Shiga Toxin (Verotoxin)-producing *Escherichia coli* and Foodborne Disease: A Review

Jun Terajima¹, Hidemasa Izumiya², Yukiko Hara-Kudo¹, Makoto Ohnishi²

¹ Division of Microbiology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

² Department of Bacteriology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

Shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) is an important cause of foodborne disease. Since outcomes of the infections with STEC have a broad range of manifestation from asymptomatic infection or mild intestinal discomfort, to bloody diarrhea, hemolytic uremic syndrome (HUS), end-stage renal disease (ESRD), and death, the disease is a serious burden in public health and classified as a notifiable infectious disease in many countries. Cattle and other ruminants are considered to be the major reservoirs of STEC though isolation of STEC from other animals have been reported. Hence, the source of contamination extends to a wide range of foods, not only beef products but also fresh produce, water, and environment contaminated by excretes from the animals, mainly cattle. A low-infectious dose of STEC makes the disease relatively contagious, and causes outbreaks with unknown contamination sources and, therefore, as a preventive measure against STEC infection, it is important to obtain characteristics of prevailing STEC isolates in the region through robust surveillance. Analysis of the isolates by pulsed-field gel electrophoresis (PFGE) and multiple-locus variable-number tandem repeat analysis (MLVA) could help finding unrecognized foodborne outbreaks due to consumption of respective contaminated sources. However, though the results of molecular analysis of the isolates could indicate linkage of sporadic cases of STEC infection, it is hardly concluded that the cases are related via contaminated food source if it were not for epidemiological information. Therefore, it is essential to combine the results of strain analysis and epidemiological investigation rapidly to detect rapidly foodborne outbreaks caused by bacteria. This article reviews STEC infection as foodborne disease and further discusses key characteristics of STEC including pathogenesis, clinical manifestation, prevention and control of STEC infection. We also present the recent situation of the disease in Japan based on the surveillance of STEC infection.

Keywords: Shiga toxin-producing *E. coli*, foodborne disease, infection, HUS, Japan

Received: 22 December 2016; Accepted: 31 March 2017; Published online: 3 June 2017

Corresponding author: Jun Terajima, D.V.M., Ph.D. Division of Microbiology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan (terajima@nihs.go.jp)

The contents of this article reflect solely the view of the author(s).

Abbreviations: A/E: attaching and effacing, CDC: Centers for Disease Control and Prevention, CFU: colony forming unit, CNS: central nervous system, DAEC: diffusely adherent *E. coli*, EAEC: enteroaggregative *E. coli*, EC: European Commission EFSA: European Food Safety Authority, EHEC: enterohemorrhagic *E. coli*, EIEC: enteroinvasive *E. coli*, EPEC: enteropathogenic *E. coli*, ER: endoplasmic reticulum, ESRD: end-stage renal disease, ETEC: enterotoxigenic *E. coli*, EU: European Union, FDA: U.S. Food and Drug Administration, FoodNet: Foodborne Diseases Active Surveillance Network, FSIS: Food Safety and Inspection Service, Gb3: glycosphingolipid globotriaosylceramide, HUS: hemolytic uremic syndrome, IDSC: Infectious Disease Surveillance Center, LD₅₀: median lethal dose, LEE: locus of enterocyte effacement, MHLW: Ministry of Health, Labour and Welfare, MLVA: Multiple-locus variable-number tandem repeat analysis, NESID: National Epidemiological Surveillance of Infectious Diseases, NIID: National Institute of Infectious Diseases, PFGE: Pulsed-field gel electrophoresis, STEC: Shiga toxin-producing *E. coli*, Stx: Shiga toxin, Stx1: Shiga toxin type 1, Stx2: Shiga toxin type 2, USDA: U.S. Department of Agriculture

1. General introduction

As an important cause of foodborne disease, it is estimated by searching references published between January 1, 1990 and April 30, 2012 that Shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) causes 2,801,000 acute illnesses annually, and leads to 3,890 cases of hemolytic uremic syndrome (HUS), 270 cases of ESRD, and 230 deaths globally¹⁾. Similar estimation for STEC global burden, that is 2.5 million illnesses and 1.2 million foodborne illnesses annually, has been given by WHO, although Norovirus was the leading cause of foodborne illness, causing 125 million cases and *Campylobacter* spp. caused 96 million foodborne illnesses^{2,3)}.

According to food poisoning statistics by the Ministry of Health, Labour and Welfare (MHLW) in Japan, the average number of food poisoning incidents and cases for the decade from 1981 to 1990 and from 2005 to 2014 were 967 and 35,618 for the former and 1,207 and 25,852 for the latter, respectively⁴⁾. However, approximately 28% of reduction was observed in the number of the cases as a whole and the number of cases per incidents decreased from 36.8 to 21.4 between the two decades. On the other hand, as the number of incidents in the latter grew 1.25 times of that of the former, large foodborne outbreaks have been decreasing in number and small outbreaks and/or sporadic cases have been increasing. There are two different reporting systems for surveillance of STEC infections in Japan. One is based on

the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections (the Infectious Diseases Control Law) and its purpose is to collect and compile reports of nationally notifiable infectious diseases, including STEC infections regardless of the route of infection. Since STEC infection is defined by isolation of STEC from the person except in the case of HUS, when serodiagnosis was possible, asymptomatic patients are also found in this system. The other is based on the Food Sanitation Law that collects reports of foodborne illness from municipal public health agencies and the system focuses on collecting symptomatic patients of food poisoning. Though the major cause of STEC infections is considered to be foodborne, there is a quite big difference between the numbers of STEC infection cases and the numbers of STEC food poisoning reported through each surveillance system (**Fig. 1**). The number of patients in food poisoning due to *Salmonella* spp. or *Vibrio parahaemolyticus* has gradually decreased since 2001 but that of patients due to *Campylobacter jejuni/coli* has remained relatively high compared to that of *Staphylococcus* spp. and STEC. The average number of the cases of STEC infection in the 11-year period from 2005 to 2015 was 3,995 but that of food poisoning due to STEC was only 364 for the same period. Although the number of cases of STEC infection includes 34% of asymptomatic patients during the period of 2009 to 2015⁵⁻¹⁰⁾, number of patients with STEC food poisoning is by far too small to compare to that of the cases of STEC infection. In this article we focus on STEC and STEC infection as foodborne illness discussing potential

Fig. 1. The number of patients of food poisoning by various causal agents in Japan from 2001 to 2015 and the number of notified STEC infection during the same period. Note that the number of patients of food poisoning by STEC is strikingly smaller than that of notified STEC infection. The number of patients with *Salmonella* species and *Vibrio parahaemolyticus* are in decline.

preventive measures against STEC infection.

2. Shiga toxin (verotoxin)-producing *E. coli* (STEC) and STEC infection

2-1. Introduction

Diarrheagenic *E. coli* that are capable of causing disease in healthy individuals can be categorized into six well-described categories: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC)¹¹. The categories of diarrheagenic *E. coli* are differentiated on the basis of pathogenic features reflecting profile of virulence factors of the isolate. We will use the term STEC to denote strains possessing Shiga toxin independent of accompanying virulence factors.

STEC was first recognized as a human pathogen in 1982, when *E. coli* O157:H7 caused two outbreaks of hemorrhagic colitis associated with consumption of undercooked ground beef^{12,13}. Since then, a number of foodborne outbreaks of hemorrhagic colitis and HUS due to not only STEC O157:H7 but also other serotypes of STEC have been reported worldwide. Since STEC resides in the gastrointestinal tract of cattle and other ruminants, contamination of meat with STEC during slaughter is a principal route by which these pathogens enter the food supply. However, a variety of foods have been identified as vehicles of STEC-associated illnesses; these include ground beef¹⁴, roast beef¹⁵, salami¹⁶, raw milk^{17,18}, cheese^{19,20}, ice-cream²¹, yogurt²², Romaine lettuce²³, lettuce²⁴, unpasteurized apple cider or juice^{25–28}, cantaloupe²⁹, spinach³⁰, radish sprouts^{31,32}, and alfalfa sprouts³³. STEC O157:H7 have been predominant serotype of STEC associated with human illness and the serotype had been the major target of most detection methods. But as Shiga toxin and/or genes for Shiga toxin have become the main target of most detection methods in STEC surveillance, more cases of non-O157 STEC are reported. STEC infection remains a leading cause of gastroenteritis among notifiable disease in Japan and is sometimes followed by a severe life-threatening complication such as HUS.

2-2. Pathogenesis

2-2-1 Shiga toxins

Shiga toxins (Stxs) are key virulence factors produced by *Shigella dysenteriae* serotype 1 and STEC. Stxs have been shown to be responsible for exacerbating intestinal damage, and cause systemic complications involving the kidneys and central nervous system (CNS). The toxin is named after Dr. Kiyoshi Shiga, who identified the causative agent of dysen-

tery, Shiga's bacillus, in an outbreak of dysentery in Japan in 1897³⁴. In 1977, Konowalchuk et al. showed that some strains of *E. coli* produced a cytotoxin capable of killing Vero cell, and the cytotoxin was referred to as Vero cytotoxin or Verotoxin³⁵. In addition to the findings with the purification of Shiga toxin from *S. dysenteriae* serotype 1 in 1980^{36,37}, it was reported that a Shiga-like toxin was produced by *E. coli* O157:H7 strain that had caused an outbreak of hemorrhagic colitis in the United States³⁸ and that this toxin was the same as the verotoxin shown to be produced by *E. coli* O157:H7³⁹. Now, the terms Shiga toxins or Verotoxins are used to describe the same toxin⁴⁰.

There are two types of Stxs produced by STEC, Shiga toxin type 1 (Stx1) and Shiga toxin type 2 (Stx2), based on their antigenic characteristics compared to the prototypical Shiga toxin produced by *S. dysenteriae* serotype 1^{41–43}. It has been shown that purified Stx2 has 400-fold lower median lethal dose (LD₅₀) in mice⁴⁴ and is 1,000 times more toxic to human renal endothelial cells than Stx1⁴⁵. Epidemiological evidence also shows that Stx2-producing strains of STEC O157:H7 are more frequently associated with HUS than are strains producing Stx1^{46,47}.

These Shiga toxin family members have a monomeric A and pentameric B molecular configuration, as revealed by X-ray crystallography^{48,49}. A catalytic A subunit is non-covalently associated with a pentamer of identical B fragments that form the B subunit, which is responsible for binding to cell surface receptors, glycosphingolipid globotriaosylceramide (Gb3; also known as CD77 or the Pk blood group antigen)^{50–52}. After binding to Gb3 receptors, the toxins are internalized and undergo retrograde intracellular transport to the endoplasmic reticulum (ER). During transport to the ER, catalytic A subunits dissociate from B subunits by proteolysis and disulfide bond reduction^{53–56}. Since catalytic A subunit has a specific RNA N-glycosidase activity that cleaves an adenine base at position 4,324 of 28S ribosomal RNA of eukaryotic ribosomes^{57,58}, it inhibits elongation factor-dependent amino-acyl tRNA binding and subsequent chain elongation⁵⁹. However, delivery of the toxins to the ER and following retrotranslocation of the catalytic A subunits into the cytoplasm result in not only host cell protein synthesis inhibition, but activation of the ribotoxic stress and ER stress response and, in some cases, the induction of apoptosis, cytokines and chemokines⁶⁰.

The *stx* genes in STEC strains are encoded by the genomes of prophages of the lambdoid family and are located downstream of the phage late gene promoter⁶¹. While *stx1* is under control of the iron-regulated authentic promoter, which will result in induction of Stx1 expression at low concentration of iron⁶², the expression of *stx2* depends primarily on the

late promoter^{63,64}). DNA-damaging agents, such as mitomycin C have been shown to increase Stx production through prophage induction^{65,66}). Stx production in STEC strains, therefore, is intimately correlated with the Stx-encoding phages.

2-2-2 Adhesins

Locus of enterocyte effacement

The ability of STEC to induce attaching and effacing (A/E) lesions of intestinal epithelia is shared by EPEC, *Escherichia albertii* (previously classified as *Hafnia alvei*), and *Citrobacter rodentium*⁶⁷). The A/E lesions were typical histopathological observations in intestinal biopsy specimens from patients and infected animals originally reported with EPEC infection, which are characterized by effacement of microvilli and intimate adherence between the bacterium and the surface of epithelial cells^{68,69}).

The bacterial genes involved in formation of A/E lesion were shown to be located on a 35-kb locus of the chromosome of EPEC and STEC isolates⁶⁷). This locus, called locus of enterocyte effacement (LEE) is not present in non-pathogenic strains of *E. coli* but is found in EPEC and STEC strains capable of producing the A/E lesion. LEE-positive STEC serotypes have been referred to as enterohemorrhagic *E. coli* (EHEC)⁷⁰) and LEE-positive STEC serotypes (such as O157:H7, O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM) are much more commonly associated with HUS and with epidemic diseases than are LEE-negative serotypes^{11,70,71}). The LEE consists of five major operons, which encode a type III secretion system, multiple secreted proteins, a bacterial adhesin called intimin, and a translocated receptor for intimin, Tir^{72–75}). Intimin is a 95-kDa outer membrane protein that is encoded by *eae* gene (*E. coli* attaching and effacing) and necessary for the formation of A/E lesions^{69,76}). The delivery of Tir into the host cell through type III secretion system is followed by binding of intimin to Tir that was recruited to the surface of the host cell membrane, which initiates formation of A/E lesions⁷⁷). Although intimin is the primary adhesin in STEC, there are other adhesins contributing to the adhesive capabilities of STEC, including fimbrial adhesin proteins such as long-polar fimbriae⁷⁸), autotransporters, flagella, and other adhesin proteins reviewed in reference⁷⁹).

2-2-3 Acid tolerance

The infectious dose of STEC is estimated to be as low as or less than 100 organism^{80,81}), which is attributed to its acid-resistant nature^{82–85}). In addition to increasing the possibility of survival of the bacterium under gastric acid environment, the acid tolerance has enabled the pathogen to

survive in various acidic food; apple cider (pH 3.7 to 4.0)⁸⁶), buttermilk (pH 4.1)⁸⁷), yogurt (pH 4.17 to 4.39)^{87,88}), and sour cream (pH 4.3)⁸⁷). Acid resistance mechanism in *E. coli* includes a glucose-repressed system, glutamate- and arginine-dependent systems⁸⁹). While *rpoS* (encoding sigma factor) is essential for expression of glucose-repressed system, glutamate- and arginine decarboxylase are required in amino acid-dependent systems. The two decarboxylase systems are believed to consume protons during the decarboxylation of glutamate or arginine, thereby preventing internal pH of the cell from decreasing to lethal levels⁹⁰).

2-3. Animal Reservoir of STEC

Cattle and other ruminants^{91,92}) are considered to be the major reservoirs of STEC, though STEC has also been isolated from other animals, such as dogs, cats, swine⁹³), and horses⁹⁴). Animal reservoirs for STEC O157:H7 including amphibians and fish, as well as invertebrates, such as insects and mollusks, were reviewed elsewhere^{95,96}). Aquatic species such as finfish and shellfish as dead-end hosts⁹⁶) could transmit the organism to other animals, when they are consumed^{97–99}).

In a survey performed on rectal content samples from 250 beef cattle on 25 beef farms and 250 dairy cows on 25 dairy farms during summer in 2011 in Japan, STEC O157 was isolated from 16 (6.4%) beef cattle on 7 (28%) beef farms, but not obtained from any dairy cows tested¹⁰⁰), and the previous investigation performed by the same authors four years apart showed very similar prevalence of STEC O157 (8.9%)¹⁰¹). In another study, prevalence of STEC strains in 932 healthy dairy cows from 123 farms was 12%, and 31 different O-serogroups, including O26 but not O157, were identified¹⁰²). 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%. Hussein¹⁰³) reviewed published reports and summarized that the prevalence rates of *E. coli* O157 ranged from 0.3 to 19.7% in feedlots and from 0.7 to 27.3% on pasture with regard to beef cattle and that corresponding prevalence rates of non-O157 STEC were 4.6 to 55.9% and 4.7 to 44.8%, respectively.

2-4. Sources of Human Infection

2-4-1. Undercooked Contaminated Beef Products

Since the most common source of STEC infection in human is consumption of contaminated foods, consumption of raw or undercooked foods of bovine origin has been the most common means of transmitting STEC infection. Ground beef is an especially efficient transmission vehicle of STEC and a multistate outbreak was traced to hamburgers distributed by a restaurant chain in 1993¹⁰⁴), and undercooked hamburg-

ers were implicated in a number of other outbreaks^{105–108}. Hamburgers prepared at home were also implicated^{109,110}. Needless to say, raw or undercooked beef products have a higher risk of transmitting contaminated bacteria to human and, in fact, the STEC O157 infections due to consumption of raw beef liver in 2010⁶, a large STEC O111 outbreak due to consumption of Yukhoe, a Korean dish of raw beef and egg yolk⁷, and a diffuse outbreak from a restaurant chain due to cubically assembled meat¹¹¹ were reported in Japan.

2-4-2. Contaminated Fresh Produce

Because STEC can attach to raw or processed fruits and vegetables, produce has also been a vehicle for transmission of the bacteria. Major outbreaks were linked to lettuce¹¹², including a multistate outbreak; sprouts³² and spinach¹¹³ are implicated in numerous HUS cases, and the large outbreak of O104:H4 in 2011 in the European Union (EU) also implicated the consumption of sprouts¹¹⁴. Produce-associated outbreak surveillance data from the Centers for Disease Control and Prevention (CDC, U.S.A.) for the period from 2000 to 2009 showed that, among produce commodities, leafy greens were the most frequently linked to outbreaks¹¹⁵. Typically, produce-mediated outbreaks were linked to foliage contaminated by irrigation/spray water¹¹⁶; STEC O157:H7 were shown to adhere to and penetrate roots¹¹⁷.

Fermentation of food products can reduce the viability of STEC. STEC O157:H7 declined up to 3.5 logs in *soudjouk* sausage^{118,119}, but fermented products may provide a vehicle for infection if curing conditions are inadequate; an outbreak in Sweden was traced to improperly processed sausage¹²⁰. Pickled vegetables⁷ and lightly salted vegetables^{8,121,122}, which were eaten fresh, as salad, have also been implicated in the outbreaks. Salmon roe that was lightly salted as a topping of sushi (*ikura*-sushi) was contaminated with STEC O157:H7 and caused an outbreak in Japan¹²³.

2-4-3. Environment-mediated Transmission

Manure is a good vehicle of STEC and some outbreaks have been associated with public events held on grazing areas, presumably strewn with manure. A scouting event held in Scotland on a muddy field grazed by sheep resulted in an outbreak with Pulsed-field gel electrophoresis (PFGE) indistinguishable isolates from patients, the field, and sheep feces¹²⁴; culturable bacteria were isolated for 15 weeks from the field soil after the outbreak¹²⁵. In a sporadic case of STEC O157 infection in Minnesota, the isolates in garden soil linked to the case could survive on manure-amended soil for more than two months¹²⁶.

Agricultural fairs exhibiting livestock are often implicated in human STEC outbreaks^{127–129}. An investigation of a fair-

associated STEC O157 outbreak suggested that infections can be caused by widespread contamination of a building, since there was no evidence implicating specific food or beverage sources but STEC O157 was recovered from the rafters of the building¹³⁰. In an outbreak of STEC O157:H7 infection associated with attendance at multiple rodeos that had used bulls from the same cattle supplier, isolates from all 14 patients showed indistinguishable PFGE pattern and isolates from nine patients had identical multiple-locus variable-number tandem repeat analysis (MLVA) patterns and five had minor differences, and an isolate of STEC O157 identified from a dirt sample collected from the bullpens of one of the attended rodeos was indistinguishable by PFGE and MLVA from the main outbreak strain¹³¹. STEC O157:H7 was recovered from 3.5% of leafy green samples of the plot at 60 m away from a cattle feedlot, whereas that was recovered from 1.8% of the samples at 180 m away from the same feedlot, suggesting airborne contamination with STEC O157:H7 from cattle production area¹³².

Waterborne STEC infection has been implicated in a number of sporadic cases and outbreaks^{133,134}. STEC outbreaks were associated with swimming in lakes^{135,136} and pools¹³⁷, and consumption of water from a private water supply^{138–140}.

2-4-4. Direct Contact Transmission

Direct human-animal contact can transmit STEC, and the most important prevention step to reduce transmission resulting from human-animal contact is hand-washing¹⁴¹. STEC outbreaks have often been associated with animals in public settings. Children are at most risk, as highlighted by HUS cases in petting zoo¹⁴² or farm^{143,144} visit and in participation in lamb feeding event¹⁴⁵.

STEC can be transmitted to humans through person-to-person transmission. Through National Outbreak Reporting system in USA, estimated 40 foodborne outbreaks of STEC were reported in 2009, and five additional STEC outbreaks were reported as transmitted by person-to-person contact¹⁴⁶. Person-to-person transmission of STEC is a recognized cause of outbreaks in childcare settings^{147–150}, which may be related to close contact of children with immature immune systems and underdeveloped personal hygiene skills.

2-5. Epidemiology

2-5-1. Clinical course

STEC infection has a broad spectrum of clinical manifestation; asymptomatic infection, nonbloody diarrhea, bloody diarrhea (hemorrhagic colitis), and HUS. Typical clinical course of STEC O157:H7 infection begins with ingestion of the organisms, followed by a 3-to-4-day incubation period before the first loose stool. Illness then begins with nonbloody

Fig. 2. Annual incidence rate per 100,000 population of STEC infection in Japan, EU, United States, Australia, New Zealand, and Canada, 2006 to 2014, except for the EU, for which data was available up to 2012.

diarrhea and abdominal cramps. Most persons who seek medical attention develop bloody diarrhea, a typical feature of STEC O157:H7 infection, in the second or third day of illness¹⁵¹. Symptoms of infection with STEC O157:H7 usually subside in about a week, with no obvious sequelae. However, about 6 percent of patients develop HUS¹⁵² and it is usually diagnosed two to 14 days after the onset of diarrhea¹⁵³. HUS is most likely to occur in young children and elderly¹⁵². In addition to age, risk factors for development of HUS include bloody diarrhea, fever, an elevated leukocyte count, antibiotic administration, and use of antimotility agents^{154–156}. Although an association between non-O157 STEC and milder clinical symptoms have been reported^{157–159}, further investigation is needed on whether features of the clinical illness vary among serotypes and how difference in virulence factors might result in differences in clinical outcomes. Although limited data are available on dose response, some findings indicate that the infectious doses of STEC are relatively low. For example, from an outbreak of O111 STEC in beef sausage in Australia, investigators extrapolated a dose range of 1 to 10 organisms, given as few as 1 cell per 10 g of sausage⁸⁰. Using the concentrations of STEC O145 in contaminated ice cream in an outbreak in Belgium, the estimated infective dose was 400 colony forming unit (CFU)²¹. This is comparable to illness from STEC O157:H7, which can result from infection with as few as 10 cells⁸¹.

2-5-2. Surveillance

In the United States, all STEC infections that cause human illness are notifiable to the Nationally Notifiable Diseases Surveillance System. Incidence rate for STEC are shown in **Fig. 2**, and the mean incidence rate for STEC in the period

from 2010 to 2014 was 1.98 in the United States¹⁶⁰. On the other hand, in 2014, the Centers for Disease Control and Prevention (CDC) Emerging Infections Program analyzed the data gathered from the Foodborne Diseases Active Surveillance Network (FoodNet). A total of 697 laboratory-confirmed cases of STEC non-O157 and 444 of STEC O157 infections were identified, with incidence rates of 1.43 and 0.91 per 100,000 persons, respectively¹⁶¹. An examination of STEC cases from Michigan demonstrated an increase in non-O157 STEC¹⁶² and similar increases in non-O157 STEC were reported in other studies^{163–165}. In both the Ontario and British Columbia sentinel sites in Canada, a total of 61 cases of STEC infections were reported between 2011 and 2012, representing an incidence rate of 3.1 cases/100,000 person-years¹⁶⁶. In comparison, the annual combined incidence rate for STEC infection as notifiable disease in Canada for both years was 1.9 cases/100,000 person-years (**Fig. 2**). While a slight decrease in the incidence rate has been observed in Canada, the apparent increasing trend of the incidence rate was reported from New Zealand¹⁶⁷. Except for the sudden increase of the incidence rate in the EU in 2011--probably due to a large outbreak of STEC O104 in Germany--it remained less than 1.5 cases/100,000 in the EU, though the data was available only up to 2012¹⁶⁸, and the incidence rate in Australia has been about 0.5 since 2007^{169–173}.

In Japan, STEC infection is a category III notifiable infectious disease, along with other bacterial infections caused by *Vibrio cholerae* O1 or O139, *Shigella* species, *Salmonella enterica* serovar Typhi, and *Salmonella enterica* serovar Paratyphi A in the National Epidemiological Surveillance of Infectious Diseases (NESID) under the Infectious Diseases Control Law enacted in April 1999. Despite control measures

instituted since 1996, including designating STEC infection as a notifiable disease, and the disease being monitored effectively through nationwide surveillance, the annual incidence rate remains around 3.0 per 100,000 population (**Fig. 2**). Under a surveillance system for food poisoning based on the Food Sanitation Law, an STEC 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 MHLW. During the investigation of the outbreaks, family members and colleagues of the patients were encouraged to have stool examination and it was revealed that approximately 35% of STEC 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 groups¹⁷⁴.

Apart from NESID, results of characterization (serotypes, Stx types, etc.) of STEC isolates at prefectural and municipal PHIs are reported to the Infectious Disease Surveillance Center (IDSC) at the National Institute of Infectious Diseases (NIID). The summary showed that STEC O157 serogroup is the predominant one, followed by O26, O111, O103, O121, O145, and others. However, as seen in the United States¹⁶³ and continental Europe^{175,176}, the percentage of non-STEC O157 serogroups among all STEC isolates from human infection has been increasing slightly; the rate of isolation frequency of STEC O157 has declined from approximately 70% of all STEC isolates in 2000 to about 60% in 2015^{177,178}. As a collaborating laboratory surveillance system between prefectural and municipal PHIs and NIID, PulseNet Japan, a national laboratory network that connects foodborne illness cases to detect outbreaks by the use of DNA fingerprinting of the isolates, has been established¹⁷⁹. It constitutes a part of PulseNet International¹⁸⁰, and has contributed to investigations of domestic¹⁸¹ and international STEC O157 outbreaks¹⁸².

2-5-3. Outbreaks in Japan

In the outbreaks with more than 10 culture-positive patients reported to the IDSC from 2000 to 2012, 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 unknown¹⁷⁴. One major setting of these outbreaks was nursery schools, which may account for the high proportion of person-to-person transmissions of the infection in the outbreaks. The most prevalent serogroup of STEC in the outbreaks in nursery schools was O26 (52%), followed by O157 (27%), O111 (9%), O103 (4%), O121 (4%), O145 (3%), and OUT¹⁷⁴, which may account for relatively

mild clinical manifestations, including asymptomatic cases and, consequently, frequent person-to-person transmission observed in these outbreaks. Increased STEC outbreaks in childcare facilities due to non-O157 serogroups, particularly O26 and O111 during 2010 to 2013, were also reported by another group in Japan¹⁵⁰. There were 13 STEC outbreaks that had more than 100 culture positive cases between 2000 and 2015 (**Table 1**)^{183,184}. All 13 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 products¹²¹; lightly salted cucumber¹²²; *Koumi-ae* consisting of boiled spinach and steamed chicken meat seasoned with welsh onion, ginger, and soy sauce¹²²; boxed meals¹⁸⁵; lettuce⁵; school lunches⁶; *Yukhoe* (raw beef)⁷, and Japanese rice cakes⁷.

Low infectious doses of STEC--possibly fewer than 10 organisms^{80,81}--is a critical factor in the transmission of the STEC, when people consume raw or lightly cooked foods such as sushi and vegetables. Because sushi and raw or lightly cooked meat are popular foods in Japan, there have been outbreaks associated with consumption of salmon roe sushi in 1998¹²³ and "rare" roast beef contaminated with STEC O157 in 2001¹²¹. In an STEC O111 outbreak associated with consumption of *Yukhoe* at Yakiniku chain restaurants, STEC 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 died⁷. HUS occurred most frequently in individuals aged 5–9 years, and this age group was significantly associated with acute encephalopathy¹⁸⁶. STEC O111:H8 was also isolated from the conserved part of the original meat preparations distributed to the chain restaurants.

Some of the outbreaks were associated with consumption of vegetables. In addition to two large outbreaks in 2011 (**Table 1**), there were four outbreaks associated with consumption of vegetables⁷; cabbage was identified as a vehicle of STEC O26:H11 in an outbreak and STEC O157:H7 was isolated from pickled eggplant and green perilla, green perilla served with grated radish, and cucumber in three outbreaks, respectively. A large outbreak of STEC O157:H7 infection traced to a brand of lightly salted vegetables occurred in Sapporo, Hokkaido in 2012⁸. STEC O157:H7 was isolated from the implicated product. Since the products were widely distributed, 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 STEC O157:H7--with indistinguishable PFGE patterns and identical MLVA type--was isolated. STEC O157:H7 was isolated from 73 of 169 patients, eight of whom, mostly elderly,

Table 1 Foodborne Outbreaks Caused by STEC in Japan

Year	Prefecture/City	Setting (reference)	Serotype	Stx type	Symptomatic cases	Culture positives	Likely mode of transmission
2001	Chiba P.	Patient's home (113)	O157:H7	Stx1&2	195	257	beef products (a)
2002	Fukuoka C.	Nursery school (114)	O157:H-	Stx2	74	112	lightly salted cucumber (a)
2002	Utsunomiya C.	Hospital and home for the elderly (114)	O157:H7	Stx1&2	123	111	Koumi-ae (a)
2003	Yokohama C.	Kindergarten (183)	O26:H11	Stx1	141	449	Foodborne
2004	Ishikawa P.	High school (184)	O111:H-	Stx1&2	110	103	Foodborne
2007	Tokyo M.	School refectory (173)	O157:H7	Stx2	467	204	Foodborne
2007	Miyagi P., Sendai C. & Akita C.	Restaurant (173)	O157:H7	Stx1&2	314	173	boxed meals (a)
2009	Saga P.	Nursery school (5)	O26:H11	Stx1	N.D.	133	lettuce (a)
2010	Mie P.	High school (6)	O157:H7	Stx2	138	164	school lunch (a)
2011	Toyama P.	Chain restaurants (7)	O111:H8	Stx2, Stx-	181	102	Yukhoe (raw beef) (a)
			O157:H7	Stx1, Stx2, Stx1&2		38	
2011	Yamagata P.	Festival (7)	O157:H7	Stx1&2	287	189	Japanese rice cakes (a)
2012	Osaka C.	Nursery school (8)	O26:H-	Stx1	68	115	Foodborne
2014	Shizuoka C.	Street stall (10)	O157:H7	Stx1&2	510	193	Foodborne

(a) Confirmed microbiologically; ND, no data.

died. In August 2014, there was STEC O157 food poisoning of 510 cases, who consumed contaminated lightly pickled cucumbers sold at food stands during a fireworks display in Shizuoka Prefecture¹⁰.

2-6. HUS

HUS is a life-threatening illness characterized by hemolytic anemia, thrombocytopenia, and renal failure, and it is the most common cause of acute renal failure among children in the United States¹⁸⁷. Foodborne Diseases Active Surveillance Network reported that among 3464 STEC O157 infections in the period of 2000-2006, 218 persons (6.3%) developed HUS and the highest proportion of HUS cases (15.3%) occurred among children aged less than 5 years and that death occurred in 0.6% of all patients with STEC O157 infection and in 4.6% of those with HUS¹⁸⁸. In Italy, an average of 33 sporadic cases of HUS per year were observed between 1988 and 2012, with a mean annual incidence of 0.4 cases per 100,000 residents aged 0–15 years¹⁷⁶. In 15 EU countries, A total of 382 (6.6%) confirmed STEC cases (n=5746) developed HUS in 2012 and 59 per cent of HUS cases (n=226) were reported in 0–4 year-old children with O157 and O26 as dominant serogroups followed by 5–14 year

old children with O157 as a dominant serogroup (74%)¹⁶⁸. In Argentina, postdiarrheal HUS is endemic and approximately 400 HUS cases were reported annually between 2002 and 2011. The incidence ranged from 10 to 17 cases per 100,000 children less than 5 years of age, and lethality was between 1 and 4%¹⁸⁹.

In Japan, due to an amendment of the case definition of STEC in the Infectious Diseases Control Law in 2006, HUS patient should be reported as having STEC infection if Stx was detected in feces, or O-antigen agglutinating antibody or anti-Stx antibody was detected in the serum of the patient. From 2006 to 2015, the average annual number of HUS cases (including serodiagnosed cases) was 99 and the incidence rate of HUS (HUS cases/symptomatic cases) was 3.6% (**Fig. 3**)^{111,190–197}. As reported in previous studies, increased rates of HUS in children less than 10 years old and the elderly^{198,199} are shown in **Fig. 3**.

From 2006 to 2015, 65% of the 985 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 Stx in the stool samples of the patients.

STEC O157 was the predominant serogroup, occupying

Fig. 3. Incidence rate of HUS in STEC infection by age groups in Japan, 2006 to 2015. Incidence rate (%) was calculated as (number of patients with HUS) ÷ (number of symptomatic cases) × 100%.

85% of all isolates in culture-confirmed HUS cases, followed by O111 (4.4%), O26 (2.7%), O121 (2.3%), O165 (1.2%), O145 (0.6%), and the rest of the O serogroups, including O55, O74, O76, O115, O174, O183, and unknown serogroup samples. Although non-O157 serogroup strains were isolated in the culture-confirmed HUS cases, 94% of all STEC isolates in the culture-confirmed HUS cases were either Stx2 or both Stx1 and Stx2 producers, which is consistent with epidemiological evidence that Stx2-containing STEC O157:H7 strains are more frequently associated with HUS than the strains containing Stx1^{46,47,71}).

2-7. Infection Control

Although it is rare to be able to find the source of infection in sporadic cases of STEC infection, many outbreaks of STEC infection have been associated with foods that become contaminated through direct or indirect environmental exposure to waste products of cattle. Therefore, implementing effective measures to reduce or eliminate STEC from all stages of the food chain, starting from production to consumption would lead to reducing STEC infection in humans. All steps ranging from reducing carriage of STEC by cattle used in food production to proper food preparation to kill STEC before consumption should be included. Certain farm management practices, especially those related to prevention of contamination and multiplication of STEC in feed and water, may provide practical means to reduce the prevalence of STEC in cattle on farms and in slaughter plants²⁰⁰. Good

hygienic practices during food production are essential to keep microbiological contamination to a minimum. The most effective method of eliminating STEC from foods is to introduce a bactericidal treatment, such as heating^{201,202} or irradiation^{203–206}. Since person-to-person contact is an important mode of transmission through the oral-fecal route in STEC infection, good hygiene practice is especially important in settings such as child-care facilities, where persons at high risk for STEC infection spend considerable time together. In addition, as the median duration of shedding reported from previous outbreaks in childcare facilities has been found to be between 20 and 50 days^{147,207–209}, exclusion of ill persons from the facilities until the diarrhea has resolved should be considered. In general, routine handwashing before eating and after diaper changes and toileting is the best way to prevent the spread of infection in child-care facilities.

3. Control Measures Against Foodborne Disease Due to STEC

Among preharvest food safety interventions to reduce the prevalence and shedding level of STEC O157 by cattle, vaccines have been the most effective interventions documented to date.

Currently, only two commercially available vaccines against *E. coli* O157:H7 in cattle exist: a type III secreted proteins (Bioniche Life Sciences Inc., Belleville, Ontario, Canada), and a siderophore receptor and porin protein (Epi-

topix, LLC, Wilmar, MN, USA). Several systematic reviews and meta-analyses suggested that both vaccines effectively reduce the probability of feedlot cattle to shed STEC O157 in feces^{210–213}.

Some of the lessons learned from STEC outbreaks have resulted in long-term improvements in food safety. In 1994, the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) declared STEC O157:H7 an adulterant in ground beef in response to the multistate outbreak caused in 1993 by undercooked hamburgers contaminated with STEC O157²¹⁴. Following these outbreaks, the U.S. Food and Drug Administration (FDA) issued more stringent guidelines for the internal temperature of cooked hamburgers²¹⁵. The pronouncement from FSIS was extended in 1999 to all nonintact raw beef products²¹⁶. Since the number of infections and outbreaks due to STEC non-O157 has increased, FSIS documented risk profile for pathogenic non-O157 STEC and concluded that STEC O157 was not the only STEC representing a hazard²¹⁷. Furthermore, FSIS decided on the implementation of sampling and testing manufacturing trim and other raw ground beef product components for STEC non-O157 in 2012²¹⁸. STEC strains of serogroups O26, O111, O103, O145, O121, and O45 were declared as adulterant in these food commodities and included in the sampling plans in addition to STEC O157.

In the European Union (EU), although the limitations exist in categorizing the level of danger associated with STEC from nonhuman sources, the seropathotypes A and B of Karmali's scheme²¹⁹ formed a large consensus in the scientific community and were endorsed by the European Food Safety Authority (EFSA). The scheme was based on the evaluation of the virulence and serological features of the strains combined with their association with severe disease and epidemic outbreaks. STEC strains belonging to serogroups O157, O26, O111, O103, and O145 were included in the seropathotypes A and B of the scheme. EFSA recommended focusing food testing for STEC on the seropathotype A and B groups^{220,221}. However, the massive outbreak caused by an enteroaggregative STEC O104:H4 strain in 2011 in Germany and other 12 European countries forced the European Commission (EC) to take measures against the possibility of other STEC crises in the EU. EFSA was asked to assess the public health risk caused by STEC and other pathogenic bacteria that may contaminate both seeds and sprouted seeds intended for direct human consumption^{222,223}. Finally, the European Commission issued Regulation (EU) 209/2013, containing the microbiological criteria for STEC in sprouts and amending Regulation (EC) 2073/2005, which introduced for the first time in EU legislation a specific criterion for STEC regarding the presence in sprouts of the five STEC

serogroups, i.e. O157, O26, O111, O103, and O145 plus STEC O104:H4²²⁴.

In Japan, STEC infections have been routinely notifiable since 1996. They are also reported as food poisoning by physicians or judged as such by the director of the health center under a surveillance system for food poisoning. Furthermore, the Abattoir Law Enforcement Regulation (Ministry of Health and Welfare Ordinance No. 44, September 28, 1953) was amended by the MHLW so that the measures for prevention and reduction of STEC contamination at every step of the processes such as tying the rectum before evisceration at slaughter houses could be fulfilled. The MHLW has also formulated standards of a hygienic control manual for large cooking facilities based on the HACCP (Hazard Analysis and Critical Control Point) to prevent food poisoning due to food provided by these facilities. Although the official detection methods for STEC O26, O111, and O157²²⁵ was in use, a newer detection method for STEC O26, O103, O111, O121, O145, and O157 in food²²⁶ has been established and validated,²²⁷ reflecting a steady increase in non-O157 STEC infections. The method involved a combination of various chromogenic agars, targeting particular STEC serogroups, and molecular approaches such as real-time PCR based on the methods used by the USDA²²⁸ and EFSA²²¹. In response to persistent food poisonings caused by raw beef, the MHLW revised the standards of beef product quality for raw-eating and put them into operation in October 2011. Further, after STEC O157 was detected in the inner part of cattle livers, the MHLW banned the marketing of cattle liver intended to be eaten raw. Probably as a consequence of these preventive measures, the incidence of STEC O157 cases related to consumption of raw meat decreased by almost half in one year, from 2011 to 2012⁸.

4. Conclusions

STEC infections continue to occur due to a variety of foods contaminated by this bacterium. Although foods of bovine origin contaminated with this pathogen have been a major source for infection, evidence of disease linked to other sources, including contaminated produce, water, and other environmental exposures, indicates a necessity for a comprehensive approach to STEC reduction or elimination at all levels of food production. However, despite various control measures taken today, reported incidence of non-O157 STEC has been gradually increasing, partially due to recent improved detection methods, and massive outbreak due to a rare category of STEC that belonged to enteroaggregative *E. coli* occurred in Europe, indicating the complexity of securing food safety when dealing with STEC.

Surveillance of STEC infections, especially laboratory investigation of the implicated isolates based on molecular analytical methods, have revealed that complex ecology and genetics of this pathogen existed and indicated that combining the results of isolate analysis with epidemiological information were important for accurate determination of the infectious source. Therefore, cooperative interplay of relevant authorities referring to public health, food, and veterinary science are indispensable for establishment of effective control measures and prevention against STEC infection.

Acknowledgements

We thank all investigators of prefectural and municipal public health institutes for providing us samples and information, and the IDSC staff for data collection and analysis. We also thank the Food Safety Committee of Japan for its support in writing the manuscript.

Conflict of interest statement

The authors had no conflicts of interest to declare in this article.

References

1. Majowicz SE, Scallan E, Jones-Bitton A, et al. Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. *Foodborne Pathog Dis*. 2014; **11**: 447–455. PMID: 24750096 doi: 10.1089/fpd.2013.1704
2. Kirk MD, Pires SM, Black RE, et al. Correction: World Health Organization Estimates of the Global and Regional Disease Burden of 22 Foodborne Bacterial, Protozoal, and Viral Diseases, 2010: A Data Synthesis. *PLoS Med*. 2015; **12**: e1001940. PMID: 26701262 doi: 10.1371/journal.pmed.1001940
3. Kirk MD, Pires SM, Black RE, et al. World Health Organization Estimates of the Global and Regional Disease Burden of 22 Foodborne Bacterial, Protozoal, and Viral Diseases, 2010: A Data Synthesis. *PLoS Med*. 2015; **12**: e1001921. PMID: 26633831 doi: 10.1371/journal.pmed.1001921
4. Japanese Ministry of Health Labour and Welfare Food Poisoning Statistics. Available at : http://www.mhlw.go.jp/file/06-Seisakujouhou-11130500-Shokuhinanzendu/nenji_3.xls. Food Poisoning Statistics. 2015.
5. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of May 2010. *Infec Agen Surv Rep*. 2010; **31**: 152–153.
6. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2011. *Infec Agen Surv Rep*. 2011; **32**: 125–126.
7. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2012. *Infec Agen Surv Rep*. 2012; **33**: 115–116.
8. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2013. *Infec Agen Surv Rep*. 2013; **34**: 123–124.
9. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2014. *Infec Agen Surv Rep*. 2014; **35**: 117–118.
10. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2015. *Infec Agen Surv Rep*. 2015; **36**: 73–74.
11. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev*. 1998; **11**: 142–201. PMID: 9457432
12. Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med*. 1983; **308**: 681–685. PMID: 6338386 doi: 10.1056/NEJM198303243081203
13. Centers for Disease Control and Prevention Isolation of *E. coli* O157:H7 from sporadic cases of hemorrhagic colitis—United States. *MMWR Morb Mortal Wkly Rep*. 1982; **31**: 585–588. PMID: 6817063
14. Erickson MC, Doyle MP. Food as a vehicle for transmission of Shiga toxin-producing *Escherichia coli*. *J Food Prot*. 2007; **70**: 2426–2449. PMID: 17969631 doi: 10.4315/0362-028X-70.10.2426
15. Rodrigue DC, Mast EE, Greene KD, et al. A university outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. *J Infect Dis*. 1995; **172**: 1122–1125. PMID: 7561194 doi: 10.1093/infdis/172.4.1122
16. Centers for Disease Control and Prevention (CDC) *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami--Washington and California, 1994. *MMWR Morb Mortal Wkly Rep*. 1995; **44**: 157–160. PMID: 7870024
17. Guh A, Phan Q, Nelson R, et al. Outbreak of *Escherichia coli* O157 associated with raw milk, Connecticut, 2008. *Clin Infect Dis*. 2010; **51**: 1411–1417. PMID: 21058911 doi: 10.1086/657304
18. Centers for Disease Control and Prevention (CDC) *Escherichia coli* O157:H7 infections in children associated with raw milk and raw colostrum from cows--California, 2006. *MMWR Morb Mortal Wkly Rep*. 2008; **57**: 625–628. PMID: 18551097
19. Gaulin C, Levac E, Ramsay D, et al. *Escherichia coli* O157:H7 outbreak linked to raw milk cheese in Quebec, Canada: use of exact probability calculation and casecase study approaches to foodborne outbreak investigation. *J Food Prot*. 2012; **75**: 812–818. PMID: 22564928 doi: 10.4315/0362-028X.JFP-11-385

20. Gill A, Oudit D. Enumeration of *Escherichia coli* O157 in Outbreak-Associated Gouda Cheese Made with Raw Milk. *J Food Prot.* 2015; **78**: 1733–1737. PMID: 26319728 doi: 10.4315/0362-028X.JFP-15-036
21. Buvens G, Possé B, De Schrijver K, De Zutter L, Lauwers S, Piérard D. Virulence profiling and quantification of verocytotoxin-producing *Escherichia coli* O145:H28 and O26:H11 isolated during an ice cream-related hemolytic uremic syndrome outbreak. *Foodborne Pathog Dis.* 2011; **8**: 421–426. PMID: 21166561 doi: 10.1089/fpd.2010.0693
22. Morgan D, Newman CP, Hutchinson DN, Walker AM, Rowe B, Majid F. Verotoxin producing *Escherichia coli* O 157 infections associated with the consumption of yoghurt. *Epidemiol Infect.* 1993; **111**: 181–187. PMID: 8405146 doi: 10.1017/S0950268800056880
23. Taylor EV, Nguyen TA, Machesky KD, et al. Multistate outbreak of *Escherichia coli* O145 infections associated with romaine lettuce consumption, 2010. *J Food Prot.* 2013; **76**: 939–944. PMID: 23726187 doi: 10.4315/0362-028X.JFP-12-503
24. Doyle MP, Erickson MC. Summer meeting 2007 - the problems with fresh produce: an overview. *J Appl Microbiol.* 2008; **105**: 317–330. PMID: 18284485
25. Besser RE, Lett SM, Weber JT, et al. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA.* 1993; **269**: 2217–2220. PMID: 8474200 doi: 10.1001/jama.1993.03500170047032
26. Centers for Disease Control and Prevention (CDC) Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider--Connecticut and New York, October 1996. *MMWR Morb Mortal Wkly Rep.* 1997; **46**: 4–8. PMID: 9011776
27. Tamblyn S, deGrosbois J, Taylor D, Stratton J. An outbreak of *Escherichia coli* O157:H7 infection associated with unpasteurized non-commercial, custom-pressed apple cider--Ontario, 1998. *Can Commun Dis Rep.* 1999; **25**: 113–117, discussion 117–120. PMID: 10448225
28. Hilborn ED, Mshar PA, Fiorentino TR, et al. An outbreak of *Escherichia coli* O157:H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider. *Epidemiol Infect.* 2000; **124**: 31–36. PMID: 10722127 doi: 10.1017/S0950268899003258
29. Beuchat LR. Pathogenic Microorganisms Associated with Fresh Produce. *Journal of Food Protection.* 1996; **59**: 204–216. doi: 10.4315/0362-028X-59.2.204
30. Centers for Disease Control and Prevention (CDC) Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach--United States, September 2006. *MMWR Morb Mortal Wkly Rep.* 2006; **55**: 1045–1046. PMID: 17008868
31. Watanabe Y, Ozasa K, Mermin JH, et al. Factory outbreak of *Escherichia coli* O157:H7 infection in Japan. *Emerg Infect Dis.* 1999; **5**: 424–428. PMID: 10341179 doi: 10.3201/eid0503.990313
32. Michino H, Araki K, Minami S, et al. Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *Am J Epidemiol.* 1999; **150**: 787–796. PMID: 10522649 doi: 10.1093/oxfordjournals.aje.a010082
33. Centers for Disease Control and Prevention Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts-Michigan and Virginia, June-July 1997. *MMWR Morb Mortal Wkly Rep.* 1997; **46**: 741–744.
34. Trofa AF, Ueno-Olsen H, Oiwa R, Yoshikawa M. Dr. Kiyoshi Shiga: discoverer of the dysentery bacillus. *Clin Infect Dis.* 1999; **29**: 1303–1306. PMID: 10524979 doi: 10.1086/313437
35. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. *Infect Immun.* 1977; **18**: 775–779. PMID: 338490
36. O'Brien AD, LaVeck GD, Griffin DE, Thompson MR. Characterization of *Shigella dysenteriae* 1 (Shiga) toxin purified by anti-Shiga toxin affinity chromatography. *Infect Immun.* 1980; **30**: 170–179. PMID: 7002787
37. Olsnes S, Eiklid K. Isolation and characterization of *Shigella shigae* cytotoxin. *J Biol Chem.* 1980; **255**: 284–289. PMID: 7350160
38. O'Brien AO, Lively TA, Chen ME, Rothman SW, Formal SB. *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (SHIGA) like cytotoxin. *Lancet.* 1983; **1**: 702. PMID: 6132054 doi: 10.1016/S0140-6736(83)91987-6
39. Johnson W. M., Lior H., Bezanson G. S. Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada. *Lancet.* 1983; **1**(8314-5): 76.
40. Kaper JB, O'Brien AD. Overview and Historical Perspectives. *Microbiol Spectr.* 2014; **2**: 3–13. PMID: 25590020 doi: 10.1128/microbiolspec.EHEC-0028-2014
41. Scheutz F, Teel LD, Beutin L, et al. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *J Clin Microbiol.* 2012; **50**: 2951–2963. PMID: 22760050 doi: 10.1128/JCM.00860-12
42. Strockbine NA, Marques LR, Newland JW, Smith HW, Holmes RK, O'Brien AD. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infect Immun.* 1986; **53**: 135–140. PMID: 3522426
43. Scheutz F. Taxonomy Meets Public Health: The Case of Shiga Toxin-Producing *Escherichia coli*. *Microbiol Spectr.* 2014; **2**. PMID: 26103973 doi: 10.1128/microbiolspec.EHEC-0019-2013
44. Tesh VL, Burris JA, Owens JW, et al. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. *Infect Immun.* 1993; **61**: 3392–3402. PMID: 8335369
45. Louise CB, Obrig TG. Specific interaction of *Escherichia coli* O157:H7-derived Shiga-like toxin II with human renal endothelial cells. *J Infect Dis.* 1995; **172**: 1397–1401. PMID: 7594687 doi: 10.1093/infdis/172.5.1397
46. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. *J Infect Dis.* 1989; **160**: 994–998. PMID: 2685131 doi: 10.1093/infdis/160.6.994
47. Scotland SM, Willshaw GA, Smith HR, Rowe B. Properties of strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2. *Epidemiol Infect.* 1987; **99**: 613–624. PMID: 3322851 doi: 10.1017/S0950268800066462

48. Fraser ME, Chernaia MM, Kozlov YV, James MN. Crystal structure of the holotoxin from *Shigella dysenteriae* at 2.5 Å resolution. *Nat Struct Biol.* 1994; **1**: 59–64. PMID: 7656009 doi: 10.1038/nsb0194-59
49. Stein PE, Boodhoo A, Tyrrell GJ, Brunton JL, Read RJ. Crystal structure of the cell-binding B oligomer of verotoxin-1 from *E. coli*. *Nature.* 1992; **355**: 748–750. PMID: 1741063 doi: 10.1038/355748a0
50. Jacewicz M, Clausen H, Nudelman E, Donohue-Rolfe A, Keusch GT. Pathogenesis of shigella diarrhea. XI. Isolation of a shigella toxin-binding glycolipid from rabbit jejunum and HeLa cells and its identification as globotriaosylceramide. *J Exp Med.* 1986; **163**: 1391–1404. PMID: 3519828 doi: 10.1084/jem.163.6.1391
51. Lindberg AA, Brown JE, Strömberg N, Westling-Ryd M, Schultz JE, Karlsson KA. Identification of the carbohydrate receptor for Shiga toxin produced by *Shigella dysenteriae* type 1. *J Biol Chem.* 1987; **262**: 1779–1785. PMID: 3543013
52. Waddell T, Cohen A, Lingwood CA. Induction of verotoxin sensitivity in receptor-deficient cell lines using the receptor glycolipid globotriaosylceramide. *Proc Natl Acad Sci USA.* 1990; **87**: 7898–7901. PMID: 2236008 doi: 10.1073/pnas.87.20.7898
53. Garred O, van Deurs B, Sandvig K. Furin-induced cleavage and activation of Shiga toxin. *J Biol Chem.* 1995; **270**: 10817–10821. PMID: 7738018 doi: 10.1074/jbc.270.18.10817
54. Garred O, Dubinina E, Polesskaya A, Olsnes S, Kozlov J, Sandvig K. Role of the disulfide bond in Shiga toxin A-chain for toxin entry into cells. *J Biol Chem.* 1997; **272**: 11414–11419. PMID: 9111051 doi: 10.1074/jbc.272.17.11414
55. Garred O, Dubinina E, Holm PK, et al. Role of processing and intracellular transport for optimal toxicity of Shiga toxin and toxin mutants. *Exp Cell Res.* 1995; **218**: 39–49. PMID: 7737376 doi: 10.1006/excr.1995.1128
56. LaPointe P, Wei X, Gariépy J. A role for the protease-sensitive loop region of Shiga-like toxin 1 in the retrotranslocation of its A1 domain from the endoplasmic reticulum lumen. *J Biol Chem.* 2005; **280**: 23310–23318. PMID: 15817449 doi: 10.1074/jbc.M414193200
57. Endo Y, Tsurugi K, Yutsudo T, Takeda Y, Ogasawara T, Igarashi K. Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. *Eur J Biochem.* 1988; **171**: 45–50. PMID: 3276522
58. Saxena SK, O'Brien AD, Ackerman EJ. Shiga toxin, Shiga-like toxin II variant, and ricin are all single-site RNA N-glycosidases of 28 S RNA when microinjected into *Xenopus* oocytes. *J Biol Chem.* 1989; **264**: 596–601. PMID: 2642481
59. Hale TL, Formal SB. Cytotoxicity of *Shigella dysenteriae* 1 for cultured mammalian cells. *Am J Clin Nutr.* 1980; **33**(Suppl): 2485–2490. PMID: 7001884
60. Johannes L, Römer W. Shiga toxins--from cell biology to biomedical applications. *Nat Rev Microbiol.* 2010; **8**: 105–116. PMID: 20023663
61. Waldor MK, Friedman DI. Phage regulatory circuits and virulence gene expression. *Curr Opin Microbiol.* 2005; **8**: 459–465. PMID: 15979389 doi: 10.1016/j.mib.2005.06.001
62. Calderwood SB, Mekalanos JJ. Iron regulation of Shiga-like toxin expression in *Escherichia coli* is mediated by the fur locus. *J Bacteriol.* 1987; **169**: 4759–4764. PMID: 3308853 doi: 10.1128/jb.169.10.4759-4764.1987
63. Wagner PL, Waldor MK. Bacteriophage control of bacterial virulence. *Infect Immun.* 2002; **70**: 3985–3993. PMID: 12117903 doi: 10.1128/IAI.70.8.3985-3993.2002
64. Tyler JS, Mills MJ, Friedman DI. The operator and early promoter region of the Shiga toxin type 2-encoding bacteriophage 933W and control of toxin expression. *J Bacteriol.* 2004; **186**: 7670–7679. PMID: 15516581 doi: 10.1128/JB.186.22.7670-7679.2004
65. al-Jumaili I, Burke DA, Scotland SM, al-Mardini H, Record CO. A method of enhancing verocytotoxin production by *Escherichia coli*. *FEMS Microbiol Lett.* 1992; **72**: 121–125. PMID: 1505736 doi: 10.1111/j.1574-6968.1992.tb05077.x
66. Wagner PL, Neely MN, Zhang X, Acheson DW, Waldor MK, Friedman DI. Role for a phage promoter in Shiga toxin 2 expression from a pathogenic *Escherichia coli* strain. *J Bacteriol.* 2001; **183**: 2081–2085. PMID: 11222608 doi: 10.1128/JB.183.6.2081-2085.2001
67. McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc Natl Acad Sci USA.* 1995; **92**: 1664–1668. PMID: 7878036 doi: 10.1073/pnas.92.5.1664
68. Moon HW, Whipp SC, Argenzio RA, Levine MM, Giannella RA. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. *Infect Immun.* 1983; **41**: 1340–1351. PMID: 6350186
69. Jerse AE, Yu J, Tall BD, Kaper JB. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc Natl Acad Sci USA.* 1990; **87**: 7839–7843. PMID: 2172966 doi: 10.1073/pnas.87.20.7839
70. Kaper JB, Mellies JL, Nataro J. Pathogenicity islands and other mobile genetic elements of diarrheagenic *Escherichia coli*. Washington, D.C.: ASM Press; 1999.
71. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J Clin Microbiol.* 1999; **37**: 497–503. PMID: 9986802
72. Elliott SJ, Wainwright LA, McDaniel TK, et al. The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic *Escherichia coli* E2348/69. *Mol Microbiol.* 1998; **28**: 1–4. PMID: 9593291 doi: 10.1046/j.1365-2958.1998.00783.x
73. Perna NT, Mayhew GF, Pósfai G, et al. Molecular evolution of a pathogenicity island from enterohemorrhagic *Escherichia coli* O157:H7. *Infect Immun.* 1998; **66**: 3810–3817. PMID: 9673266
74. Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell.* 1997; **91**: 511–520. PMID: 9390560 doi: 10.1016/S0092-8674(00)80437-7
75. Stevens M. P., Frankel G. M. The Locus of Enterocyte Effacement and Associated Virulence Factors of Enterohemorrhagic *Escherichia coli*. *Microbiol Spectr.* 2014;2(4):EHEC-0007-2013.

76. Yu J, Kaper JB. Cloning and characterization of the *eae* gene of enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol.* 1992; **6**: 411–417. PMID: 1552854 doi: 10.1111/j.1365-2958.1992.tb01484.x
77. DeVinney R, Stein M, Reinscheid D, Abe A, Ruschkowski S, Finlay BB. Enterohemorrhagic *Escherichia coli* O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated. *Infect Immun.* 1999; **67**: 2389–2398. PMID: 10225900
78. Ross BN, Rojas-Lopez M, Cieza RJ, McWilliams BD, Torres AG. The Role of Long Polar Fimbriae in *Escherichia coli* O104:H4 Adhesion and Colonization. *PLoS One.* 2015; **10**: e0141845. PMID: 26517878
79. McWilliams BD, Torres AG. Enterohemorrhagic *Escherichia coli* Adhesins. *Microbiol Spectr.* 2014; **2**. PMID: 26103974 doi: 10.1128/microbiolspec.EHEC-0003-2013
80. Paton AW, Ratcliff RM, Doyle RM, et al. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. *J Clin Microbiol.* 1996; **34**: 1622–1627. PMID: 8784557
81. Tilden J Jr, Young W, McNamara AM, et al. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *Am J Public Health.* 1996; **86**: 1142–1145. PMID: 8712275 doi: 10.2105/AJPH.86.8_Pt_1.1142
82. Arnold KW, Kaspar CW. Starvation- and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. *Appl Environ Microbiol.* 1995; **61**: 2037–2039. PMID: 7646045
83. Benjamin MM, Datta AR. Acid tolerance of enterohemorrhagic *Escherichia coli*. *Appl Environ Microbiol.* 1995; **61**: 1669–1672. PMID: 7747983
84. Conner DE, Kotrola JS. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. *Appl Environ Microbiol.* 1995; **61**: 382–385. PMID: 7887621
85. Cheville AM, Arnold KW, Buchrieser C, Cheng CM, Kaspar CW. *rpoS* regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7. *Appl Environ Microbiol.* 1996; **62**: 1822–1824. PMID: 8633882
86. Miller LG, Kaspar CW. *Escherichia coli* O157:H7 Acid Tolerance and Survival in Apple Cider. *Journal of Food Protection.* 1994; **57**: 460–464. doi: 10.4315/0362-028X-57.6.460
87. Dineen SS, Takeuchi K, Soudah JE, Boor KJ. Persistence of *Escherichia coli* O157:H7 in dairy fermentation systems. *J Food Prot.* 1998; **61**: 1602–1608. PMID: 9874336 doi: 10.4315/0362-028X-61.12.1602
88. Hudson LM, Chen J, Hill AR, Griffiths MW. Bioluminescence: a rapid indicator of *Escherichia coli* O157:H7 in selected yogurt and cheese varieties. *J Food Prot.* 1997; **60**: 891–897. doi: 10.4315/0362-028X-60.8.891
89. Foster JW. *Escherichia coli* acid resistance: tales of an amateur acidophile. *Nat Rev Microbiol.* 2004; **2**: 898–907. PMID: 15494746 doi: 10.1038/nrmicro1021
90. Castanie-Cornet MP, Penfound TA, Smith D, Elliott JF, Foster JW. Control of acid resistance in *Escherichia coli*. *J Bacteriol.* 1999; **181**: 3525–3535. PMID: 10348866
91. Gyles CL. Shiga toxin-producing *Escherichia coli*: an overview. *J Anim Sci.* 2007; **85**(Suppl): E45–E62. PMID: 17085726 doi: 10.2527/jas.2006-508
92. La Ragione RM, Best A, Woodward MJ, Wales AD. *Escherichia coli* O157:H7 colonization in small domestic ruminants. *FEMS Microbiol Rev.* 2009; **33**: 394–410. PMID: 19207740 doi: 10.1111/j.1574-6976.2008.00138.x
93. Beutin L, Geier D, Steinrück H, Zimmermann S, Scheut F. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J Clin Microbiol.* 1993; **31**: 2483–2488. PMID: 8408571
94. Trevena WB, Hooper RS, Wray C, Willshaw GA, Cheasty T, Domingue G. Vero cytotoxin-producing *Escherichia coli* O157 associated with companion animals. *Vet Rec.* 1996; **138**: 400. PMID: 8732197
95. Ferens WA, Hovde CJ. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog Dis.* 2011; **8**: 465–487. PMID: 21117940 doi: 10.1089/fpd.2010.0673
96. Persad A. K., LeJeune J. T. Animal Reservoirs of Shiga Toxin-Producing *Escherichia coli*. *Microbiol Spectr.* 2014; **2**(4):EHEC-0027-2014.
97. Gourmelon M, Montet MP, Lozach S, et al. First isolation of Shiga toxin 1d producing *Escherichia coli* variant strains in shellfish from coastal areas in France. *J Appl Microbiol.* 2006; **100**: 85–97. PMID: 16405688
98. Manna SK, Das R, Manna C. Microbiological quality of finfish and shellfish with special reference to shiga toxin-producing *Escherichia coli* O157. *J Food Sci.* 2008; **73**: M283–M286. PMID: 19241559 doi: 10.1111/j.1750-3841.2008.00815.x
99. Bennani M, Badri S, Baibai T, et al. First detection of Shiga toxin-producing *Escherichia coli* in shellfish and coastal environments of Morocco. *Appl Biochem Biotechnol.* 2011; **165**: 290–299. PMID: 21509602 doi: 10.1007/s12010-011-9251-x
100. Sasaki Y, Murakami M, Maruyama N, et al. Comparison of the prevalence of shiga toxin-producing *Escherichia coli* strains O157 and O26 between beef and dairy cattle in Japan. *J Vet Med Sci.* 2013; **75**: 1219–1221. PMID: 23595164 doi: 10.1292/jvms.12-0514
101. Sasaki Y, Tsujijima Y, Kusakawa M, Murakami M, Katayama S, Yamada Y. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* O157 and O26 in beef farms. *Vet Microbiol.* 2011; **150**: 140–145. PMID: 21292409 doi: 10.1016/j.vetmic.2010.12.024
102. Kobayashi H, Kanazaki M, Ogawa T, Iyoda S, Hara-Kudo Y. Changing prevalence of O-serogroups and antimicrobial susceptibility among STEC strains isolated from healthy dairy cows over a decade in Japan between 1998 and 2007. *J Vet Med Sci.* 2009; **71**: 363–366. PMID: 19346709 doi: 10.1292/jvms.71.363
103. Hussein HS. Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J Anim Sci.* 2007; **85**(Suppl): E63–E72. PMID: 17060419 doi: 10.2527/jas.2006-421
104. O'Brien AD, Melton AR, Schmitt CK, McKee ML, Batts ML, Griffin DE. Profile of *Escherichia coli* O157:H7 pathogen responsible for hamburger-borne outbreak of hemorrhagic colitis and hemolytic uremic syndrome in Washington. *J Clin Microbiol.* 1993; **31**: 2799–2801. PMID: 8253989

105. Roberts CL, Mshar PA, Cartter ML, et al. The role of heightened surveillance in an outbreak of *Escherichia coli* O157:H7. *Epidemiol Infect.* 1995; **115**: 447–454. PMID: 8557076 doi: 10.1017/S095026880005860X
106. Shefer AM, Koo D, Werner SB, et al. A cluster of *Escherichia coli* O157:H7 infections with the hemolytic-uremic syndrome and death in California. A mandate for improved surveillance. *West J Med.* 1996; **165**: 15–19. PMID: 8855679
107. Cieslak PR, Noble SJ, Maxson DJ, et al. Hamburger-associated *Escherichia coli* O157:H7 infection in Las Vegas: a hidden epidemic. *Am J Public Health.* 1997; **87**: 176–180. PMID: 9103093 doi: 10.2105/AJPH.87.2.176
108. Torso LM, Voorhees RE, Forest SA, et al. *Escherichia coli* O157:H7 Outbreak Associated with Restaurant Beef Grinding. *J Food Prot.* 2015; **78**: 1272–1279. PMID: 26197277 doi: 10.4315/0362-028X.JFP-14-545
109. Mead PS, Finelli L, Lambert-Fair MA, et al. Risk factors for sporadic infection with *Escherichia coli* O157:H7. *Arch Intern Med.* 1997; **157**: 204–208. PMID: 9009977 doi: 10.1001/archinte.1997.00440230076009
110. Rivas M, Caletti MG, Chinen I, et al. Home-prepared hamburger and sporadic hemolytic uremic syndrome, Argentina. *Emerg Infect Dis.* 2003; **9**: 1184–1186. PMID: 14531383 doi: 10.3201/eid0909.020563
111. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection 2010. (in Japanese) *Infec Dis Weekly Rep.* 2011; **13**: 11–19.
112. Ackers ML, Mahon BE, Leahy E, et al. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J Infect Dis.* 1998; **177**: 1588–1593. PMID: 9607837 doi: 10.1086/515323
113. Grant J, Wendelboe AM, Wendel A, et al. Spinach-associated *Escherichia coli* O157:H7 outbreak, Utah and New Mexico, 2006. *Emerg Infect Dis.* 2008; **14**: 1633–1636. PMID: 18826833 doi: 10.3201/eid1410.071341
114. Beutin L, Martin A. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. *J Food Prot.* 2012; **75**: 408–418. PMID: 22289607 doi: 10.4315/0362-028X.JFP-11-452
115. Erickson MC, Doyle MP. Plant food safety issues: linking production agriculture with One Health, In Improving food safety through a One Health approach—workshop summary. Institute of Medicine, National Academies Press, Washington, DC. 2012:140–175.
116. Solomon EB, Pang HJ, Matthews KR. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. *J Food Prot.* 2003; **66**: 2198–2202. PMID: 14672213 doi: 10.4315/0362-028X-66.12.2198
117. Wachtel MR, Whitehand LC, Mandrell RE. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *J Food Prot.* 2002; **65**: 18–25. PMID: 11808792 doi: 10.4315/0362-028X-65.1.18
118. Porto-Fett AC, Hwang CA, Call JE, et al. Viability of multi-strain mixtures of *Listeria monocytogenes*, *Salmonella typhimurium*, or *Escherichia coli* O157:H7 inoculated into the batter or onto the surface of a soudjouk-style fermented semi-dry sausage. *Food Microbiol.* 2008; **25**: 793–801. PMID: 18620971 doi: 10.1016/j.fm.2008.04.012
119. Hwang CA, Porto-Fett AC, Juneja VK, Ingham SC, Ingham BH, Luchansky JB. Modeling the survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella Typhimurium* during fermentation, drying, and storage of soudjouk-style fermented sausage. *Int J Food Microbiol.* 2009; **129**: 244–252. PMID: 19157610 doi: 10.1016/j.ijfood-micro.2008.12.003
120. Sartz L, De Jong B, Hjertqvist M, et al. An outbreak of *Escherichia coli* O157:H7 infection in southern Sweden associated with consumption of fermented sausage; aspects of sausage production that increase the risk of contamination. *Epidemiol Infect.* 2008; **136**: 370–380. PMID: 17445322 doi: 10.1017/S0950268807008473
121. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection as of April 2002. *Infec Agen Surv Rep.* 2002; **23**: 137–138.
122. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection as of May 2003. *Infec Agen Surv Rep.* 2003; **24**: 129–130.
123. Terajima J, Izumiya H, Iyoda S, Tamura K, Watanabe H. Detection of a multi-prefectural *E coli* O157:H7 outbreak caused by contaminated Ikura-Sushi ingestion. *Jpn J Infect Dis.* 1999; **52**: 52–53. PMID: 10816617
124. Strachan NJ, Fenlon DR, Ogden ID. Modelling the vector pathway and infection of humans in an environmental outbreak of *Escherichia coli* O157. *FEMS Microbiol Lett.* 2001; **203**: 69–73. PMID: 11557142 doi: 10.1111/j.1574-6968.2001.tb10822.x
125. Ogden ID, Hepburn NF, MacRae M, et al. Long-term survival of *Escherichia coli* O157 on pasture following an outbreak associated with sheep at a scout camp. *Lett Appl Microbiol.* 2002; **34**: 100–104. PMID: 11849503 doi: 10.1046/j.1472-765x.2002.01052.x
126. Mukherjee A, Cho S, Scheftel J, Jawahir S, Smith K, Diez-Gonzalez F. Soil survival of *Escherichia coli* O157:H7 acquired by a child from garden soil recently fertilized with cattle manure. *J Appl Microbiol.* 2006; **101**: 429–436. PMID: 16882151 doi: 10.1111/j.1365-2672.2006.02913.x
127. Keen JE, Wittum TE, Dunn JR, Bono JL, Durso LM. Shiga-toxicogenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerg Infect Dis.* 2006; **12**: 780–786. PMID: 16704838 doi: 10.3201/eid1205.050984
128. Keen JE, Durso LM, Meehan TP. Isolation of *Salmonella enterica* and Shiga-toxicogenic *Escherichia coli* O157 from feces of animals in public contact areas of United States zoological parks. *Appl Environ Microbiol.* 2007; **73**: 362–365. PMID: 17071798 doi: 10.1128/AEM.01563-06
129. Centers for Disease Control and Prevention (CDC) Notes from the field: *Escherichia coli* O157:H7 gastroenteritis associated with a State Fair - North Carolina, 2011. *MMWR Morb Mortal Wkly Rep.* 2012; **60**: 1745–1746. PMID: 22217625

130. Varma JK, Greene KD, Reller ME, et al. An outbreak of *Escherichia coli* O157 infection following exposure to a contaminated building. *JAMA*. 2003; **290**: 2709–2712. PMID: 14645313 doi: 10.1001/jama.290.20.2709
131. Lanier WA, Hall JM, Herlihy RK, et al. Outbreak of Shiga-toxigenic *Escherichia coli* O157:H7 infections associated with rodeo attendance, Utah and Idaho, 2009. *Foodborne Pathog Dis*. 2011; **8**: 1131–1133. PMID: 21657937 doi: 10.1089/fpd.2011.0884
132. Berry ED, Wells JE, Bono JL, et al. Effect of proximity to a cattle feedlot on *Escherichia coli* O157:H7 contamination of leafy greens and evaluation of the potential for airborne transmission. *Appl Environ Microbiol*. 2015; **81**: 1101–1110. PMID: 25452286 doi: 10.1128/AEM.02998-14
133. Chalmers RM, Aird H, Bolton FJ. Waterborne *Escherichia coli* O157. *Symp Ser Soc Appl Microbiol*. 2000; **29**: 124S–132S. PMID: 10880187 doi: 10.1111/j.1365-2672.2000.tb05340.x
134. Muniesa M, Jofre J, García-Aljaro C, Blanch AR. Occurrence of *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli* in the environment. *Environ Sci Technol*. 2006; **40**: 7141–7149. PMID: 17180960 doi: 10.1021/es060927k
135. Friedman MS, Roels T, Koehler JE, Feldman L, Bibb WF, Blake P. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. *Clin Infect Dis*. 1999; **29**: 298–303. PMID: 10476731 doi: 10.1086/520204
136. Samadpour M., Stewart J., Steingart K., Addy C., Louderback J., McGinn M., et al. Laboratory investigation of an *E. coli* O157:H7 outbreak associated with swimming in Battle Ground Lake, Vancouver, Washington. *Journal of environmental health*. 2002; **64**: 16–20, 26, 25.
137. Verma A, Bolton FJ, Fiefield D, et al. An outbreak of *E. coli* O157 associated with a swimming pool: an unusual vehicle of transmission. *Epidemiol Infect*. 2007; **135**: 989–992. PMID: 17288639 doi: 10.1017/S0950268807007947
138. Olsen SJ, Miller G, Breuer T, et al. A waterborne outbreak of *Escherichia coli* O157:H7 infections and hemolytic uremic syndrome: implications for rural water systems. *Emerg Infect Dis*. 2002; **8**: 370–375. PMID: 11971769 doi: 10.3201/eid0804.000218
139. Bopp DJ, Sauders BD, Waring AL, et al. Detection, isolation, and molecular subtyping of *Escherichia coli* O157:H7 and *Campylobacter jejuni* associated with a large waterborne outbreak. *J Clin Microbiol*. 2003; **41**: 174–180. PMID: 12517844 doi: 10.1128/JCM.41.1.174-180.2003
140. Hrudey SE, Payment P, Huck PM, Gillham RW, Hrudey EJ. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. *Water Sci Technol*. 2003; **47**: 7–14. PMID: 12638998
141. National Association of State Public Health Veterinarians Inc (NASPHV); Centers for Disease Control and Prevention (CDC). Compendium of measures to prevent disease associated with animals in public settings, 2011: National Association of State Public Health Veterinarians, Inc. *MMWR Recomm Rep*. 2011; **60**: 1–24.
142. Goode B, O'Reilly C, Dunn J, et al. Outbreak of *Escherichia coli* O157: H7 infections after Petting Zoo visits, North Carolina State Fair, October–November 2004. *Arch Pediatr Adolesc Med*. 2009; **163**: 42–48. PMID: 19124702 doi: 10.1001/archpediatrics.2008.525
143. Waguri A, Sakuraba M, Sawada Y, et al. Enterohemorrhagic *Escherichia coli* O157 infection presumably caused by contact with infected cows, Aomori Prefecture, Japan. *Jpn J Infect Dis*. 2007; **60**: 321–322. PMID: 17881879
144. Ihekweazu C, Carroll K, Adak B, et al. Large outbreak of verocytotoxin-producing *Escherichia coli* O157 infection in visitors to a petting farm in South East England, 2009. *Epidemiol Infect*. 2012; **140**: 1400–1413. PMID: 22093751 doi: 10.1017/S0950268811002111
145. Rowell S, King C, Jenkins C, et al. An outbreak of Shiga toxin-producing *Escherichia coli* serogroup O157 linked to a lamb-feeding event. *Epidemiol Infect*. 2016; **144**: 2494–2500. PMID: 27297133 doi: 10.1017/S0950268816001229
146. Wikswo ME, Hall AJ. Centers for Disease Control and Prevention Outbreaks of acute gastroenteritis transmitted by person-to-person contact--United States, 2009–2010. *MMWR Surveill Summ*. 2012; **61**: 1–12. PMID: 23235338
147. Brown JA, Hite DS, Gillim-Ross LA, et al. Outbreak of shiga toxin-producing *Escherichia coli* serotype O26: H11 infection at a child care center in Colorado. *Pediatr Infect Dis J*. 2012; **31**: 379–383. PMID: 22189536 doi: 10.1097/INF.0b013e3182457122
148. Gallagher L, Soyemi K, Conover C, et al. Outbreak of *Escherichia coli* O157:H7 in a child care center in Cook County, Illinois, with prolonged shedding and household transmission. *Am J Infect Control*. 2013; **41**: 936–938. PMID: 23870793 doi: 10.1016/j.ajic.2013.03.312
149. MacDonald E, Dalane PK, Aavitsland P, Brandal LT, Wester AL, Vold L. Implications of screening and childcare exclusion policies for children with Shiga-toxin producing *Escherichia coli* infections: lessons learned from an outbreak in a daycare centre, Norway, 2012. *BMC Infect Dis*. 2014; **14**: 673. PMID: 25518922
150. Kanayama A, Yahata Y, Arima Y, et al. Enterohemorrhagic *Escherichia coli* outbreaks related to childcare facilities in Japan, 2010–2013. *BMC Infect Dis*. 2015; **15**: 539. PMID: 26589805
151. Ostroff SM, Kobayashi JM, Lewis JH. Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. *JAMA*. 1989; **262**: 355–359. PMID: 2661870 doi: 10.1001/jama.1989.03430030043031
152. Griffin PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In: Blaser MJ SP, Ravdin JI, Greenberg HB, Guerrant RL, editor. *Infections of the gastrointestinal tract*. New York: Raven Press; 1995. p. 739–761.
153. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis*. 1985; **151**: 775–782. PMID: 3886804 doi: 10.1093/infdis/151.5.775
154. Pavia AT, Nichols CR, Green DP, et al. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. *J Pediatr*. 1990; **116**: 544–551. PMID: 2181098 doi: 10.1016/S0022-3476(05)81600-2

155. Bell BP, Griffin PM, Lozano P, Christie DL, Kobayashi JM, Tarr PI. Predictors of hemolytic uremic syndrome in children during a large outbreak of *Escherichia coli* O157:H7 infections. *Pediatrics*. 1997; **100**: E12. PMID: 9200386 doi: 10.1542/peds.100.1.e12
156. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med*. 2000; **342**: 1930–1936. PMID: 10874060 doi: 10.1056/NEJM200006293422601
157. Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a two-year prospective study. *J Infect Dis*. 1988; **157**: 1054–1057. PMID: 3283256 doi: 10.1093/infdis/157.5.1054
158. Huppertz HI, Busch D, Schmidt H, Aleksic S, Karch H. Diarrhea in young children associated with *Escherichia coli* non-O157 organisms that produce Shiga-like toxin. *J Pediatr*. 1996; **128**: 341–346. PMID: 8774501 doi: 10.1016/S0022-3476(96)70278-0
159. Hedican EB, Medus C, Besser JM, et al. Characteristics of O157 versus non-O157 Shiga toxin-producing *Escherichia coli* infections in Minnesota, 2000–2006. *Clin Infect Dis*. 2009; **49**: 358–364. PMID: 19548834 doi: 10.1086/600302
160. Adams DA, Thomas KR, Jajosky RA, et al. Nationally Notifiable Infectious Conditions Group Summary of Notifiable Infectious Diseases and Conditions - United States, 2014. *MMWR Morb Mortal Wkly Rep*. 2016; **63**: 1–152. PMID: 27736829 doi: 10.15585/mmwr.mm6354a1
161. Centers for Disease Control and Prevention Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2014 (Final Report). Atlanta, Georgia: U.S. Department of Health and Human Services, CDC; 2014.
162. Tseng M, Sha Q, Rudrik JT, et al. Increasing incidence of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) in Michigan and association with clinical illness. *Epidemiol Infect*. 2016; **144**: 1394–1405. PMID: 26584572 doi: 10.1017/S0950268815002836
163. Gould LH, Mody RK, Ong KL, et al. Emerging Infections Program Foodnet Working Group Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog Dis*. 2013; **10**: 453–460. PMID: 23560425 doi: 10.1089/fpd.2012.1401
164. Manning SD, Madera RT, Schneider W, et al. Surveillance for Shiga toxin-producing *Escherichia coli*, Michigan, 2001–2005. *Emerg Infect Dis*. 2007; **13**: 318–321. PMID: 17479902 doi: 10.3201/eid1302.060813
165. Stigi KA, Macdonald JK, Tellez-Marfin AA, Lofy KH. Laboratory practices and incidence of non-O157 shiga toxin-producing *Escherichia coli* infections. *Emerg Infect Dis*. 2012; **18**: 477–479. PMID: 22377034 doi: 10.3201/eid1803.111358
166. Government of Canada Canadian National Enteric Pathogen Surveillance System (FoodNet Canada) 2015. Guelph, ON: Public Health Agency of Canada; 2015.
167. Research Institute of Environmental Science and Notifiable disease; N002 - Trend rates by year since 1997 2016 [cited 2016; Available at: <http://www.nzpho.org.nz/NotifiableDisease.aspx>.
168. European Centre for Disease Prevention and Control Annual epidemiological report 2014 –food- and waterborne diseases and zoonoses. Stockholm: ECDC; 2014.
169. Vally H, Hall G, Dyda A, et al. Epidemiology of Shiga toxin producing *Escherichia coli* in Australia, 2000–2010. *BMC Public Health*. 2012; **12**: 63. PMID: 22264221
170. NNDSS Annual Report Writing Group Australia's notifiable disease status, 2011: annual report of the National Notifiable Diseases Surveillance System. *Commun Dis Intell Q Rep*. 2013; **37**: E313–E393. PMID: 24882235
171. NNDSS Annual Report Writing Group Australia's notifiable disease status, 2013: Annual report of the National Notifiable Diseases Surveillance System. *Commun Dis Intell Q Rep*. 2015; **39**: E387–E478. PMID: 26620352
172. NNDSS Annual Report Writing Group Australia's notifiable disease status, 2012: Annual report of the National Notifiable Diseases Surveillance System. *Commun Dis Intell Q Rep*. 2015; **39**: E46–E136. PMID: 26063098
173. NNDSS Annual Report Working Group Australia's notifiable disease status, 2014: Annual report of the National Notifiable Diseases Surveillance System. *Commun Dis Intell Q Rep*. 2016; **40**: E48–E145. PMID: 27080029
174. Terajima J, Iyoda S, Ohnishi M, Watanabe H. Shiga Toxin (Verotoxin)-Producing *Escherichia coli* in Japan. *Microbiol Spectr*. 2014; **2**. PMID: 26104366 doi: 10.1128/microbiolspec.EHEC-0011-2013
175. Bielaszewska M, Mellmann A, Bletz S, et al. Enterohemorrhagic *Escherichia coli* O26:H11/H-: a new virulent clone emerges in Europe. *Clin Infect Dis*. 2013; **56**: 1373–1381. PMID: 23378282 doi: 10.1093/cid/cit055
176. Germinario C, Caprioli A, Giordano M, et al. all participants of the Outbreak investigation team Community-wide outbreak of haemolytic uraemic syndrome associated with Shiga toxin 2-producing *Escherichia coli* O26:H11 in southern Italy, summer 2013. *Euro Surveill*. 2016; **21**: 1–9. PMID: 27684204 doi: 10.2807/1560-7917.ES.2016.21.38.30343
177. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2001. *Infect Agen Surv Rep*. 2001; **22**: 135–136.
178. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2016. *Infect Agen Surv Rep*. 2016; **37**: 85–86.
179. Watanabe H, Terajima J, Izumiya H, Iyoda S, Tamura K. PulseNet Japan: surveillance system for the early detection of diffuse outbreak based on the molecular epidemiological method. *Kansenshogaku Zasshi*. 2002; **76**: 842–848. PMID: 12448842 doi: 10.11150/kansenshogakuzasshi1970.76.842
180. Swaminathan B, Gerner-Smidt P, Ng LK, et al. Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. *Foodborne Pathog Dis*. 2006; **3**: 36–50. PMID: 16602978 doi: 10.1089/fpd.2006.3.36

181. Terajima J, Izumiya H, Iyoda S, Mitobe J, Miura M, Watanabe H. Effectiveness of pulsed-field gel electrophoresis for the early detection of diffuse outbreaks due to Shiga toxin-producing *Escherichia coli* in Japan. *Foodborne Pathog Dis*. 2006; **3**: 68–73. PMID: 16602981 doi: 10.1089/fpd.2006.3.68
182. Centers for Disease Control and Prevention (CDC) *Escherichia coli* O157:H7 infections associated with ground beef from a U.S. military installation--Okinawa, Japan, February 2004. *MMWR Morb Mortal Wkly Rep*. 2005; **54**: 40–42. PMID: 15660018
183. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection as of May 2004, Japan. *Infec Agen Surv Rep*. 2004; **25**: 138–139.
184. Kato K, Shimoura R, Nashimura K, et al. Outbreak of enterohemorrhagic *Escherichia coli* O111 among high school participants in excursion to Korea. *Jpn J Infect Dis*. 2005; **58**: 332–333. PMID: 16249637
185. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2008. *Infec Agen Surv Rep*. 2008; **29**: 117–118.
186. Yahata Y, Misaki T, Ishida Y, et al. E. coli O111 Outbreak Investigation Team Epidemiological analysis of a large enterohaemorrhagic *Escherichia coli* O111 outbreak in Japan associated with haemolytic uraemic syndrome and acute encephalopathy. *Epidemiol Infect*. 2015; **143**: 2721–2732. PMID: 25600435 doi: 10.1017/S0950268814003641
187. Siegler RL. The hemolytic uremic syndrome. *Pediatr Clin North Am*. 1995; **42**: 1505–1529. PMID: 8614598 doi: 10.1016/S0031-3955(16)40096-9
188. Gould LH, Demma L, Jones TF, et al. Hemolytic uremic syndrome and death in persons with *Escherichia coli* O157:H7 infection, foodborne diseases active surveillance network sites, 2000–2006. *Clin Infect Dis*. 2009; **49**: 1480–1485. PMID: 19827953 doi: 10.1086/644621
189. Rivas M, Chinen I, Miliwebsky E, Masana M. Risk Factors for Shiga Toxin-Producing *Escherichia coli*-Associated Human Diseases. *Microbiol Spectr*. 2014; **2**. PMID: 26104362 doi: 10.1128/microbiolspec.EHEC-0002-2013
190. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection 2006, 2007. *Infec Dis Weekly Rep*. 2009; **11**: 16–22. Japanese.
191. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Reported cases of hemorrhagic uremic syndrome associated with EHEC infection in 2008—NESID. *Infec Dis Weekly Rep*. 2009; **30**: 122–123. Japanese.
192. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection 2009. *Infec Dis Weekly Rep*. 2010; **12**: 11–19. Japanese.
193. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Enterohemorrhagic *Escherichia coli* infection 2011. *Infec Dis Weekly Rep*. 2012; **14**: 9–19. Japanese.
194. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Hemolytic uremic syndrome that appeared in NESID: trends in 2012. *Infec Agen Surv Rep*. 2013; **34**: 140–141.
195. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Hemolytic uremic syndrome among EHEC patients in Japan, 2013. *Infec Agen Surv Rep*. 2014; **35**: 130–132. Japanese.
196. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Hemolytic uremic syndrome among EHEC patients in Japan, 2014. *Infec Agen Surv Rep*. 2015; **36**: 84–86. Japanese.
197. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare Hemolytic uremic syndrome among EHEC patients in Japan, 2015. *Infec Agen Surv Rep*. 2016; **37**: 97–98. Japanese.
198. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N Engl J Med*. 1995; **333**: 364–368. PMID: 7609755 doi: 10.1056/NEJM199508103330608
199. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet*. 2005; **365**: 1073–1086. PMID: 15781103
200. Hancock DD, Besser TE, Rice DH. Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. In: Kaper JB, O'Brien AD, editors. *Escherichia coli* O157:H7 and other Shiga toxin-producing *E coli* strains. Washington, DC: ASM Press; 1998. p. 85–91.
201. U.S. Department of Agriculture Food Safety and Inspection Service Compliance guideline for validating cooking instructions for mechanically tenderized beef products. In: Agriculture Do, editor.; 2013.
202. Gill CO, Devos J, Badoni M, Yang X. Inactivation of *Escherichia coli* O157:H7 in Beef Roasts Cooked in Conventional or Convection Ovens or in a Slow Cooker under Selected Conditions. *J Food Prot*. 2016; **79**: 205–212. PMID: 26818980 doi: 10.4315/0362-028X.JFP-15-116
203. Thayer DW, Boyd G. Elimination of *Escherichia coli* O157:H7 in meats by gamma irradiation. *Appl Environ Microbiol*. 1993; **59**: 1030–1034. PMID: 8476281
204. Thayer DW, Rajkowski KT, Boyd G, Cooke PH, Soroka DS. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* by gamma irradiation of alfalfa seed intended for production of food sprouts. *J Food Prot*. 2003; **66**: 175–181. PMID: 12597473 doi: 10.4315/0362-028X-66.2.175
205. Rezende AC, Igarashi MC, Destro MT, Franco BD, Landgraf M. Effect of gamma radiation on the reduction of *Salmonella* strains, *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* and sensory evaluation of minimally processed spinach (*Tetragonia expansa*). *J Food Prot*. 2014; **77**: 1768–1772. PMID: 25285495 doi: 10.4315/0362-028X.JFP-14-108

206. Sommers C, Rajkowski KT, Scullen OJ, Cassidy J, Fratamico P, Sheen S. Inactivation of Shiga toxin-producing *Escherichia coli* in lean ground beef by gamma irradiation. *Food Microbiol.* 2015; **49**: 231–234. PMID: 25846936 doi: 10.1016/j.fm.2015.02.013
207. Shah S, Hoffman R, Shillam P, Wilson B. Prolonged fecal shedding of *Escherichia coli* O157:H7 during an outbreak at a day care center. *Clin Infect Dis.* 1996; **23**: 835–836. PMID: 8909859 doi: 10.1093/clinids/23.4.835
208. Wahl E, Vold L, Lindstedt BA, Bruheim T, Afset JE. Investigation of an *Escherichia coli* O145 outbreak in a child day-care centre--extensive sampling and characterization of eae- and stx1-positive *E. coli* yields epidemiological and socioeconomic insight. *BMC Infect Dis.* 2011; **11**: 238. PMID: 21902833 doi: 10.1186/1471-2334-11-238
209. Dabke G, Le Menach A, Black A, et al. Duration of shedding of Verocytotoxin-producing *Escherichia coli* in children and risk of transmission in childcare facilities in England. *Epidemiol Infect.* 2014; **142**: 327–334. PMID: 23672954 doi: 10.1017/S095026881300109X
210. Snedeker KG, Campbell M, Sargeant JM. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the faeces of domestic ruminants. *Zoonoses Public Health.* 2012; **59**: 126–138. PMID: 21824378
211. Varela NP, Dick P, Wilson J. Assessing the existing information on the efficacy of bovine vaccination against *Escherichia coli* O157:H7--a systematic review and meta-analysis. *Zoonoses Public Health.* 2013; **60**: 253–268. PMID: 22856462
212. Vogstad AR, Moxley RA, Erickson GE, Klopfenstein TJ, Smith DR. Assessment of heterogeneity of efficacy of a three-dose regimen of a type III secreted protein vaccine for reducing STEC O157 in feces of feedlot cattle. *Foodborne Pathog Dis.* 2013; **10**: 678–683. PMID: 23692077 doi: 10.1089/fpd.2012.1374
213. Vogstad AR, Moxley RA, Erickson GE, Klopfenstein TJ, Smith DR. Stochastic simulation model comparing distributions of STEC O157 faecal shedding prevalence between cattle vaccinated with type III secreted protein vaccines and non-vaccinated cattle. *Zoonoses Public Health.* 2014; **61**: 283–289. PMID: 23826923 doi: 10.1111/zph.12069
214. Barrett TJ, Lior H, Green JH, et al. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. *J Clin Microbiol.* 1994; **32**: 3013–3017. PMID: 7883892
215. U.S. Department of Health and Human Services. Food Code 1997. 1997;section 3-401.11.
216. U.S. Department of Agriculture Food Safety and Inspection Service. FSIS Policy on Nonintact Raw Beef Products Contaminated with *E. coli* O157:H7. 1999.
217. U.S. Department of Agriculture Food Safety and Inspection Service Risk Profile for Pathogenic Non-O157 Shiga Toxin-Producing *Escherichia coli* (non-O157 STEC). In: Agriculture Do, editor.; 2012.
218. U.S. Department of Agriculture Food Safety and Inspection Service Shiga toxin-producing *Escherichia coli* in certain raw beef products. *Federal Register.* 2011; **76**: 58157–58165.
219. Karmali MA, Mascarenhas M, Shen S, et al. Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. *J Clin Microbiol.* 2003; **41**: 4930–4940. PMID: 14605120 doi: 10.1128/JCM.41.11.4930-4940.2003
220. European Food Safety Authority Panel on Biological Hazards (BIOHAZ) Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. *EFSA Journal.* 2007; **579**: 1–61.
221. European Food Safety Authority Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food) on request of EFSA. *EFSA Journal.* 2009; **7**: 1366–1408. doi: 10.2903/j.efsa.2009.1366
222. European Food Safety Authority Panel on Biological Hazards (BIOHAZ) Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. *EFSA Journal.* 2013; **11**: 3138–3243. doi: 10.2903/j.efsa.2013.3138
223. European Food Safety Authority Panel on Biological Hazards (BIOHAZ) Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal.* 2011; **9**: 2424–2524. doi: 10.2903/j.efsa.2011.2424
224. THE EUROPEAN COMMISSION. COMMISSION REGULATION (EU) No 209/2013 of 11 March 2013. Official Journal of the European Union. 2013;12.3:L 68/19-23.
225. Ministry of Health Labour and Welfare. Notification No. 1217 (17 December 2012). 2012.
226. Ministry of Health Labour and Welfare. Notification No. 1120 (20 November 2014). 2014.
227. Hara-Kudo Y, Konishi N, Ohtsuka K, et al. An interlaboratory study on efficient detection of Shiga toxin-producing *Escherichia coli* O26, O103, O111, O121, O145, and O157 in food using real-time PCR assay and chromogenic agar. *Int J Food Microbiol.* 2016; **230**: 81–88. PMID: 27153219 doi: 10.1016/j.ijfoodmicro.2016.03.031
228. U.S. Department of Agriculture. Laboratory guidebook MLG 5B appendix1.01. Primer and probe sequences and reagent concentrations for non-O157 Shiga toxin-producing *Escherichia coli* (STEC) real-time PCR assay. 2012