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A cluster of Typhoid fever in Hsinchu, April 2011

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Abstract

The first cluster of typhoid fever this year (2011) was identified in Hsinchu area involving four cases with a common history of dietary exposure. The epidemiological investigations on correlation among persons, time and places, as well as test for samples collected from the catering staff were conducted. *Salmonella typhi* was detected by laboratory from the samples on May 8 that confirmed the catering staff was an asymptomatic carrier. Based on the results of epidemiological investigation and laboratory data, the source of infection in this cluster outbreak was suggested to be associated with food-related settings.

Taiwan CDC has coordinated with local health authorities to take urgent and relevant prevention and quarantine measures, to suspend the catering business till completing the treatment and testing negative for patients, as well as to enhance the surveillance for gastrointestinal infections in Hsinchu area till late June if no new case occurred.

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Original Article

Review and response of Enterohaemorrhagic *Escherichia coli* infection

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Abstract

Since May, 2011, Germany had reported a significantly increasing trend on cases of enterohaemorrhagic Escherichia *coli* (EHEC) infection and haemolytic uraemic syndrome (HUS). Afterward a number of European countries had reported cases linked to this outbreak, and most cases had travel history to Germany. As of June 15, Germany had reported 2,518 cases of EHEC infection (without HUS) and 786 HUS cases with 38 fatalities. Other European countries had reported a total of 69 EHEC cases and 36 HUS cases leading to one fatal [1]. Approximate 70% of cases were in females and most were in the group aged 20–49 years. The pathogen was identified as enteroaggregative verocytotoxin-producing Escherichia coli O104:H4 bacterium. According to epidemiological findings, German officials announced that the vehicle of infection was contaminated bean and seed sprouts.

The current event represents the largest outbreak of EHEC infection in recent years that resulted in dozens of deaths, as well as made great economic loss due to difficulties in epidemiological investigations and fail in prompt determination of the infective source. This article will introduce the domestic and international situation in epidemiology and surveillance of enterohaemorrhagic Escherichia *coli* infection.

A. Introduction of Enterovirulent *E.coli*

E. coli (*Escherichia coli*) is an aerobic bacterium which holds the largest number of normal human intestinal bacteria. These *E. coli* does not contain any virulence factors but plays an important role in maintaining normal immune functions by assisting other intestinal anaerobic bacteria, and prevents infection or invasion from virulent bacteria [2]. However, there are many serotypes among the *E. coli* family which contain virulence factors; according to the different characteristics of the strains, *E. coli* can be categorized as either an extraintestinal pathogenic *E. coli* (ExPEC), or an *E. coli* which causes intestinal infection. The former is the most common cause of urinary tract infection among humans, and can often be the cause for pneumonia and meningitis; the latter can be categorized into five categories according to their virulence factors and clinical symptoms. These five categories consist of enterotoxigenic *E.coli* (ETEC), enteropathogenic *E.coli* (EPEC), enteroinvasive *E.coli* (EIEC), enterohemorrhagic *E.coli* (EHEC), and enteroaggregative *E.coli* (EAEC). Among

these five categories, enterohemorrhagic *E.coli* (EHEC) causes the most serious illness and is further discussed in the review.

B. Shiga Toxin-Producing *E. coli* (STEC/VTEC) / Enterohemorrhagic *E.coli* (EHEC)

EHEC has many names. Shiga toxin-producing *E. coli* (STEC) is named according to its virulence factor since this type of *E. coli* contains bacteriophage which carries virulent genes. This leads to the production of a Shiga-like toxin which is similar to the Shiga toxin produced by *Shigella spp*. The toxins produced consist of Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*). These types of toxins were first found in *E. coli* strains isolated from pediatric patients. Because the toxin produced by these isolates can cause the death of monkey kidneys cells (or called Vero cells), the toxin was named as verotoxin, and thus STEC is also called verotoxigenic *E.coli* (VTEC) [3]. Enterohemorrhagic *E. coli* (EHEC) refers to a subgroup of STEC with a unique virulence factor, *eae* gene [2]. However, since bloody diarrhea is a hallmark of STEC infection; EHEC is commonly used to refer to STEC. Here we will refer to the Shiga-like toxin producing *E. coli* as EHEC.

EHEC is the main cause of intestinal bacterial infection. In developed countries, it is the most common cause of bloody diarrhea [4]. EHEC mainly infects ruminants such as cows or sheep. Humans could become infected by coming into contact with infected animals, ingesting contaminated food or water, or coming into contact with an infected person. The infective dose is as low as ten to several hundreds of bacteria [5]. In accordance with the O antigens on the surface, EHEC can be divided into over 250 different serotypes, among which several hundred have already been reported to be pathogenic to humans [6]. It is estimated that there are over 100,000 cases of EHEC in the United States each year, of which 3,000 were hospitalized and 90 resulted in death [7].

C. EHEC and Hemolytic Uremic Syndrome (HUS): O157 vs non-O157

The main symptoms after EHEC infection include abdominal pain, bloody or non-bloody diarrhea, and sometimes fever and vomiting. The incubation period is 3 to 8 days. The highest incidences of EHEC infection occur among elderly persons and children, of which are mostly composed of self-limiting infections that can be cured within a week. However, among 10% to 15% of infected patients, the toxins released into the bloodstream may result in hemolytic uremic syndrome (HUS) and lead to death. The clinical presentations of HUS include acute renal failure, hemolytic anemia, and thrombocytopenia. Once HUS has occurred, the patient usually needs dialysis and a number of invasive treatments due to acute renal failure, with a mortality rate between 3-5%. Approximately 50% of the surviving patients will suffer from the sequelae of chronic renal insufficiency. HUS caused by the EHEC infection is also one of the most common causes of acute renal failure among children [2].

In 1982, an outbreak of bloody diarrhea occurred in the states of Michigan and Oregon in the US. After investigation, it was

discovered that the cause was due to the infection of *E. coli* O157:H7 by consumption of undercooked beef. Because of its biochemical characteristics, this unique strain of EHEC is easier to isolate than other types of EHEC. In addition, due to its higher virulence, it is the main serotype that causes HUS and also the most extensively studied. After this outbreak, other countries have also reported O157:H7 outbreaks caused by contaminated food, including unpasteurized milk and dairy products, lettuce, sausage, and venison [2].

Due to the fact that there are hundreds of non-O157 EHEC strains and the great variability of clinical symptoms, it is impossible to distinguish infections caused by non-O157 EHEC from those caused by O157 or other enteric pathogens through symptoms only. Infected persons may be asymptomatic, present with mild diarrhea, watery diarrhea, or even bloody diarrhea or HUS. The results of a Canadian study on over five thousand diarrhea patients show, in comparison to O157 infections, non-O157 infections had longer durations of diarrhea (average of 9.1 days vs 5.7 days) but fewer cases of bloody diarrhea (42% vs 97%). On the other hand, other symptoms such as abdominal pain, vomiting, and fever showed no significant difference [8]. The percentage of non-O157 infections in EHEC cases differs between countries. A study in Denmark shows 75% of EHEC were caused by non-O157 infections, yet only 20% to 40% non-O157 infections among EHEC cases were found in England, USA, and Canada [5]. The virulence and probability of causing HUS caused by non-O157 serotypes is no less than by O157., including the O26 that caused outbreaks in Germany and Italy and the O111 that caused outbreaks in Australia and Japan [5]. The outbreak in Germany caused by O104, however, is the first large-scale outbreak cased by this serotype.

D. Updates on the outbreak in Europe

The recent O104 outbreak has drawn global attention, and according to the latest WHO data on June 16, 2011, currently 2,587 cases of EHEC infection, 825 cases of HUS with 39 deaths have been reported globally,. All of the cases had travel history to Germany or contact with other confirmed cases.

After the outbreak the German government launched an epidemiologic investigation and case control study in May in hopes of early identification of the infection source. The investigation results show an incubation period of 3 to 4 days (a range of 2 to 10 days) from exposure to the first display of symptoms. Early case control studies only indicated the relevance of consumption raw tomatoes, cucumbers, and lettuce with the infection, but did not permit a more precise identification of the source of infections. Therefore, in the second stage of investigation, further cohort studies were made on the 112 customers who dined at the same restaurant (19 of which were confirmed EHEC cases). The results of the study showed customers who had ate sprouts had an 8.6 fold increased risk of illness compared to those who did not (95% CI 1.5-∞). In addition, all cases had in fact consumed sprouts; which confirms the infection source to be contaminated sprouts. However, so far O104 has not been detected in any food product [9].

To this date, the number of reported cases has already started to decline and the German government has advised the public to avoid the consumption of raw sprouts in addition to general food safety awareness.

E. Current status of Epidemiology in Taiwan

The infection of enterohemorrhagic *E.coli* (EHEC) has been listed as a category II notifiable infectious disease since 1999. Due to the fact that O157 is the most common and important of all EHECs, only testing of O157 has been conducted. Hospitals are required to report cases of suspected symptoms or with positive isolation. From 1999 till now, a total of 118 cases have been reported and only one case of a six-year-old boy was confirmed in 2001. This child is of US nationality and lives in the US and started to display symptoms when on vacation in Taiwan. The isolated tested positive for toxin gene and was identified as serotype O157. According to gene sequence data, the case was thus confirmed as an imported case [10]. Since then, no other confirmed O157 cases occurred.

In accordance with the epidemiology of intestinal pathogenic *E. coli*, Taiwan CDC conducted a study in 2000-2001. *E. coli* isolated from the diarrhea cases reported through the National Notifiable Disease Surveillance System were collected. Together with isolates collected from diarrhea patients in three hospitals in northern, central and southern Taiwan, biochemistry and molecular tests were performed on more than 500 *E.coli* isolates. It was discovered that ETEC accounted the highest percentage of 7.08%, followed by EIEC (4.9%). Apart from the O157 strain from the first confirmed case mentioned earlier, no other EHEC isolates were found [11].

Afterwards, the Research and Diagnostic Center at Taiwan CDC collaborated with more than 30 hospitals in northern Taiwan during the period between 2005 and 2006 in collecting fecal samples from diarrhea patients in hospitals and passengers passing through airport fever screening stations who showed symptoms of diarrhea. Apart from routine bacterial cultures and *E. coli* serotyping, molecular methods were used. PCR targeting on toxin genes of different intestinal pathogenic *E. coli*, including *stx1, stx2, 1t,* and *st* were performed. Among the 261 specimens collected, 137 cases (52%) had *E. coli* isolated. As of the results for the toxin gene testing, among the 137 isolates, 10 were ETEC, 3 EIEC, and 2 EPEC; none of which were found to be EHEC carrying *stx1* or *stx2* [2]. The findings of this study were consistent with the surveillance from National Notifiable Disease Reporting System and other domestic studies [13]. Furthermore, the cause of domestic HUS cases is mainly *Streptococcus pneumonia*e infection which is less frequently seen in Western countries [14]. We hypothesize that the difference of prevalence of EHEC in our country and Western countries resulted from our habit of eating hot and cooked food.

As of today, no case of domestic EHEC infection has been reported. However, O157 and non-O157 EHEC have been isolated from both domestic livestock and imported meat. In 1994, Pingtung University of

Science once isolated O157 carrying toxin genes from cattle in the southern part of Taiwan[15]. A study conducted between 2006 and 2009 by the National Chung Hsing University Department of Veterinary Medicine on milk samples or bovine diarrhea samples found that among 1,674 milk samples, 2 EHEC strains were isolated (a positive rate of 0.001%) and 5 strains of EHEC were found among the 24 bovine diarrhea samples (a positive rate of 20.8%) [16]. Another study by the same department conducted in 2008 shows that imported beef from the US has a 4.4% testing positive for EHEC, whereas Australian beef has a 1.8% positivity [17]. The EHEC detected in the two previous studies were non-O157. These studies show that the threat of EHEC infection as a zoonosis exists; whether in domestic livestock or imported meat products, there is a possibility of resulting in a human case or even an outbreak.

F. **Response to current outbreak**

After the German outbreak, Taiwan CDC adopted a number of immediate measures, including:

1. Issue a press release to remind people to avoid eating raw foods when traveling and a ”Letter to Doctors” to the medical community to alert physicians.
2. Obtain the pathogenic strain (O104) and its antiserum to establish diagnosis ability.
3. Maintain close contact with Taiwan Food and Drug Administration and exchange relevant information.
4. Amend the definition of EHEC in National Notifiable Disease Reporting System.

   After the outbreak, Taiwan CDC immediately reviewed the case definition of “enterohemorrhagic *E. coli* infection”. In order to increase sensitivity of the surveillance system and grasp infection cases of both O157 and non-O157, amendments of the clinical conditions and testing conditions of Escherichia coli infections are made (Table 1).
5. The specimen collection list is shown in Table 2.

Table 1. Comparison of amendments of case definition of EHEC

	Before Amendment	After Amendment
Clinical criteria	Patients with suspected symptoms such as diarrhea (mostly bloody), abdominal cramps. May combine with hemolytic uremic syndrome or thrombotic thrombocytopenic purpura. Asymptomatic infection may also occur.	Patients with intestinal symptoms, with serious cases showing bloody diarrhea, hemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP).
Laboratory criteria	*E. coli* O157:H7 or non-motile enterotoxigenic *E. coli* O157: NM are isolated and identified from clinical specimens (stool or rectal swabs).	Shiga toxin-producing (shiga-like toxin) *E. coli* are isolated and identified from clinical specimens (stool or rectal swabs).

6. EHEC testing flowchart

Because most EHEC cases were caused by O157, samples of EHEC were only tested for the presence of *E.coli* O157. Isolates will be tested in O157-specific antiserum, follow by PCR to determine the presence of toxin genes. In accordance with the amendment of the case definition of "enterohemorrhagic *E. coli* infection", the testing procedure is changed. PCR targeting the two toxin genes, *stx1* and *stx2* will be performed first. PCR positive isolates are then tested in a number of antiserum to determine the serotype. Therefore, all EHEC carrying toxin genes can be diagnosed (Figure).

Table 2. Specimen collection list

Category	Enterohemorrhagic *E. coli* infection	
Specimen types	Stool	Isolates
Purpose of specimen collection	Pathogen detection (isolation, identification); toxins and anti-serum test	Pathogen detection (identification); toxins and anti-serum test
Timing of specimen collection	Immediate collection	As soon as possible
Specimen collection requirements	Use sterile cotton swab to collect mucus, blood, or pus of the stool and place in Cary-Blair transport medium	Place loopful purified isolate in a Cary-Blair transport medium.
Transport conditions	Low temperature	
Notes	1. See Appendix 2.7.2 Remarks; stool specimen collection procedures refer to Appendix I section 3.5. 2. Specimen collection bottle should be sealed with a paraffin film to prevent leakage or drying out of samples. 3. If patient has been treated or is currently under treatment, please note on the report form. 4. If the sample is from a food poisoning case, a copy of the "food poisoning investigation form" should be attached.	

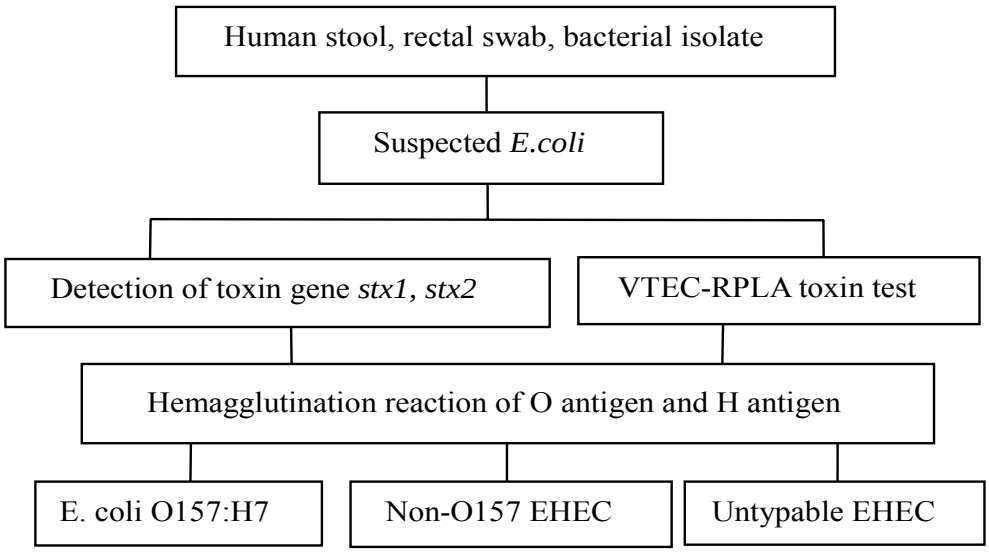

Figure. Testing Procedure of EHEC

Note：There are currently 51 types of O antiserum, which include EHEC serotypes such as O26

7. Strengthening the symptom surveillance of incoming passengers

International airport quarantine strengthening mechanisms is activated and travelers are alerted for health measures. Travelers with a European travel history and suspected symptoms should be reported through the quarantine system and specimens should be collected and sent for testing in order to facilitate timely diagnosis.

So far this year, four cases were reported with none confirmed.

G. Public health recommendations

1. To the public

So far, no evidence has shown that the suspected contaminated food in this outbreak has been exported to other areas apart from Europe; thus there is no need for the public to panic. However, the general food safety principles for everyday life are as following:

(1) Wash hands: particularly before meals, after going to the toilet, and before and after handling food.

(2) Separate raw and cooked: raw food and cooked food should be separated when being prepared. It is advised to avoid using the same cutlery and utensils to reduce the chance of cross contamination.

(3) Cook thoroughly: this type of bacteria can be killed by being heated to 75℃ for over 1 minute; thus it is essential for food to be cook thoroughly.

(4) Keep food at safe temperatures: consume food as soon as possible after cooking or place it in the refrigerator.

(5) Using safe water and raw materials: this includes paying attention to the source of drinking water and fresh food produce, and avoids consuming uncooked vegetables and unsterilized milk [18].

If a person has travel history to an epidemic area, ingesting raw food and is starting to show symptoms of diarrhea, please seek medical care as soon as possible and notify the physician of the relevant information.

2. To medical personnel

Medical personnel should have high index of suspicion of patients with suspected symptoms and travel history to epidemic areas. They should be reported with specimens collected for testing as soon as possible. Proper treatment also has to be administered immediately.

H. Conclusion

This outbreak highlights the importance of continuous monitoring of EHEC infection. Today, in a world where infectious diseases know no borders, it is important not only to maintain the most complete surveillance system and advanced testing technology, but even more so in grasping the newest global epidemic and being at a standby to make professional preparations to achieve the purpose of ensuring public health for all.

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