CJ, Smith RP, McNally A, Toszeghy M, Futter R, Kay A, Paiba GA.** 2008. Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. Epidemiol. Infect. **136**:739–751.
35. **Feder I, Wallace FM, Gray JT, Fratamico P, Fedorka-Cray PJ, Pearce RA, Call JE, Perrine R, Luchansky JB.** 2003. Isolation of *Escherichia coli* O157:H7 from intact colon fecal samples of swine. Emerg. Infect. Dis. **9**:380–383. http://dx.doi.org/10.3201/eid0903.020350.

# 小中高生への感染症予防教育を充実して戴きたい

Promoting Education to Prevent Infectious Diseases Aimed at Schoolchildren

五十嵐　　隆
Takashi Igarashi

学校保健研究　第55巻　第2号　別刷

(2013年6月20日発行)

学校保健研究 *Jpn J School Health* 55：2013

巻頭言

# 小中高生への感染症予防教育を充実して戴きたい

五十嵐　隆

# Promoting Education to Prevent Infectious Diseases Aimed at Schoolchildren

Takashi Igarashi

感染症は現在のわが国の子どもにとっても，極めて重要な健康問題の一つである．抗生物質や抗ウイルス薬の開発が進み今では様々な感染症に対する治療法が向上している．しかしながら，感染症の発症予防を目指した様々な方策は現在でも重要性を失っていない．そのなかでも予防接種は極めて有効な感染症予防策である．

では，わが国の現行の小中高校用「体育」「保健体育」の教科書で，感染症と予防接種に関して，どのような教育がなされているのだろうか？実際に教科書を調べてみると，感染症とその予防対策（予防接種）についての記載は小学生用教科書２社いずれも４頁，中学生用教科書２社いずれも４頁，高校生用教科書３社いずれも２頁であった．記載内容を詳細に検証すると，子どもが罹患しやすい感染症あるいは子どもが罹患すると様々な健康被害を受ける感染症，それらの感染症を予防する手段としての予防接種の意義と限界，実際にわが国あるいは世界で行われている予防接種の現状に関する記述が極めて乏しいとの印象を持った．

子どものこころと体の健全な育成のために必要とされる最低限の知識を子どもに教えるという観点からすると，現行の教科書に記載されている感染症と予防接種の内容は不十分である．学校教育で十分な知識を与えられないままに成人となり自分の子どもを持ったとき，ほとんどの保護者は自治体からの予防接種（国民が無料で受けることの出来る「定期接種」）実施の案内を受け取ってはじめて予防接種を子どもに受けさせる行動に移る．子どもにとって接種が必要であるのにわが国では「定期接種」になっていない水痘，おたふくかぜ，Ｂ型肝炎ウイルスなどに対する予防接種（現時点では国民が接種費用を負担する「任意接種」）の存在を知らない保護者も少なくない．重篤な病気ではないので水痘やおたふくかぜに罹患した方が免疫が自然について良いと誤解している保護者が少なくない．肺炎で死亡する水痘患者や難聴になるおたふくかぜ患者が毎年多数出ていることをほとんどの保護者は知らないからである．これらの予防接種はほとんどの先進諸国では「定期接種」としてすべての子どもに接種されている．一方わが国では水痘とおたふくかぜの予防接種の接種率は約３割でしかない．

死亡を含めた感染症による健康被害の実情とその予防策としての予防接種の有効性と限界について子どもに直接啓発することも子どものヘルスプロモーションを図るために必要である．その際に，過去に接種を受けた予防接種記録が残されている自分の母子健康手帳を子どもの手に触れさせ，子どもに自分が過去に受けた予防接種の記録を確認させることが子どもに予防接種を直接啓発する手段として有効である．

私見であるが感染症と予防接種について具体的に以下の内容を子どもに教えることが必要と考える．

小学生には，子どもが罹患しやすい感染症について簡単に解説する．また，これらの感染症には有効な予防接種があることを明示する．予防接種にて抗体が形成され，その抗体が感染予防として有効に作用する免疫のしくみについて解説する．

中学生には，わが国で実施されている予防接種のリストを示し，麻疹，水痘，おたふくかぜなどの流行する感染症を減らすには国民の95%以上がワクチンを接種し抗体を獲得しておくことが必要なこと，インフルエンザは抗原が簡単に変化しやすいウイルスであるため毎年抗原が変化したウイルスが流行することを解説する．また，子どもが罹患しやすい感染症の他に，Ｂ型肝炎ウイルス感染症やパピローマウイルス感染症についても解説する．性感染症としてのエイズ，Ｂ型肝炎，淋菌，梅毒などについて教えることも必要である．

高校生にはわが国の予防接種が定期接種と任意接種とに分かれていること，任意接種とされている予防接種も本来は子どもが受けるべき予防接種であること，しかしながら，任意接種の接種率が実際には低いためにわが国では他の先進諸国に比べ予防接種にて予防できる子どもの感染症患者が多いことを解説する．わが国と欧米諸国で行われている予防接種のリスト（定期接種，任意接種についても明示する）を呈示してわが国の予防接種体制の実態を理解させる．さらに，中学生と同様に，性感染症としてのエイズ，Ｂ型肝炎，淋菌，梅毒などについて教えることが必要である．

なお，予防接種には副反応があること，副反応の中には避けることの出来ない重篤な副反応があること，重篤な副反応を受けた場合には政府からの補償を受ける制度が整備されていることも説明する．

今後，以上の様な小中高生への感染症予防教育を充実化することを可能とする学習指導要領の改革がなされることをこころより願う．

（日本小児科学会会長　国立成育医療研究センター総長）

プラタナス

ヒポクラテスは、プラタナスの樹の下で弟子たちに医学を説いたといわれる

# 小児患者の成人への移行を支援しよう


五十嵐　隆

◎いがらし たかし
国立成育医療研究センター総長・理事長

1978年東大卒。2000年同大小児科教授、2012年より現職。小児腎臓病が専門で、尿細管機能異常症の分子レベルでの原因解明に取り組む。日本小児科学会理事長、日本学術会議第20、21、22期会員。


かつて我が国では、血液悪性腫瘍、先天性心疾患、神経筋疾患などの慢性疾患を持つ子どもや、低出生体重児の生命予後が悪く、成人にまで到達できないことが多かったが、近年、医療の進歩により生命予後が改善され、成人に至る患者が増加している。しかしながら、これらの患者の中には成人になっても評価・治療が必要な者や、新たな合併症が出現する者が少なくない。さらに、長期間にわたる入院生活や治療のために学校・社会生活を送る上で何らかの障害を持つ患者も少なくない。一方、小児期・思春期に気管支喘息、肥満、糖尿病、高血圧を発症したり、メンタルヘルスに障害をきたす患者も増加している。

慢性的に身体・発達・行動・精神状態に障害を持ち、何らかの医療や支援が必要な子どもと青年（children and youth with special health care needs；CSHCN）が、米国では17歳の子どもの17%、英国では12%に及ぶとされる。同様の問題が我が国でも生じており、以下のような現状にある。

①小児科医は成人患者の医療を十分に担当できないにもかかわらず、成人医療提供者側の受け入れ体制が整備されていないために、患者を移管できない。

②人工呼吸管理を必要とする患者、知的障害の強い患者などが入院治療を必要とする場合、成人医療提供者側が患者を受け入れてくれないことがある。

③長期間にわたり治療中心の生活をしてきた子どもは精神的に未熟で社会性に乏しく、保護者や医療提供者などに依存的になっていることが少なくない。

④家族、特に母親が手厚く面倒をみる結果、子どもが自立していないことがある。

今後こうした問題を解決するためには、以下の対応が必要である。

1）小児患者の成人への移行が医療上の大きな問題になっていることを社会に啓発する。

2）小児医療提供者から成人医療提供者への働きかけにより、移行患者への理解を深めるとともに、学会レベルでも協力体制を組む。

3）成人に移行する小児慢性疾患について、小児科医、内科医そして看護師や保健師などの医療提供者が協力して移行患者を診療する「移行プログラム」を整備する。

4）小学生には自分で病気を管理する意識を持たせ、中学生になったら1人で外来受診できるよう、日頃から患児や保護者を教育する。

CSHCNの人たちがself esteemを持って社会の一員として活躍できるようにするために、関係者が協力して彼らを支援する医療・保健・福祉を充実させたい。

Clin Exp Nephrol (2014) 18:4–9
DOI 10.1007/s10157-013-0911-8

SPECIAL ARTICLE

# Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the joint committee of the Japanese society of nephrology and the Japan pediatric society

**Toshihiro Sawai · Masaomi Nangaku · Akira Ashida · Rika Fujimaru · Hiroshi Hataya · Yoshihiko Hidaka · Shinya Kaname · Hirokazu Okada · Waichi Sato · Takashi Yasuda · Yoko Yoshida · Yoshihiro Fujimura · Motoshi Hattori · Shoji Kagami**

Published online: 18 December 2013


**Abstract** Atypical hemolytic uremic syndrome (aHUS) is rare and comprises the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury. Recently, abnormalities in the mechanisms underlying complement regulation have been focused upon as causes of aHUS. The prognosis for patients who present with aHUS is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis. If treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, we have developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment. We hope these diagnostic criteria will be disseminated to as many clinicians as possible and that they will be used widely.

**Keywords** Atypical hemolytic uremic syndrome · Thrombotic microangiopathy · Complement dysregulation · Alternative complement pathway · ADAMTS13

This article has been jointly published in *Clinical and Experimental Nephrology* and *Pediatrics International* by the Japanese Society of Nephrology and the Japan Pediatric Society.

T. Sawai
Department of Pediatrics, Shiga University of Medical Science, Otsu, Japan

M. Nangaku
Division of Nephrology and Endocrinology, The University of Tokyo School of Medicine, Tokyo, Japan

A. Ashida
Department of Pediatrics, Osaka Medical College, Takatsuki, Japan

R. Fujimaru
Department of Pediatrics, Osaka City General Hospital, Osaka, Japan

H. Hataya
Department of Nephrology, Tokyo Metropolitan Children's Medical Center, Fuchu, Japan

Y. Hidaka
Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Japan

S. Kaname
First Department of Internal Medicine, Kyorin University School of Medicine, Mitaka, Japan

H. Okada
Department of Nephrology, Faculty of Medicine, Saitama Medical University, Saitama, Japan

W. Sato
Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan

T. Yasuda
Division of Nephrology and Hypertension, Department of Medicine, St. Marianna University School of Medicine, Kawasaki, Japan

Y. Yoshida · Y. Fujimura
Department of Blood Transfusion Medicine, Nara Medical University, Kashihara, Japan

M. Hattori
Department of Pediatric Nephrology, Tokyo Women's Medical University, Tokyo, Japan

## Introduction

Hemolytic uremic syndrome (HUS) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI) [1]. Approximately 90 % of pediatric patients develop this syndrome after infection with *Shigella dysenteriae*, which produces true Shiga toxins, or *Escherichia coli*, some strains of which produce Shiga-like toxins. Shiga toxin was originally called verotoxin because Vero cells derived from the kidney epithelial cells of the African green monkey are hypersensitive to this toxin [2]. Subsequently, other toxins were called Shiga-like toxin because of their similarities to Shiga toxin in terms of their antigenicity and structure. Shiga-like toxin-1 differs from Shiga toxin by only 1 amino acid, whereas Shiga-like toxin-2 shares 56 % sequence homology with Shiga-like toxin-1. Although Shiga-like toxin-producing *E. coli*-HUS (STEC-HUS) strains most often trigger HUS, certain Shiga toxin-secreting strains of *S. dysenteriae* can also cause HUS. They are currently known as the Shiga toxin family, and the terms are often used interchangeably. HUS occurring from infection with STEC-HUS was formerly called diarrhea + HUS (D + HUS) or typical HUS.

In contrast, HUS that is not related to Shiga toxins and accounts for ~10 % of all HUS cases, is called atypical HUS (aHUS). Although STEC-HUS is relatively common in children, aHUS occurs in individuals of all ages and is often familial. The prognosis is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis [3].

In recent years, abnormalities in the mechanisms underlying complement regulation have been focused on as causes of aHUS. Various genetic abnormalities in complement regulatory factors, including complement factor H, have been noted in 50–60 % of patients. The analysis of the pathology underlying this condition is currently progressing rapidly [4].

The differential diagnosis of aHUS from STEC-HUS or thrombotic thrombocytopenic purpura (TTP), another form of thrombotic microangiopathy (TMA) caused by a deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), is not necessarily easy at the early stages of disease onset. However, if treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society (JSN/JPS) has developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment [5, 6]. We hope that the diagnostic criteria presented in this report will become familiar to as many clinicians as possible and that they will be used widely.

### Definition of aHUS

aHUS is a type of TMA that differs from STEC-HUS and TTP, with the latter being caused by markedly reduced ADAMTS13 activity. aHUS is a syndrome characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI, which is similar to STEC-HUS.

## Guidelines for the diagnosis of aHUS

### Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI is present. The disease should not be associated with Shiga toxins, and TTP should also be excluded.

The Joint Committee of the JSN/JPS defined microangiopathic hemolytic anemia based on a hemoglobin (Hb) level of <10 g/dL. The presence of microangiopathic hemolytic anemia should be confirmed based on increased serum lactate dehydrogenase levels, a marked decrease in serum haptoglobin levels, and the presence of red blood cell fragments in a peripheral blood smear.

Thrombocytopenia is defined as a platelet (PLT) count of <150,000/μL.

The definition of AKI has been updated, with the most recent definition given by the international guidelines group, the Kidney Disease: Improving Global Outcomes that integrates both the Risk, Injury, Failure, Loss, End-stage kidney disease and the Acute Kidney Injury Network classifications to facilitate identification. Thus, we recommend diagnosis based on the most recent guidelines, along with the following definitions. For pediatric cases, the serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7]. For adult cases, the diagnostic criteria for AKI should be used.

## Guidelines for the diagnosis of aHUS

### Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia,

S. Kagami (✉)
Department of Pediatrics, Graduate School of Medical Sciences, Tokushima University, Tokushima, Japan
e-mail: kagami@tokushima-u.ac.jp

**Table 1** Definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that have been established by the joint committee of the JSN/JPS

| Microangiopathic hemolytic anemia | Thrombocytopenia | Acute kidney injury |
|---|---|---|
| Defined as an Hb level <10 g/dL<br>Presence confirmed based on:<br>Increased serum LDH levels<br>Marked decreases in serum haptoglobin levels<br>The presence of red blood cell fragments in a peripheral blood smear | Defined as a PLT count <150,000/μL | The most recent AKI definition is provided by the international guideline group, the KDIGO, integrating the RIFLE and AKIN classifications to facilitate identification. Thus, diagnosis should be based on the most recent guidelines, and the following definitions should be used.<br>Pediatric cases: Serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7].<br>Adult cases: Diagnostic criteria for AKI should be used |

*Hb* hemoglobin, *LDH* lactate dehydrogenase, *PLT* platelet, *AKI* acute kidney injury, *KDIGO* kidney disease: improving global outcomes, *RIFLE* risk, injury, failure, loss, end-stage kidney disease, *AKIN* acute kidney injury network

and AKI is present. The disease should have no association with Shiga toxins, and TTP should also be excluded. Table 1 presents the definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that are established by the Joint Committee of the JSN/JPS.

Probable diagnosis

A probable diagnosis of aHUS is made when 2 of the following 3 conditions are found: microangiopathic hemolytic anemia, thrombocytopenia, and AKI. The disease should have no association with Shiga toxins and TTP should be excluded.

## Applicability of these diagnostic criteria

When we applied these diagnostic criteria to the Nara Medical University (NMU) TMA cohort, 15 out of 37 individuals who had all the data required for the assessment were diagnosed as having definitive aHUS. Since the data were recorded at one time point only, we speculate that the sensitivity of the diagnostic criteria would increase if we could assess data from multiple time points. The cut-off value for anemia, defined as an Hb level of <10 g/dL, and the cut-off value for thrombocytopenia, defined as a PLT count of <150,000/μL, are equivalent to those employed by the International Registry of Recurrent and Familial HUS/TTP [8]. We had considered using a cut-off value of a PLT count <100,000/μL for thrombocytopenia to reflect that used in the diagnostic criteria for STEC-HUS by the Japanese Society for Pediatric Nephrology (2000), but we only found 1 patient with a PLT count between 100,000 and 150,000/μL in the NMU cohort. Therefore, it is likely that this difference will not have a large impact on the sensitivity or specificity of our diagnostic criteria. Our diagnostic criteria include the category of "Probable" aHUS because we believe that this tentative diagnosis will help in the early diagnosis of aHUS and avoid delays in developing appropriate therapeutic approaches for patients with aHUS.

## Evaluation of inappropriate complement activation

Abnormalities in complement regulation are among the main causes of aHUS. The diagnosis of aHUS that is caused by inappropriate complement activation has become more critical because eculizumab, a humanized anti-C5 monoclonal antibody, has been shown to be an effective therapeutic modality [9] that has been approved for the treatment of aHUS patients in Europe and the United States. Recently, Fan and colleagues evaluated genotype–phenotype relationships in 10 Japanese patients with aHUS and identified potentially causative mutations in complement factor H, C3, membrane cofactor protein, and thrombomodulin in 8 of the patients [10]. However, the definitive diagnosis of inappropriate complement activation in aHUS patients is difficult because some patients show normal serum levels of complement components [11] and there are a number of complement regulatory proteins, making it difficult to decide which complement regulatory protein is responsible for a particular patient developing aHUS.

## Excluding Shiga toxin-producing *E. coli* infection

STEC-HUS is characterized by diarrhea accompanied by bloody stools. However, diarrhea may also be present in some aHUS cases. Diarrhea in aHUS can be a manifestation of ischemic colitis. In addition, enteritis that is not caused by STEC can trigger aHUS. Therefore, a diagnosis of STEC-HUS cannot be made based on symptoms alone, and the earlier nomenclature that used "D + HUS" to correspond with STEC-HUS and "D-HUS" to correspond

with aHUS is not used at present [11]. The involvement of Shiga toxins should be confirmed by stool culture, the direct detection of Shiga toxins, or the detection of anti-lipopolysaccharide-IgM antibodies.

### Excluding TTP

Conventionally, TTP has been diagnosed based on the classic pentad (microangiopathic hemolytic anemia, thrombocytopenia, labile psychoneurotic disorder, fever, and renal failure). However, the discovery of ADAMTS13 led to the finding that 60–90 % of patients with TTP have a marked reduction in the activity of ADAMTS13, to a level of <5 %, regardless of race. Therefore, when diagnosing aHUS, patients who have markedly reduced levels of ADAMTS13 activity (<5 %) should be diagnosed as having TTP, thereby ruling out a diagnosis of aHUS. However, some patients may show the classic TTP pentad and have normal or slightly reduced levels of ADAMTS activity. Therefore, if a patient has levels of ADAMTS13 activity ≥5 %, a differential diagnosis of aHUS or TTP may be necessary to account for other clinical symptoms.

### Excluding TMA caused by other distinct factors

Diseases that evidently cause a clinical state of TMA, including disseminated intravascular coagulation, sclerodermatous kidney, and malignant hypertension, should be excluded when diagnosing aHUS.

### When a probable case of aHUS is suspected

When a probable case of aHUS is suspected, samples that are necessary to determine the appropriate diagnosis should be collected, and the therapeutic strategy should be established after consultation with an institution that has extensive experience of managing aHUS cases.

### Cases where aHUS should be strongly suspected

If there are features that are characteristic of HUS, aHUS should be strongly suspected if the following criteria are fulfilled, regardless of the presence of diarrhea: the patient is younger than 6 months of age; time of onset is unclear (latent onset); the patient has a history of HUS (recurrent case); the patient has a history of anemia of unknown cause; recurrent HUS after kidney transplantation; the patient has a family history of HUS (excluding cases of food poisoning); and, the patient has no diarrhea or bloody stools.

### Classification of aHUS causes, excluding TTP caused by the ADAMTS13 defect

Table 2 classifies the causes of aHUS and presents methods to determine the causes.

## Discussion

Nineteen years after Gasser et al. [1] reported HUS, an interesting report was published in the *Lancet* [10]. This report indicated that although C3-predominant activity is initiated in the blood vessels in TMA patients, this is not observed in typical cases of HUS, suggesting that complement activation is involved in aHUS onset [12]. Subsequently, numerous researchers have elucidated further information on the pathology of aHUS. At present, the reported causes of aHUS include, complement regulation abnormalities, cobalamin metabolism disorder, infection with *Streptococcus pneumoniae* and other microorganisms, drugs, pregnancy, and autoimmune diseases.

The complement system plays an important role as part of the immune systems of living organisms. It is activated via 3 pathways, the classical, alternative, and lectin pathways. As a result of the activation of the host's alternative and classical pathways, C5b-9, a membrane attack complex, is generated and destroys cells by forming transmembrane pores. The alternative pathway is involved in the onset of aHUS. Unlike the classical and lectin pathways, activation of the alternative pathway does not require initiators; it is continuously activated by the spontaneous hydrolysis of C3.

When complement proteins are inappropriately activated, there is a risk of inducing cell dysfunction within the host itself. Thus, humoral factors in the circulating plasma and several plasma membrane-bound factors are involved in the regulation of complement activation and act at various stages, such as the inactivation of C3b or C4b, and the inhibition of the generation of membrane attack complexes. The regulators involved in the alternative pathway include complement factors H and I, which are humoral factors, and membrane cofactor protein and thrombomodulin, which are membrane-bound factors. If these factors are abnormal, the subsequent failure of regulation will hyperactivate the complement proteins, leading to the onset of aHUS. Some cases of aHUS develop after trigger events, for example, infections of the respiratory tract and the gastrointestinal tract, and it is likely that activation of the complement cascade by these trigger events and the

Table 2 Classification and determination of the causes of aHUS, excluding TTP caused by the ADAMTS13 defect

| Cause of aHUS | Method to determine the cause |
|---|---|
| Complement regulation abnormality | Hemolysis test, quantification of complement proteins and complement regulatory proteins, and gene analysis. Even if the amounts of complement proteins and complement regulatory proteins are within the normal ranges, it does not serve as a basis for excluding complement-related aHUS |
| (i) Congenital | |
| Genetic mutations of complement proteins, factor H, factor I, membrane cofactor protein, C3, factor B, and thrombomodulin | |
| (ii) Acquired | |
| Production of autoantibodies, including anti-factor H antibody | Detection of anti-factor H antibody by ELISA, western blot, etc. |
| (2) Cobalamin metabolism disorder | Age at onset should be considered (<6 months old), and hypomethioninemia or hyperhomocysteinemia is detected on plasma amino acid analysis |
| (3) Infection | Definitive diagnosis by identification of pathogenic microorganisms and serological examination |
| (i) Pneumococcus | |
| (ii) Human immunodeficiency virus | |
| (iii) Pertussis | |
| (iv) Influenza | |
| (v) Varicella | |
| (4) Drug-induced | Identification of the drug |
| (i) Anticancer drugs | |
| (ii) Immunomodulatory drugs | |
| (iii) Antiplatelet drugs | |
| (5) Pregnancy-related | |
| (i) Hemolysis, elevated liver enzymes, low platelet counts (HELLP) syndrome | |
| (ii) Eclampsia | |
| (6) Autoimmune disease, collagen disease | Definitive diagnosis by autoantibody test, antiphospholipid antibody test, and serological examination |
| (i) Systemic lupus erythematosus | |
| (7) Bone-marrow transplant, organ transplant-related | |
| (8) Others | |

*aHUS* atypical hemolytic uremic syndrome, *ELISA* enzyme-linked immunosorbent assay

subsequent amplification of complement activation by the alternative pathway cannot be regulated in patients with deficiencies in complement regulation. Gain-of-function mutations in C3 and complement factor B, which are complement-activating factors, also cause hyperactivation of complement proteins and, ultimately, aHUS.

It has been reported that ~50 % of aHUS patients have genetic abnormalities in complement regulatory factors, including complement factor H. The frequency of the presence of certain mutations among aHUS cases, responsiveness to plasma therapy, prognosis of kidney function, and the recurrence rate after kidney transplantation, vary depending on the type of genetic abnormalities present [13]. Although plasmapheresis within 24 h of confirmation of the diagnosis has been recommended as the initial treatment for aHUS [14], its effects are not always satisfactory. The mortality or incidence of end-stage renal disease is considered to be between 70 and 80 %, and the recurrence rate after kidney transplantation may be as high as 80–90 %, particularly in patients with abnormal complement factor H, which is the most frequent abnormality [15].

In 2011, eculizumab (Soliris®, Alexion Pharmaceuticals), a terminal complement inhibitor, was approved as a new drug for the treatment of aHUS in Europe and the US. Eculizumab is a humanized recombinant immunoglobulin G2/4 monoclonal antibody directed against the complement component C5, which was developed as a treatment for paroxysmal nocturnal hemoglobinuria. By binding to complement component C5, the drug inhibits the generation of C5a and C5b-9, and thus subsequently inhibits the complement system.

There are a number of reports stating that only HUS that is associated with complement regulation abnormalities is defined as aHUS. On the basis of the current diagnostic criteria, we have defined aHUS to include all types of HUS that are not related to Shiga toxins or other distinct causes. In cases where aHUS is associated with complement

dysregulation, the introduction of eculizumab may markedly change therapeutic strategies. It should be noted, however, that recommendations of specific therapeutic modalities are beyond the scope of the current diagnostic criteria. However, in cases where complement dysregulation is confirmed as the cause, treatment with eculizumab is established. Thus, it may be desirable to assign HUS associated with complement dysregulation a separate disease name rather than it being classified as "aHUS", as in the case of definitive "complement-mediated TMA".

As described in previous reports, aHUS is a disease that may frequently cause renal failure and be fatal if it is not appropriately diagnosed and treated at the early stages of disease onset. In Japan, aHUS may be misdiagnosed as HUS caused by Shiga toxins because clinicians are not sufficiently aware of aHUS, and consequently, treatment may be delayed. Thus, our diagnostic criteria include the category of "Probable" aHUS to ensure that the clinicians consider aHUS during diagnosis. Many issues should be addressed in the future, including the development of diagnostic strategies to diagnose cases of suspected aHUS, the establishment of insurance coverage for ADAMTS13 activity measurement testing that is necessary to differentiate aHUS from TTP, and the development of treatment guidelines. We hope that our diagnostic criteria will be used widely and will contribute to the diagnosis and treatment of aHUS patients.

**Acknowledgments** These diagnostic criteria for aHUS were proposed by the Joint Committee of the Japanese Society of Nephrology (JSN) (President: Seiichi Matsuo) and the Japan Pediatric Society (JPS) (President: Takashi Igarashi). The members of the committee are Shoji Kagami (Chair), Akira Ashida, Rika Fujimaru, Hiroshi Hataya, Motoshi Hattori, Yoshihiko Hidaka, Shinya Kaname, Masaomi Nangaku, Hirokazu Okada, Waichi Sato, Toshihiro Sawai, Takashi Yasuda, Yoko Yoshida (Adviser) and Yoshihiro Fujimura (Adviser). This study was supported by the JPS and JSN.

**Conflict of interest** Advisory role: Yoshihiro Fujimura (Baxter Bioscience and Alexion Pharmaceuticals).Honoraria: Masaomi Nangaku (Kyowa Hakko Kirin Co. Ltd and Daiichi Sankyo Co. Ltd).Subsidies: Masaomi Nangaku (Kyowa Hakko Kirin Co. Ltd, Daiichi Sankyo Co. Ltd, Astellas Pharma Inc., Mitsubishi Tanabe Pharma Corporation, Chugai Pharmaceutical Co. Ltd and Takeda Pharmaceutical Co. Ltd). The other authors have no conflicts of interest.

## References

1. Gasser C, Gautier E, Steck A, Siebenmann R, Oechslin R. Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. Schweizerische medizinische Wochenschrift. 1955;85(38–39):905–9.
2. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun. 1977;18:775–9.
3. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(17):1676–87. doi:10.1056/NEJMra0902814.
4. Kavanagh D, Goodship T. Genetics and complement in atypical HUS. Pediatric Nephrol (Berlin, Germany). 2010;25(12):2431–42. doi:10.1007/s00467-010-1555-5.
5. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T, et al. Diagnostic criteria of atypical hemolytic uremic syndrome. Jpn J Nephrol. 2013;55(2):91–3 (in Japanese).
6. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T et al. Diagnostic criteria of atypical hemolytic uremic syndrome. J Jpn Pediatr Soc. 2013; http://www.jpeds.or.jp/uploads/files/saisin_130201.pdf (in Japanese).
7. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, et al. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol. 2011;15(5):694–9. doi:10.1007/s10157-011-0452-y.
8. Noris M, Caprioli J, Bresin E, Mossali C, Pianetti G, Gamba S, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Amer Soc Nephrol. 2010;5(10):1844–59. doi:10.2215/CJN.02210310.
9. Legendre C, Licht C, Muus P, Greenbaum L, Babu S, Bedrosian C, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013;368(23):2169–81. doi:10.1056/NEJMoa1208981.
10. Fan X, Yoshida Y, Honda S, Matsumoto M, Sawada Y, Hattori M, et al. Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol. 2013;54(2):238–46. doi:10.1016/j.molimm.2012.12.006.
11. Loirat C, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011;6:60. doi:10.1186/1750-1172-6-60.
12. Stühlinger W, Kourilsky O, Kanfer A, Sraer J. Letter: haemolytic-uraemic syndrome: evidence for intravascular C3 activation. Lancet. 1974;2(7883):788–9.
13. Nester C, Thomas C. Atypical hemolytic uremic syndrome: what is it, how is it diagnosed, and how is it treated? Hematology. 2012;2012:617–25. doi:10.1182/asheducation-2012.1.617.
14. Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C, et al. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatric Nephrol (Berlin, Germany). 2009;24(4):687–96. doi:10.1007/s00467-008-0964-1.
15. Waters A, Licht C. aHUS caused by complement dysregulation: new therapies on the horizon. Pediatric Nephrol (Berlin, Germany). 2011;26(1):41–57. doi:10.1007/s00467-010-1556-4.

日腎会誌 2013；55(2)：91-93.

# 非典型溶血性尿毒症症候群　診断基準

非典型溶血性尿毒症症候群診断基準作成委員会
委員長　香美祥二　徳島大学大学院小児科
日本腎臓学会
岡田浩一　埼玉医科大学腎臓内科
要　伸也　杏林大学医学部第一内科
佐藤和一　名古屋大学腎臓内科
南学正臣　東京大学腎臓・内分泌内科
安田　隆　聖マリアンナ医科大学腎臓・高血圧内科
日本小児科学会
服部元史　東京女子医科大学腎臓小児科
芦田　明　大阪医科大学小児科
幡谷浩史　都立小児総合医療センター腎臓内科
日高義彦　信州大学小児科
澤井俊宏　滋賀医科大学小児科
藤丸季可　大阪市立総合医療センター小児総合診療科
外部委員
藤村吉博　奈良県立医科大学輸血部
吉田瑤子　奈良県立医科大学輸血部

## 疾患の定義

非典型溶血性尿毒症症候群(atypical hemolytic uremic syndrome：aHUS)は，志賀毒素による HUS と ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs, member 13)活性著減による血栓性血小板減少性紫斑病(thrombotic thrombocytopenic purpura：TTP)以外の血栓性微小血管障害(thrombotic microangiopathy：TMA)で，微小血管症性溶血性貧血，血小板減少，急性腎障害(acute kidney injury：AKI)を 3 主徴とする疾患である。

## 診断基準

Definite：

3 主徴が揃い，志賀毒素に関連するものでないこと。血栓性血小板減少性紫斑病でないこと。

・微小血管症性溶血性貧血：Hb 10 g/dL 未満

血中 Hb 値のみで判断するのではなく，血清 LDH の上昇，血清ハプトグロビンの著減，末梢血スメアでの破砕赤血球の存在を基に微小血管症性溶血の有無を確認する。

・血小板減少：PLT 15 万/$\mu$L 未満

・急性腎障害(AKI)：

小児例：年齢・性別による血清クレアチニン基準値の 1.5 倍

(血清クレアチニンは，小児腎臓病学会の基準値を用いる。)

成人例：AKI の診断基準を用いる。