ABSTRACT A series of outbreaks of infection with Shiga toxin (verotoxin)-producing Escherichia coli or enterohemorrhagic E. coli (EHEC) O157:H7 occurred in Japan in 1996, the largest outbreak occurring in primary schools in Sakai City, Osaka Prefecture, where more than 7,500 cases were reported. Although the reason for the sudden increase in the number of reports of EHEC isolates in 1996 is not known, the number of reports has grown to more than 3,000 cases per year since 1996, from an average of 105 reports each year during the previous 5-year period (1991-1995). Despite control measures instituted since 1996, including designating Shiga toxin-producing E. coli infection as a notifiable disease, and nationwide surveillance effectively monitoring the disease, the number of reports remains high, around 3,800 cases per year. Serogroup O157 predominates over other EHEC serogroups, but isolation frequency of non-O157 EHEC has gone up slightly over the past few years. Non-O157 EHEC has recently caused outbreaks where consumption of a raw beef dish was the source of the infection and some fatal cases occurred. Laboratory surveillance comprised prefectural and municipal public health institutes, and the National Institute of Infectious Diseases has contributed to finding not only multiprefectural outbreaks but also recognizing sporadic cases that could have been missed as an outbreak without the aid of molecular subtyping of EHEC isolates. This short overview presents recent information on the surveillance of EHEC infections in Japan.

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Correspondence: Haruo Watanabe, haruwata@nih.go.jp © 2014 American Society for Microbiology. All rights reserved.
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SURVEILLANCE AND REPORTING

In 1966, EHEC infection was designated as a disease requiring mandatory reporting regardless of source. EHEC infection is one of category III notifiable infectious diseases along with other bacterial infections caused by Vibrio cholerae O1 or O139, Shigella species, Salmonella enterica serovar Typhi, and Salmonella serovar Paratyphi A in the National Epidemiological Surveillance of Infectious Diseases (NESID) under the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections (the Infectious Diseases Control Law) enacted in April 1999. Under a surveillance system for food poisoning based on the Food Sanitation Law, an EHEC infection is reported as food poisoning by physicians or judged as such by the director of the health center and reported as such by the local government to the Ministry of Health, Labour and Welfare (MHLW). Active surveillance of the population surrounding the outbreak, including checking food-intake and stool specimens of infected cases and persons engaged in food preparation, revealed that approximately 35% of EHEC infections were asymptomatic and that the proportion of asymptomatic infection was high among the middle-aged group whereas symptomatic cases were more frequent in young and old age groups. The public health importance of symptomatic shedding (3) and asymptomatic shedding (4) in transmission among preschool children is well established, but asymptomatic shedding in adults is not yet well understood though high rates of culture-positive, asymptomatic adults with EHEC O157:H7 infection were reported in 1998 (5), and the tendency remains similar with EHEC infection currently (6). Apart from NESID, results of characterization (serotypes, VT types, etc.) of EHEC isolates at prefectural and municipal PHIs are reported to the Infectious Disease Surveillance Center (IDSC) at the National Institute of Infectious Diseases. The summary showed that EHEC O157 serogroup is the predominant one, followed by O26, O111, O103, O121, O145, and others. However, as seen in the United States (7), the percentage of non-EHEC O157 serogroups among all EHEC isolates from human infection has been growing slightly; the rate of isolation frequency of EHEC O157 has declined from approximately 70% of all EHEC isolates in 2000 to less than 60% in 2012, though the situation of EHEC infection in Japan is not exactly the same as in continental Europe where non-O157 EHEC serotypes are more common than infection with O157:H7 EHEC (8, 9). The number of EHEC isolates reported to IDSC is about half the number of EHEC infection cases reported to NESID, because only a small proportion of isolates in hospitals or commercial laboratories are sent to PHIs.

LABORATORY SURVEILLANCE

Stool samples from suspected cases of diarrhea should be routinely screened for EHEC by plating on sorbitol-MacConkey agar containing cefixime and tellurite in addition to conventional E. coli isolation agar. Rhamnosel/
sorbos-MacConkey agar containing cefixime and tellurite and chromogenic media could also be used for O26/ O111 isolates as these serogroups are major ones following O157. The use of an enrichment culture step or immunomagnetic separation available for *E. coli* O157, O26, O111, O103, and O145 facilitates isolation of these pathogens. It is essential that *E. coli* isolates be examined for production of VT or the presence of VT genes regardless of serogroup of the isolates since these are prerequisite results for diagnosis of EHEC infection. It could be important to examine production of VT or the presence of VT genes in all isolates on selective agar plates with and without cefixime and tellurite to detect various serogroups of EHEC (10). Molecular subtyping techniques, including pulsed-field gel electrophoresis (PFGE), have sufficient sensitivity and discriminatory power for use in epidemiological investigations. The establishment of the molecular subtyping network, PulseNet, in the United States (11) encouraged us to construct an equivalent molecular subtyping network based on PFGE analysis, PulseNet Japan (12). It participates in the Asia and Pacific regional network, PulseNet Asia Pacific, which constitutes a part of PulseNet International (13), and contributed in investigations of domestic outbreaks (14) and an international EHEC O157 outbreak that occurred between Japan and the United States (15). Although PFGE is still in wide use in outbreak investigations for its high discrimination power, there are additional DNA-based methods for strain analysis that are simpler, faster to perform, and equivalent to PFGE in strain discrimination. Among these, multilocus sequence typing was shown to have insufficient discriminatory power for EHEC O157:H7 (16, 17). On the other hand, multilocus variable-number tandem-repeat analysis (MLVA) has proven to be useful for genetic fingerprinting of EHEC O157:H7 (13, 18-21), O26, and O111 (22) and pathogenic bacteria (23). For strain analysis, in addition to MLVA, an insertion element (IS)-printing system that is a PCR-based strain-typing method for EHEC O157 (24) has been applied to outbreak investigations. The IS-printing system showed that it had equivalent capacity to subtype the isolates in the outbreak. It is based on the variability in genomic location of IS629 among EHEC O157 strains and can produce the results in a much shorter time than other subtyping methods such as PFGE. The method has another advantage: if standard protocol is established and quality assurance is achieved, then the results are suitable for creating a database since PCR results can be expressed as presence or absence of the amplicon for the sample, which is easily digitized, and the information can readily be shared with other laboratories involved in the outbreak investigation. Characterization of EHEC isolates using DNA-based methods could be critical for linking simultaneously occurring sporadic cases that would otherwise have been unrecognized as a diffuse outbreak (14). It is important to note that a prompt epidemiological investigation is necessary to confirm that a cluster of genetically indistinguishable isolates may represent an outbreak with a common source. Also, it is noteworthy that epidemiological links are not established for all the clusters; many remain unresolved.

**HEMOLYTIC-UREMIC SYNDROME (HUS)**

The case definition of EHEC infection was partly amended in April 2006 as follows: if VT is detected in feces, or O-antigen agglutinating antibody or anti-VT antibody is detected in serum of a patient with HUS, the patient should be reported as having EHEC infection. From 2006 to 2012, the average annual number of HUS cases (including serodiagnosed cases) was 100 and the incidence rate of HUS (HUS cases/symptomatic cases) was 3.7% (Fig. 2) (25–29). Increased rates of HUS in children less than 10 years old and the elderly (30, 31) are shown in Fig. 2, and a rise in incidence rate of HUS for the age group older than 65 in 2012 reflects an outbreak of EHEC O157 where five HUS cases occurred in a facility for the elderly (32). A rise in incidence rate for the age group 15 to 64 years in 2011 was due to a large outbreak of EHEC O111 in which the age of 21 HUS cases ranged from 15 to 64 years among 34 HUS cases in the outbreak. From 2006 to 2012, 67% of the 605 HUS cases were culture-confirmed by laboratory testing, and the rest of the cases were diagnosed by detecting antilipopolysaccharide antibody of *E. coli* in the serum of the patients or VT in the stool samples of the patients. EHEC O157 was the predominant serogroup, occupying 85% of all isolates in culture-confirmed HUS cases, followed by O111 (5%), O121 (2.1%), O26 (1.7%), O165 (1%), O145 (1%), and the rest of the O serogroups, including O55, O174, O183, and unknown serogroup samples. Although non-O157 serogroup strains were isolated in the culture-confirmed HUS cases, 94% of all EHEC isolates in the culture-confirmed HUS cases were either VT2 or both VT1 and VT2 producers, which is consistent with epidemiological evidence that VT2-containing EHEC O157:H7 strains are more frequently associated with HUS than the strains containing VT1 (33, 34). Some non-O157 EHEC strains that were also classified as enteroaggregative *E. coli* have been isolated in HUS cases due to O104:H4 infection in Germany (35, 36) as well as an outbreak due to O111:H2 in France (37), but there has been only one such case...
reported in Japan, where a 3-year-old boy in Kagoshima Prefecture developed HUS, developed encephalopathy, and died due to the infection of VT2-producing and entericaggregative E. coli O86:HNM (38).

OUTBREAKS

In the 197 outbreaks with more than 10 culture-positive patients, which were reported to IDSC from 2000 to 2012 (6, 32, 39–50), the main mode of transmission of the infection was person to person (41%), food borne (29%), and water borne (3%), and in about one-third of the outbreaks, the mode of transmission of the infection remains unknown. The most frequent serogroup of EHEC associated with the outbreaks was EHEC O157 (47%), followed by O26 (35%), O111 (8%), O103 (4%), O121 (3%), O145 (2%), and others. The predominance of EHEC O157 serogroup and other representative serogroups that had caused the outbreaks is fairly consistent with the laboratory surveillance results obtained by PHIs for EHEC infection that includes outbreaks and sporadic cases.

Among 197 outbreaks, 101 outbreaks have occurred in nursery schools, which may account for the high proportion of person-to-person transmissions of the infection in the outbreaks. Interestingly, the most prevalent serogroup of EHEC in the outbreaks in nursery schools was O26 (52%), followed by O157 (27%), O111 (9%), O103 (4%), O121 (4%), O145 (3%), and OUT, which may account for relatively mild clinical manifestations, including asymptomatic cases and, consequently, frequent person-to-person transmission. More O26-associated cases may have occurred; however, such cases would not be detected as sporadic due to mild symptoms among adults and children. Larger outbreaks resulted from consumption of contaminated foods. Between 2000 and 2012, there were 12 EHEC outbreaks that had more than 100 culture-positive cases (Table 1). All 12 outbreaks appear to have resulted from consumption of contaminated foods, and in some of these outbreaks, microbiological testing confirmed the implicated foods. These included beef products (39); lightly salted cucumber (40); Koumi-ae consisting of boiled spinach and steamed chicken meat seasoned with Welsh onion, ginger, and soy sauce (40); boxed meals (43); lettuce (44); school lunch (45); Yukhoe (raw beef) (6), and Japanese rice cakes (6). The infectious dose of EHEC O157 is very low, probably fewer than 100 organisms (51, 52), and this is a critical factor in the transmission of the EHEC when people consume raw or lightly cooked foods such as sushi and vegetables. Because sushi and raw or lightly cooked meat are favorite food items in Japan, outbreaks were associated with consumption of salmon roe sushi in 1998 (53) and “rare” roast beef contaminated with EHEC O157 in 2001 (39). In 2011, a large EHEC O111 outbreak due to consumption of Yukhoe, a Korean dish of raw beef and egg yolk, was identified at Yakiniku chain restaurants. EHEC O111:H8 was isolated from 85 of 181 patients (median age 20 years); in 34 of those patients HUS developed; encephalopathy developed in 21 patients; and 5 patients died (6). EHEC O111:H8 was also isolated from the conserved part of the original meat preparations distributed to the chain restaurants. In response to persistent food poisonings caused by raw beef, MHLW revised the standards of beef product quality for...
raw-eating and put them into operation in October 2011. Further, after the EHEC O157 was detected in the inner part of cattle livers, MHLW banned marketing of cattle liver for raw-eating. Probably as a consequence of these preventive measures, the incidence of EHEC O157 cases related to consumption of raw meat decreased by almost half in 1 year from 2011 to 2012 (32).

Some of the outbreaks were associated with consumption of vegetables. Among four outbreaks associated with consumption of vegetables in 2011 (6), EHEC O26:H11 was isolated from cabbage in an outbreak and EHEC O157:H7 was isolated from pickled eggplant and green perilla, green perilla served with grated radish, and cucumber in three outbreaks, respectively. In 2012, there was a large EHEC O157:H7 outbreak due to consumption of a brand of lightly salted vegetables from a company in Sapporo, Hokkaido (32). EHEC O157:H7 was isolated from the implicated product. Because of the products’ wide distribution, 169 patients were reported from five facilities for the elderly, hotels, restaurants, and families in Hokkaido and included four cases in different prefectures from which EHEC O157:H7 was isolated. Among 169 patients, EHEC O157:H7 was isolated from 73 patients, 8 of whom, mostly elderly, died.

TABLE 1 Characterization of 12 EHEC outbreaks with more than 100 culture-positive cases between 2000 and 2012

<table>
<thead>
<tr>
<th>Year</th>
<th>Prefecture/City</th>
<th>Setting (reference)</th>
<th>Serotype</th>
<th>VT type</th>
<th>Symptomatic cases</th>
<th>Culture positives</th>
<th>Likely mode of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Chiba P.</td>
<td>Patient’s home (39)</td>
<td>O157:H7</td>
<td>VT1 &amp; 2</td>
<td>195</td>
<td>257</td>
<td>Beef products*</td>
</tr>
<tr>
<td>2002</td>
<td>Fukuoka C.</td>
<td>Nursery school (40)</td>
<td>O157:H-</td>
<td>VT2</td>
<td>74</td>
<td>112</td>
<td>Lightly salted cucumber*</td>
</tr>
<tr>
<td>2002</td>
<td>Utsunomiya C.</td>
<td>Hospital and home for the elderly (40)</td>
<td>O157:H7</td>
<td>VT1 &amp; 2</td>
<td>123</td>
<td>111</td>
<td>Koumi-ae*</td>
</tr>
<tr>
<td>2003</td>
<td>Yokohama C.</td>
<td>Kindergarten (41)</td>
<td>O26:H11</td>
<td>VT1</td>
<td>141</td>
<td>449</td>
<td>Food borne</td>
</tr>
<tr>
<td>2004</td>
<td>Ishikawa P.</td>
<td>High school (42)</td>
<td>O111:H-</td>
<td>VT1 &amp; 2</td>
<td>110</td>
<td>103</td>
<td>Food borne</td>
</tr>
<tr>
<td>2007</td>
<td>Tokyo M.</td>
<td>School refectory (43)</td>
<td>O157:H7</td>
<td>VT2</td>
<td>467</td>
<td>204</td>
<td>Food borne</td>
</tr>
<tr>
<td>2007</td>
<td>Miyagi P., Sendai C., and Akita C.</td>
<td>Restaurant (43)</td>
<td>O157:H7</td>
<td>VT1 &amp; 2</td>
<td>314</td>
<td>173</td>
<td>Boxed meals*</td>
</tr>
<tr>
<td>2009</td>
<td>Saga P.</td>
<td>Nursery school (44)</td>
<td>O26:H11</td>
<td>VT1</td>
<td>ND</td>
<td>133</td>
<td>Lettuce*</td>
</tr>
<tr>
<td>2010</td>
<td>Mie P.</td>
<td>High school (45)</td>
<td>O157:H7</td>
<td>VT2</td>
<td>138</td>
<td>164</td>
<td>School lunch*</td>
</tr>
<tr>
<td>2011</td>
<td>Toyama P.</td>
<td>Chain restaurants (6)</td>
<td>O111:H8</td>
<td>VT2, VT-</td>
<td>181</td>
<td>102</td>
<td>Yukhoe (raw beef)*</td>
</tr>
<tr>
<td>2011</td>
<td>Yamagata P.</td>
<td>Festival (6)</td>
<td>O157:H7</td>
<td>VT1 &amp; 2</td>
<td>287</td>
<td>189</td>
<td>Japanese rice cakes*</td>
</tr>
<tr>
<td>2012</td>
<td>Osaka C.</td>
<td>Nursery school (32)</td>
<td>O26:H-</td>
<td>VT1</td>
<td>68</td>
<td>115</td>
<td>Food borne</td>
</tr>
</tbody>
</table>

*Confirmed microbiologically; ND, no data.

Routes of Transmission of EHEC Infections

Consumption of contaminated foods is the most common source of EHEC infection, and because cattle are considered to be major reservoirs of EHEC, consumption of raw or undercooked foods of bovine origin has been the most common means of transmitting EHEC infection. Laboratory investigations have played a critical role in the investigation of EHEC infections since some cases were geographically dispersed, and without the aid of DNA-based fingerprinting of the isolates, it was difficult to recognize whole cases as a diffuse outbreak. For example, in 2009, there was a diffuse outbreak from a restaurant chain that spread all over Japan. Implicated restaurants were providing cubically assembled meat, a type of processed meat made by mixing and filling different parts of chopped meat, that is frozen and cut into cubes to make the appearance of cube-cut steak. EHEC O157 contaminating the process probably survived in the product, and its intake caused illness. Initially, some cases were reported as sporadic cases by different prefectures, but the results of PFGE and MLVA of EHEC O157 isolates of the cases as well as the product showed identical genotype and strongly suggested that these isolates were part of the outbreak (44). Other examples are the EHEC O157 infections due to consumption of raw beef liver in Aichi Prefecture in 2010 (45). There were four successive food poisoning incidents due to EHEC O157 in Nagoya City, Aichi Prefecture, and all were related to consumption of raw beef liver. During the same period, food poisonings also caused by EHEC O157 occurred in multiple restaurants serving grilled meat in Aichi Prefecture. Laboratory investigation of the cases showed identical genotypes of the isolates, and investigation of the marketing routes revealed that the apparently diffuse incidents were actually the same-sourced food poisoning cases affecting a wide area.
Prevalence of EHEC strains in beef cattle in Japan between November 2007 and March 2008 was reported to be 8.9% and 0.4% for EHEC O157 and O26, respectively, among 2,436 beef cattle reared on 406 farms (54). In another study, prevalence of EHEC strains in 932 healthy dairy cows from 123 farms was 12%, and 31 different O-serogroups, including O26 but not O157, were identified (55). Using stx-PCRs for screening, the same study also found that the prevalence of the stx gene-positive samples among the dairy cows was 30.4%. Although EHEC O157 was found in retail meat at a low frequency (0.1%) in a national food surveillance (56), relatively high prevalence of EHEC in cattle on farms could account for sporadic cases of EHEC and outbreaks when people have contact with these animals at farms (57–59).

Raw vegetables, such as lettuce (44, 46), cabbage (6), and cucumber (6), have been implicated in EHEC outbreaks. Pickled vegetables (6) and lightly salted vegetables (32, 39, 40), which were fresh as salad, have also been implicated in the outbreaks. Trace-back studies of an EHEC O104 outbreak associated with consumption of fenugreek sprouts in Germany (60) and France (61) showed that two outbreaks shared fenugreek seeds imported from Egypt as the most likely common link (62), and studies of a spinach outbreak due to EHEC O157 in the United States also showed that environmental samples including river water, cattle feces, and wild pig feces from the field adjacent to the spinach-growing field were contaminated with identical pulsotype isolates of EHEC O157 (63); however, few trace-back studies have been conducted in Japanese outbreaks associated with consumption of vegetables.

Person-to-person transmission of EHEC is the most likely mode of transmission in the outbreaks reported from nursery schools, and prolonged fecal shedding of EHEC that was similar to that reported in the literature (64–66) was seen in the outbreaks due to EHEC O26 (67), O157 (68), and O103 (69). Because both symptomatic and asymptomatic patients are usually among members of the staff of the daycare and family members of the children at daycare, extended stool culture examination of all children attending daycare (4) and family members would facilitate in taking control measures to prevent further dissemination of EHEC infection.

CONCLUSIONS

The large outbreak in Sakai City and the subsequent outbreaks that occurred domestically in 1996 illustrate some of the serious public health problems associated with EHEC O157 infections. The government initiated a series of preventive control measures against the disease, including enacting the new infectious disease control law in 1999. Since then, however, a gradual increase in infection caused by EHEC has been reported, partly because there have been improvements in methodologies for isolating these organisms and more laboratories were practicing enhanced screening for these organisms. Although EHEC O157 has been isolated predominantly in the EHEC infection, increased rates of non-O157 EHEC cases are being reported with a variety of non-O157 EHEC serotypes. Especially among the outbreaks between 2000 and 2012, one-half of the outbreaks were due to EHEC O157 and the other half were due to O26, O111, O121, O103, O145, and other serogroups; some serious cases were caused by O111. Precautions need to be taken not only for non-O157 EHEC in addition to EHEC O157 but also for emerging types of Shiga toxin-producing enteropathogenic E. coli, such as O104:H4 in Germany; a fatal case due to O86:HNM with similar phenotype to the O104 E. coli was reported in Japan in 1999 (38). Nursery schools have become frequent outbreak settings where EHEC infection was prolonged by person-to-person transmission involving family members of the child. To reduce the risk of outbreaks, it is important to improve the sanitary controls in the nursery schools according to the guideline presented by MHLW to prevent EHEC dissemination.

Laboratory investigations employing DNA-based methods are critically important components in finding and controlling diffuse outbreaks as early as possible while simultaneously conducting epidemiological investigations of the outbreak. It is also important for relevant organizations and bodies to communicate effectively and work together to control and prevent EHEC infection.

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