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ORIGINAL ARTICLE

Enteropathogenic *Escherichia coli* Outbreak and its Incubation Period: Is it Short or Long?

Dong-Woo Lee [a,*], Jin Gwack [b], Seun-Ki Youn [b]

[a]*Division of Public Health Crisis Responses, Korea Centers for Disease Control and Prevention, Osong, Korea.*
[b]*Division of Epidemic Intelligence Service, Korea Centers for Disease Control and Prevention, Osong, Korea.*

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Abstract
Objectives: The aim of this study is to determine the incubation period of enteropathogenic *Escherichia coli* (EPEC), which creates several outbreaks in a year in South Korea.
Methods: We reviewed all water and food-borne outbreaks data reported to the Korea Centers for Disease Control and Prevention (KCDC) from 2009 to 2010 and determined their characteristics. Through this process, we can presume the incubation period of EPEC among outbreaks in South Korea.
Results: A total of 497 water and food-borne outbreaks were reported to KCDC and 66 (13.28%) are defined as *E coli*-origin outbreaks. EPEC was the most common subtype of *E coli*, being confirmed as a causative organism in 26 outbreaks. Overall attack rate was 15.85% (range 0.9–100). The subjects were eight outbreaks that have a clear history of single exposure and we can estimate the incubation time of EPEC as minimum 0.5 hours to maximum 34.0 hours with a mean 12.9 hours (range 4.5–24.0). The cases of those cannot completely rule out the chance of multiple exposure from same source or place have minimum 1.0 hour, to a maximum of 195.5 hours and a mean 30.5 (range 22.7–61.0) hours of incubation period.
Conclusions: This serial analysis suggests that EPEC has actually shorter mean incubation period as much as 12 hours. When this period is longer than 1 day or over, then the epidemiologic investigator should consider the chance of repeated or continuous exposure by making it clear whether there is any chance of any other exposure in common.

1. Introduction

Escherichia coli is the predominant nonpathogenic facultative flora of the human intestine. Most people normally carry harmless strains of *E coli* in their intestine. Both the harmless strains and those that cause diarrhea are acquired primarily through ingestion of contaminated food or water [1]. Person-to-person and animal-to-human transmission is through the oral-fecal route. However, several strains of *E coli* have usually

*Corresponding author.
E-mail: aryumput2@naver.com

caused diarrhea/gastroenteritis in human which settles within several days without specific treatment [2].

The varieties of *E coli* can be grouped into six pathotypes according to their virulence determinants whose specific nature makes each pathotype different both clinical presentation and distinctive epidemiologic characteristics: enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and diffuse adherent (DAEC) [3].

EPEC is one of the oldest recognized diarrheagenic *E coli*. EPEC cause either a watery or bloody diarrhea [2]. EPEC has been well known to be highly related to infant diarrhea in developing countries through the pioneering work of Bray [4] who established the importance of EPEC as a cause of outbreaks of infantile gastroenteritis in the UK in the 1940s. These continued until the early 1970s, but since then outbreaks caused by 'classical' EPEC strains have become very rare [5]. Infections caused by EPEC are difficult to differentiate from those with other causes; symptoms include watery diarrhea, sometimes accompanied by low-grade fever and vomiting. However, EPEC infection may be severe, vomiting may make oral rehydration difficult and life-threatening dehydration may ensue. Furthermore, disease caused by EPEC may be protracted, resulting in weight loss, malnutrition, and death [6]. Its characteristics are relatively well defined. However, there are some controversies about the incubation period both it can be as short as 6 hours or can be as long as 6 to 10 days [7,8]. As a result, it is known that EPEC has just 'variable' incubation time [9].

In South Korea, all outbreaks by water-borne and food-borne disease with two or more persons should be reported to a local public health center for epidemic investigation. When there is an outbreak of which source infectious agent is thought as EPEC, Korea Centers for Disease Control and Prevention (KCDC) epidemic intelligence service (EIS) officers estimate the causal relationship between exposure and onset of outbreak in consideration of two factors, laboratory result and epidemic nature, especially during the incubation period. Following the WHO guideline, KCDC has suggested the incubation period of EPEC as 1 to 6 days [10,11]. Nevertheless, it has caused some difficulties in presuming first exposure time during epidemic investigations when EPEC was confirmed as causative organism by laboratory result, but incubation period of the epidemic was very short, e.g., less than 6 hours.

This study is dedicated to clarifying whether the incubation period of EPEC is truly long over 1 day or whether it is shorter than several hours by analyzing recent EPEC outbreaks of South Korea.

2. Methods

The incubation period, which is the amount of time between infection with a virus or bacteria and the start of symptoms, can vary from one case to another according to the route by which the person was exposed, the dose of bacteria received, and other factors, including immune status. Estimates of the incubation period are further complicated by the fact that some patients have had opportunities for multiple exposures to the pathogens. The particular exposure that caused disease may prove impossible to determine [10].

For these reasons, the most reliable estimates of the incubation period are based on a study of cases having a single documented exposure to a known case. We reviewed all water and food-borne outbreaks data reported to KCDC from 2009 to 2010 and determined whether these outbreaks were truly defined vehicles of transmission and single exposure. With this process, we can presume the incubation period of EPEC among outbreaks in South Korea during 2009−2010.

3. Results

From January 1, 2009, to December 31, 2010, a total of 497 water and food-borne outbreaks were investigated and reported to KCDC and 66 (13.3%) were defined as *E coli*-origin outbreaks. The distribution of subtype is shown in Table 1. EPEC was the most common subtype of *E coli*, being confirmed as a causative organism in 26 outbreaks, followed by 20 of ETEC, seven of EHEC, five of EAEC, and eight of mutual or other subtypes.

EPEC outbreaks had, as a whole, 11,302 population at risk and 1791 cases were defined as EPEC related cases. Its overall attack rate was 15.9% (range 0.9−100.0). This wide range of attack rate mainly comes from the variety of outbreaks; from an outbreak of small group less than five, but all are defined cases to that of group food service in a school over 1,000 students but had small cases.

Among these 26 outbreaks, to presume a more precise incubation period of EPEC, we selected 13 outbreaks that had an obvious identified single-point

Table 1. *E coli* subtypes of outbreaks: Korea, 2009−2010

	Total[a]	
Subtypes	Cases	Percentage
EPEC	26	39.4%
ETEC	20	30.3%
EHEC	7	10.6%
EAEC	5	7.6%
Unspecified	8	12.1%
Total	66	13.3%*

[a]*Total cases and percentages of E coli origin outbreaks in Korea, 2009−2010 except *.*

**means incidence rate of E coli outbreaks among all water and food-borne outbreaks in Korea, 2009−2010.*

exposure history and which had accurate time interval information from exposure to first symptom onset. Of the 13 outbreaks, eight have a clear history and the defined cases had only one meal in common, before and after of possible exposure time. Meanwhile, five have a chance of continuous exposure despite of their assumed single exposure time by epidemiologic investigation. These were students in school who had lunch or dinner from the same 'group food catering service' at the same place, in every day.

From the outbreaks that had no possibility of repeated or continuous exposure, we can estimate the incubation period of EPEC as shown in Table 2. Minimum incubation period (MiIP) is 0.5 hour, maximum incubation period (MaIP) is 34.0 hours, and mean incubation period (MIP) is 12.87 (range 4.5−24.0) hours. However, the cases that cannot completely rule out the chance of multiple exposure from same source or place (Table 3), MiIP is 1.0 hour, MaIP is 195.5 hours, and MIP is 30.5 (range 22.7−61.0) hours.

Frequencies of clinical symptoms among cases are shown in Table 4. The most common symptom was diarrhea, followed by abdominal pain.

4. Discussion

In 2010, EPEC was one of the three most common causative organisms of water- and food-borne outbreaks in Korea, next to norovirus and salmonella. When an outbreak has occurred, sophisticated epidemiologic investigation is important to determine the source of infection, the exact pathogen, and how it is transmitted through the persons. Prompt and exact response upon investigation is crucial to prevent spread of disease to local community. In this process, to identify correct pathogen is a key point to layout a whole outbreak. However, laboratory results are not the only concern when determining the cause; epidemiologic characteristics should always be considered together.

Appropriate standards or guidelines for epidemiologic features of certain pathogens are important when the field outbreak investigation begins. Incubation period is a core-determining factor in these guidelines for inferring the causative organism of outbreak with epidemic curve. Nevertheless, the incubation period of EPEC is not well established in guidelines, including that of KCDC. The WHO guideline states that the EPEC incubation period is 1−6 days (as short as 12−36 hours) [10]. However, it is just 'variable' in the guideline of CDC [9]. Moreover, in the past reports, Nakamura and colleagues said it can be as long as two to 20 days, though it can be chiefly 1−5 days [7]. However, in Korea, when there is an EPEC outbreak, it is often as short as 6 hours or less.

Our results suggest that MIP of EPEC is not just 'variable'. 'Outbreak with clear single exposure history'

Table 2. Characteristics of 8 EPEC outbreaks with clear single exposure and without any other meal in common: South Korea, 2009−2010

Outbreak No.	Year	No. of population at risk	No. of cases	Attack rates (%)	No. of specimen		Incubation period (h)			Outbreak in group catering service
					Collected	Confirmed	Minimum	Mean	Maximum	
1	2009	16	10	62.5	10	1	3.0	4.5	12.0	No
2	2009	21	16	76.2	12	3	5.0	15.5	26.0	No
3	2009	5	5	100.0	4	2	3.0	10.0	19.0	No
4	2009	103	36	35.0	13	4	1.5	5.3	8.5	No
5	2010	6	6	100.0	5	2	3.0	5.0	8.5	No
6	2010	887	82	9.2	82	3	18.0	24.0	34.0	No
7	2010	476	71	14.9	131	41	0.5	5.2	14.5	No
8	2010	4	4	100.0	6	4	15.0	15.7	16.5	No
Total (Range)		1518	230	15.2 (9.2-100)	263	60	0.5	12.9 (4.5-24.0)	34.0	

Table 3. Characteristics of five EPEC outbreaks that cannot rule out repeated exposure from the same source: South Korea, 2009–2010

Outbreak No.	Year	No. of population at risk	No. of cases	Attack rates (%)	No. of specimens		Incubation period (h)			Outbreak in group catering service
					Collected	Confirmed	Minimum	Mean	Maximum	
1	2009	773	85	11.0	107	26	2.0	24.0	96.0	Yes (school)
2	2009	76	11	14.5	12	4	19.0	37.7	57.0	Yes (school)
3	2010	1663	203	12.2	40	24	9.5	22.7	195.5	Yes (school)
4	2010	157	43	27.4	5	2	33.0	46.0	60.0	Yes (school)
5	2010	522	46	8.8	33	6	1.0	61.0	124.0	Yes (school)
Total (range)		3191	388	12.2 (8.8–27.4)	197	62	1.0	30.5 (24.0–61.0)	195.5	

Table 4. Clinical symptom frequencies of EPEC cases: Korea, 2009–2010

Symptoms ($n = 123$)	Percentage
Diarrhea	94.3%
Abdominal pain	85.4%
Nausea	41.6%
Chilling	27.7%
Headache	24.4%
Fever	20.2%

means outbreak occurred among persons who had only one meal together and had no other chance of common exposure before and after suspected event. MIP of EPEC outbreaks in this setting is only 12.9 hours. It is shorter than 1 day. With this trait, outbreaks of EPEC should show a different epidemic curve from that of the norovirus, which usually presents a flatter curvature.

MIP of Outbreaks of 'group catering service' in school, however, is three times longer as 32 hours and MaIP extends up to 195 hours, which is about 8 days. In this situation, persons in school (including teachers and other workers) had same meals in the same place in every day. There was higher chance of repeated or continuous exposure for EPEC. As a result, without subgroup analysis considering characteristics of exposure, intermingled incubation period of EPEC outbreaks looks much longer and widely dispersed.

Recent studies have suggested, however, EPEC is not a homogeneous pathogen. 'Typical' EPEC carries adherence factor plasmid (pEAF). This plasmid encodes bundle-forming pili (BFP), which promote bacterial adherence to epithelial cells and are an essential virulence determinant [12] and a transcriptional activator (PER), that upregulates genes within a chromosomal pathogenicity island [13,14]. This pathogenecity island is thought to encode a number of essential virulence proteins, including the surface protein intimin (the product of the eae gene), which is required to produce the attaching-effacing lesions that are a key feature of EPEC-induced pathology [15]. A subset of EPEC, known as 'atypical' EPEC, does not carry pEAF and hence does not produce BFP or PER [16]. Accordingly, their role in the disease is controversial. However, there are several reports that 'atypical' EPEC show different clinical aspects; Nguyen and colleagues suggested that atypical EPEC is associated with prolonged diarrhea in children [15].

In this study, we did not collect data about specific type of EPEC in each outbreak. Therefore, we cannot infer relationship between the length of incubation period and each type of EPEC. However, KCDC is planning further study and will be combining epidemiologic investigation and specific laboratory process about typing matches to determine whether certain outbreaks are from 'typical' or 'atypical' EPEC.

5. Conclusion

The clinical and other epidemiologic characteristics of EPEC are well known. However, its incubation period is not well-established in guidelines for epidemiologic investigation. We estimated roughly the incubation period of EPEC with limited data in 2 years for 2009 and 2010. Nevertheless, this serial analysis suggests that EPEC has a relatively shorter incubation period, i.e., as much as about 12 hours. When this period is 1 day or more, then EIS officer should consider the chance of repeated or continuous exposure and focus his or her investigation on determining whether the cases truly have a single-point exposure. It has not yet been discovered whether this difference comes from the type of EPEC (typical vs. atypical), so further investigation through laboratory support should be performed in the near future.

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