Incubation period of Shiga-toxin producing Escherichia coli

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Running head: Systematic Review of the Incubation Period of STEC

ABSTRACT

Shiga toxin-producing *Escherichia coli* are pathogenic bacteria found in the gastrointestinal tract of humans. Severe infections could lead to life threatening complications especially in young children and the elderly. Understanding the distribution of the incubation period, which is currently inconsistent and ambiguous, can help in controlling the burden of disease. We have undertaken a systematic review of outbreak investigation reports, extracted individual incubation data and summary estimates, tested for heterogeneity, classified studies into subgroups with limited heterogeneity and undertook a meta-analysis to identify factors that may contribute to the distribution of incubation period.

Twenty-nine studies were identified for inclusion in the review, and the resulting value of I^2 was 77% indicating high heterogeneity. Studies were classified into five subgroups with the mean incubation period ranging from 3.5 to 8.1 days. The length of the incubation period increased with age and decreased by 7.2 hours with every 10% increase in attack rate.

KEYWORDS

Escherichia coli, Hemolytic-Uremic Syndrome, Shiga-Toxigenic *Escherichia coli*

ABBREVIATIONS

STEC - Shiga-Toxigenic *Escherichia coli*

HUS - Hemolytic-Uremic Syndrome

KS test – Kolmogorov-Smirnov Test

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a pathogenic form of the *Escherichia coli (E. coli)* bacteria. It is common and benign in many organisms, but usually causes illness in humans (1). Symptoms generally include severe stomach cramps, profuse diarrhoea which is often bloody, and vomiting (2). Symptoms could last between five to seven days (3), however severe infections can be life threatening and result in complications (2). A common complication of STEC infection is hemolytic uremic syndrome (HUS) which affects five to ten per cent of ill persons (1,3).

There are several disease-causing STEC serogroups, but the most common is STEC O157 (4),(5). Its reservoir is mainly cattle (6) and it is transmitted to humans through consumption of contaminated foods, fecal-oral transmission or cross contamination (5). Other STEC serotypes are collectively called non-O157 STEC. Numerous non-O157 strains have been identified however the most common serogroups associated with disease include O111, O26, O45, O103, and O145 (7,8).

Globally, STEC is estimated to cause nearly 3 million cases annually leading to approximately 200 deaths (9). Most reported cases of STEC O157 are sporadic (10), but due to the low infectious dose required for infection to occur (10), there is the potential for large outbreaks (11). Cases of non-O157 are relatively fewer and likewise, non-O157 related outbreaks are rare, however, large outbreaks have also been reported (12).

Incubation period is the time between exposure to the infecting pathogen and onset of clinical symptoms. Accurate knowledge of the distribution of incubation period is necessary for understanding its epidemiology, and is useful in outbreak investigations and distinguishing between primary and secondary cases which is an important factor in STEC epidemiology as person to person transmission can occur. (11). In an outbreak investigation where the date of

exposure is unknown, accurate knowledge of the incubation period distribution can help identify the potential source of contamination.

There are several conflicting reports on the incubation period distribution of STEC. The World Health Organization reports a range of three to eight days (1), the Centre for Disease Prevention and Control report three to four days after exposure (3) and cases in a few notable outbreaks have reported unexpectedly long incubation period (13,14). Due to the ambiguity around the incubation period distribution of STEC, researchers have used other methods to identify the time of exposure in outbreak investigations (15).

In order to describe the incubation period distribution of STEC, examine the ambiguity around the reported incubation period and identify possible influencing factors, the authors undertook a systematic review of published and grey literature reporting the incubation period of STEC in exposed populations. We extracted reported individual patient incubation data and summary estimates and undertook a meta-analysis and meta-regression of the extracted data. We tested for the presence of heterogeneity and attempted to explain observed variation by identifying influencing factors.

METHODS

We undertook a systematic literature review of observational studies with known point sources in order to extract data on incubation period. These data were assessed for the presence of heterogeneity and subgroups with limited evidence of heterogeneity between them were identified. We conducted a meta-regression to identify factors that influence the incubation period distribution. Fuller details on methods used have already been published (16).

Research questions and modified PICO elements

Our research questions were:

1. What is the distribution of the incubation period of STEC infections in humans?
2. What factors affect the incubation period?

The modified PICO elements and associated components are listed in Table 1.

Search strategy

A systematic literature search for peer reviewed publications and grey literature reports of observational and experimental studies reporting incubation period was conducted in bibliographic databases (PubMed and Scopus) and Google Scholar. A customised grey literature search of the websites: www.cdc.gov, www.who.int, www.opengrey.eu and open Google search (screening only the first fifty items) was undertaken using combinations of search terms (Web Table 1). All searches were carried out from 5 June 2017 to 3 July 2017. There was no restriction on the dates or language of articles returned. The reference lists of identified review papers were also screened to find other relevant studies where incubation period of STEC may have been reported.

Selection process

Following the identification of likely relevant articles from the literature review, each article went through a selection process and quality assessment. The steps involved in the selection process and quality assessment process have previously been described in Awofisayo-Okuyelu et al (16).

Data extraction

Data were extracted from all included studies according to a pre-determined proforma (Web Table 2). When available, individual incubation period data were extracted from text and tables. Where an epidemic curve was provided, the raw incubation period data were extracted using the online tool, WebPlotDigitizer (17). Summary data, such as the mean, median and mode, were also extracted or calculated from the raw data where necessary. Raw data were used in the test for heterogeneity and the subgroup analysis. The summary data were used in the meta-regression analysis as explanatory variables were only available for outbreaks and not individuals. The unit of incubation data reported and extracted was days. For point source outbreaks, or outbreaks with continuous exposures where the total number of people exposed was observed and reported, the attack rate was determined.

Descriptive analyses

The extracted data were summarized according to the reported characteristics. Frequencies and percentages were calculated for year of study, study design, geographical region of study, serotype, toxin type, mode of transmission and food vehicle, where applicable.

The extracted individual incubation period data were used to plot histograms of the incubation periods and re-create the epidemic curves of the outbreaks. The epidemic curves

were plotted on a uniform x-axis representing the incubation period from one to twenty days and an individual y-axis indicating the number of cases in each outbreak.

Statistical analyses

We tested for the presence of heterogeneity using the individual incubation data, and the pattern of heterogeneity was investigated. We also identified factors that may explain heterogeneity using the summary statistics and available outbreak characteristics. Statistical analyses were done using the statistical software R version 3.2.3 (2015-12-10) – "Wooden Christmas Tree" (18).

Testing for heterogeneity. Using the available individual data extracted from the studies, the test for heterogeneity was done by calculating the value of I^2 and performing a two-sample Kolmogorov Smirnov test (KS test) to compare the cumulative distributions between studies. We applied a bootstrapped version of the test with 10,000 samples to derive p-values that will provide improved coverage given the compared data are discretized at the point of reporting (16).

Identifying factors that explain heterogeneity. In order to examine the relationship between the outbreak characteristics and the mean incubation period, we performed a linear regression analysis. We fitted a generalized linear model, with a gamma family function and a link identity, to account for the skewness of data. The mean incubation period was the dependent variable and the outbreak or host characteristics were the explanatory variables. The association of the explanatory variables on the mean incubation period was examined first using a univariate model, and then by building a multivariable model with variables that had a significant association (p-value <0.05) to test for confounding.

Identifying subgroups. Following the confirmation of heterogeneity, we explored the data according to subgroups. Using the p-values of the bootstrapped KS test, we conducted a hierarchical cluster analysis to produce a graphical representation of the dissimilarity matrix between the studies (16).

Subgroup analyses

The individual incubation data of studies in a subgroup was collated and summary statistics and outcome measures were derived for each subgroup. A forest plot was created to show the distribution of the mean incubation period and the corresponding 95% confidence interval for studies with individual incubation data.

Risk of bias

Our data were tested for 'small-study effect' by creating a funnel plot which graphically represented the relationship between sample size and incubation period.

RESULTS

Search strategy and selection process

A total of 1,980 unique articles were retrieved from both bibliographic databases, 840 from Google Scholar and 1,279 articles were identified from the grey literature search. After screening for relevance and removing duplicates identified from all three sources, 2,059 articles were excluded (Figure 1). An additional 26 articles were identified from searching through reference list of relevant review papers resulting in 2066 articles for further screening. Excluding articles that did not report incubation period resulted in 42 articles for full text review and eligibility screening. A further fourteen articles were excluded as they did not meet the eligibility criteria (Web Table 3) resulting in 28 articles for inclusion and data extraction, one of which reported two outbreaks (Web Table 4). All of the included studies were outbreak reports, and 22 of these reported individual incubation period data available for extraction. Some outbreaks were part of larger outbreaks. Three outbreaks (19–21) within the German O104 outbreak, for which incubation time could be extracted, are included. It was not possible to include all cases in the 2011 German outbreak as incubation time was not known due to uncertain date of exposure.

Descriptive analyses

Of the 29 studies included in our review, 75.9% (22/29) were of serotype O157. All but one of the studies reported the onset of either bloody diarrhoea or HUS. Nine outbreaks (31%) each reported bloody diarrhoea alone or HUS alone and ten outbreaks (34.5%) reported both HUS and bloody diarrhoea (Table 2).

Outbreaks involving mostly children account for about half (51.7%; 15/29) of the included studies while the age distribution of one study was unknown. Outbreaks involving mostly

children reported settings such as farm visits (5/15), schools (4/15), and outdoor settings (4/15) such as swimming pools, camping and exposure to surface water. Outbreaks involving mostly adults reported settings such as private parties (6/13) and nursing homes (4/13). Foodborne transmission accounted for 69% (20/29) of the outbreaks of which 40% (8/20) were private parties, and 20% (4/20) took place in either a nursing home or a school. Non-foodborne transmission accounted for 31% (9/29) of outbreaks, of which 44% (4/9) were associated with farm visits. The most commonly identified food categories in foodborne outbreaks were vegetables (35%; 7/20) and red meat (20%; 4/20) (Table 2).

The funnel plot showed no evidence of small-study effect. It was a symmetrical funnel with small sample studies reporting both short and long incubation periods (Figure 2). The re-created epidemic curves from the extracted individual data showed variation in the distribution of the incubation period (Web Figure 1).

Test for heterogeneity

The results of the Cochran's Q statistics (<0.001) and I^2 (77%) indicated high heterogeneity between the studies. From the KS test, 56% (143/253) of the resulting p-values were less than 0.05 and the probability of obtaining this proportion by chance was <0.0001.

Factors that may explain heterogeneity

The results of the regression analysis showed outbreaks involving mostly children and attack rate as factors that may influence the mean incubation period. From the univariate analysis, outbreaks with children reported an incubation period of 2.7 days shorter than outbreaks involving adults (p-value – 0.01). A 10% rise in the attack rate resulted in a reduction in incubation period by 0.3 days (p-value – 0.03). The results were similar in the multivariable

analysis; however, the p-value indicated a borderline significant association with the mean incubation period (p-value- 0.06 for both) (Table 3).

Identifying subgroups of studies

The output of the clustering analysis produces a dendrogram visualizing the dissimilarity matrix. As a result of the pragmatic adjustment made to the significance level, the corrected p-value was 0.002, and subtracting this from 1 resulted in a cut-off point of 0.998. Applying this cut-off point to the dendrogram resulted in five subgroups of studies with limited evidence of heterogeneity between them. These consisted of a subgroup of seven studies, a subgroup with five studies, a subgroup of four studies and two subgroups with three studies each (Web Figure 2).

Summary of subgroup analysis

The mean incubation period between subgroups varied from 3.5 to 8.1 days, and differed significantly between subgroups 1 to 4 (Web Figure 3). Subgroups 2 and 4 had significantly longer mean incubation periods of 6.7 days and 8.1 days respectively compared with the other subgroups (Web Table 5). The variance, skew and kurtosis also differed, and were larger for subgroups with smaller sample sizes. Some variation was observed within subgroups particularly in outbreaks reporting symptoms of bloody diarrhoea and HUS and the outbreak setting.

Two out of three outbreaks in subgroup 2 involved mostly adults and all outbreaks in subgroup 4 involved mostly adults. The distribution of serotypes in the subgroups was diverse, although all outbreaks involving serotype O104 were clustered within subgroup 4. Severity of symptoms was similar across subgroups as outbreaks in all subgroups reported both bloody diarrhoea and HUS (Web Table 5).

DISCUSSION

We have undertaken a systematic review of published and grey literature and identified articles reporting precisely estimated data on the incubation period of STEC. We extracted the reported data and due to the presence of heterogeneity, classified studies into five subgroups for analysis among which the mean incubation time varies as well as other measures of distribution such as the median and variance. The mean incubation period of the subgroups ranged from 3.5 to 8.1 days. Age and attack rate were identified as factors that influence incubation period.

In our study, the length of incubation period increased with age as outbreaks involving mostly children reported shorter incubation periods. This contrasts with the findings of Werber et al (22) where incubation period decreased with age in a single O104 outbreak using individual patient data. Children are more at risk of STEC infections (9) for a number of reasons, some of which could also influence the incubation period. However, our study could only perform ecological analysis due to the lack of individual patient data and additionally lacked information to assess many possible explanatory factors that may explain the association of age with the distribution of incubation period.

The attack rate of a disease and incubation period have been reported to have an inversely proportional relationship such that a higher attack rate results in a shorter incubation period in a study of Salmonella outbreaks (23). Our study showed that for every ten percent increase in attack rate, the incubation period was shortened by 7.2 hours or 0.3 days. Factors such as virulence of the pathogen, the infectious dose and host susceptibility which alter the attack rate of a disease in a population (24–27) and also the incubation period of the disease (28) may have contributed to this association.

We did not find statistical evidence for association of other features with incubation time: setting, mode of transmission, symptoms, and serotype. STEC O157 is the most commonly

reported serogroup (4) and was also the dominant STEC described in our review accounting for 22 outbreaks out of 29. The O104 serogroup that caused the German outbreak accounted for 3 outbreaks, hence, the paucity of outbreaks caused by non-O157 serogroup may be responsible for the non-significant association between serogroup and incubation period. Despite this, all outbreaks associated with the O104 serogroup were clustered in one subgroup, which had the longest incubation period. Mostly reporting serotype and toxin type, the outbreaks studied lacked more detailed microbial characterization. As routine STEC genome sequencing increases, testing of a wider range of pathogen factors for association with incubation period will be possible. The observed clustering of the O104 outbreaks within the same incubation time subgroup (Web Figure 3) (19–21,29), which outbreaks shared both a pathogen lineage and a transmission route through contaminated beansprouts supports the potential importance of these factors in affecting incubation period.

The variability of incubation time distribution between outbreaks, and the large number of outbreaks with incubation times longer than those cited in reference materials (1,3) is striking. The mean incubation time across the seven outbreaks in subgroup 4 was longer than the standard ranges proposed for individual cases (1,3). Even acknowledging that three of these seven were from those outbreaks (19–21) within the large German O104 outbreak (14) with identifiable incubation times and one from a related O104 outbreak in France (29), this large cluster of outbreaks with longer than expected incubation times highlights the risks associated with restricting investigation to exposures occurring within expected incubation time distributions. A consequence is the need for outbreak investigators to consider a wide range of potential incubation times. The current work offers an evidence base of the scale of variation to inform future editions of reference materials.

Although we have been able to summarize data across the published literature our study is based on very limited data compared to the large burden of disease of STEC and the

numerous outbreaks investigated and reported (5,11). The majority of STEC cases are either sporadic (30), and some are part of continuous source outbreaks (31–34) where it is difficult to identify exposure time and therefore difficult to calculate incubation periods accurately. Many studies were excluded because, although they appear to have been gathered, these data were not reported in a way that allowed suitable data extraction. Standard approaches to reporting incubation period data in outbreak reports should be developed and would be useful for better understanding incubation periods. The same is no doubt true for other questions, including the effectiveness of control measures, where the natural experiments offered by outbreaks could contribute to evidence based practice if well reported and collated.

One reporting feature of importance to incubation time is the case definition used. Where data for both were available we observed a period of four to seven days between onset of diarrhoea and onset of HUS, similar to that reported by Razzaqq et al (35). In our review, most of the case definitions used by the authors included diarrhoea or bloody diarrhoea without HUS, while a few included all three clinical outcomes. We observed that studies reporting all clinical outcomes including HUS reported longer mean incubation period that other studies; however, we did not identify a significant association between HUS and the length of incubation period as also reported by Werber et al (22). We suspect that some of the heterogeneity we observed in the incubation period distribution across outbreaks may be explained by the differences in the case definitions. Reporting with more specific case definitions and even separation of results across cases based on different symptoms is thus one example of a feature of standard reporting that could promote better evidence synthesis. Even with detailed and standardized reporting of outbreaks there are limitations to using the published literature to study factors associated with incubation time. For these questions, individual patient data allowing analysis of host factors such as pre-morbidity, ongoing medications and dose is required alongside outbreak level characteristics such as mode of

transmission and pathogen characteristics. This raises questions on how best to capture, store, and make accessible data from individual outbreaks in a way that will allow joint analysis and align with the increasing trend toward individual patient data meta-analysis in the synthesis of randomised controlled trial evidence.

The studies included in our review were predominantly outbreak reports where incubation period was not the main goal of investigation and the population being studied were cases being investigated as part of the outbreak. Therefore, there is very little possibility of encountering publication bias or selection bias due to the reported incubation period. Furthermore, we found no evidence of small-study effect. Although protecting against publication bias this lack of focus on sharing information on incubation period no doubt contributed to the exclusion of studies, with the majority of studies we identified not meeting the quality assessment criteria for reporting of incubation period. Extracting individual data involved the use of an online data extraction tool and a manual process of selecting each data point which is open to human error and could alter the reported incubation period, but these errors are likely to be small. Results are therefore not likely to be substantially biased, although based on a restricted number of studies reporting usable data.

In conclusion, our study confirmed that the incubation period varied across outbreaks identifying several subgroups some of which had incubation periods far longer than expected based on standard estimates. We identified age and attack rate as factors that may influence the distribution of incubation period. However, there was insufficient information to explain the relationship between these factors and incubation period or to study the impact of many individual patient level factors on incubation period. Our work highlights both the opportunities afforded for information synthesis across outbreaks to support evidence-based practice and the challenges to be overcome to optimise reporting and ultimately support

combined analysis across outbreaks if the full potential of these natural experiments is to be exploited.

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FIGURE LEGENDS

Figure 1. Flowchart of Study Selection Process

Figure 2. Funnel Plot Showing Relationship Between Sample Size and Mean Incubation Period

Web Figure 1. Collated Epidemic Curves of Included Studies

Web Figure 2. Dendrogram Showing Dissimilarity Matrix and Associated Subgroups

Web Figure 3. Forest Plot Showing Mean Incubation Period and Subgroup Summary Mean

Table 1. Modified PICO Element in the Systematic Review of Studies Between 1984 and 2012

PICO Elements	Components
Population studies/Participants	Cases of STEC in a laboratory confirmed point source exposure outbreak or continuous source outbreak where date of exposure and onset is known for each case. Individual case laboratory confirmation was not required within each outbreak where cases met a clinical and epidemiological case definition.
Infectious Agent	Shiga-toxin producing *Escherichia coli*
Comparator	Host factors and outbreak characteristics: food vehicle and level of contamination/dose Clinical characteristics such as HUS and bloody diarrhoea Microbiological characteristics such as serotype ad toxin type
Outcome	Time from exposure to onset of clinical illness as described or defined by the reporting authors including diarrhoea, bloody diarrhoea, abdominal pain, vomiting, HUS

Abbreviations: PICO: Population studies, Infectious Agent, Comparator and Outcome; STEC: Shiga-toxin producing *Escherichia coli;* HUS: Hemolytic Uremic Syndrome

Table 2. Characteristics of Included Outbreaks in the Systematic Review of STEC Between 1984 and 2012

Variables	Number of outbreaks	Proportion
Total number of outbreaks	29	
Year of study		
Before year 2000	16	55.2
2000 and later	13	44.8
Study design		
Case control study	9	31.0
Case study	1	3.4
Cohort study	14	48.3
Descriptive	5	17.2
Age group		
Children	15	51.7
Adults	13	44.8
Mixed	0	0.0
Unknown	1	3.4
Region of study		
Europe	16	55.2
North America	8	27.6
Asia	5	17.2
Serotype		
O157	22	75.9
O104	4	13.8
O127	1	3.4

O145 & O26	1	3.4
O103	1	3.4
Toxin type		
VT1 & VT2	9	31.0
VT2 alone	8	27.6
VT1 alone	3	10.3
Unknown	9	31.0
Clinical outcome		
Bloody diarrhoea	9	31.0
HUS alone	9	31.0
Both	10	34.5
Unknown	1	3.4
Foodborne transmission	20	69.0
Private party	8	40.0
Nursing homes	4	20.0
School	4	20.0
Community	1	5.0
Farm visit	1	5.0
Restaurant	2	10.0
Non-foodborne transmission	9	31.0
Farm visit	4	44.4
Laboratory acquired	1	11.1
Outdoor activity	2	22.2
Surface water	1	11.1
Swimming pool	1	11.1

Food vehicle		
Vegetables	7	35.0
Red meat	4	20.0
Dairy	3	15.0
Poultry	2	10.0
Others	2	10.0
Unknown	2	10.0

Abbreviations: STEC: Shiga-toxin producing *Escherichia coli;* VT: Vero-toxin; HUS: Hemolytic Uremic Syndrome

Table 3. Generalised Linear Regression Model Identifying Factors Associated with Incubation Period in the Systematic Review of Studies Between 1984 and 2012.

Variables		Univariate model (difference in days)	p-value	Multivariable model (difference in days)	p-value
Age			0.01		0.06
	Adult	Reference			
	Children	-2.70		-2.10	
Attack rate		-0.03	0.03	-0.03	0.06
Setting					
	Other	Reference			
	Farm	1.40	0.40		
	Nursing home	3.20	0.10		
	Outdoor	1.10	0.60		
	Private party	3.10	0.10		
	School	-0.60	0.70		
	Restaurant	1.50	0.40		
Mode of transmission			0.50		
	Foodborne	Reference			
	Non-foodborne	-0.60			
Serotype			0.30		
	Non O157	Reference			
	O157	-1.20			
Toxin type					
	VT1	Reference			
	VT2	1.50	0.30		

	VT1 & VT2	1.50	0.30
Bloody diarrhoea			0.90
	No	Reference	
	Yes	-0.10	
HUS			0.70
	No	Reference	
	Yes	0.30	

Abbreviations: VT: Vero-toxin; HUS: Hemolytic Uremic Syndrome