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A 10-year analysis of VTEC microbiological clearance times, in the under-six population of the Midlands, Ireland

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SUMMARY

Verotoxin-producing *Escherichia coli* (VTEC) is a significant problem in the under-six population in the Midlands, Ireland. VTEC spreads by person-to-person transmission and children attending childcare facilities are excluded until they achieve two consecutive negative stool samples. This report analyses 10 years data on the number of days children under the age of six take to microbiologically clear VTEC. We identified from our data that the median clearance time for VTEC was 39 days, interquartile range (IQR) 27–56 days, maximum clearance time 283 days. At 70 days from onset of infection, 90% of children had cleared the infection. These findings were slightly more prolonged but consistent with international literature on VTEC clearance times for children. Asymptomatic children cleared VTEC infection significantly faster (median time 25 days IQR 13–43 days) than symptomatic children (median time 43 days IQR 31–58 days). Symptomatic children older than 1 year of age cleared VTEC infection significantly faster (median time 42 days IQR 31–57) than symptomatic children year under 1 year (median time 56 days IQR 35–74 days). This report identifies clear data which can be used to more accurately advise parents on time periods required to achieve microbiological clearance from VTEC.

Key words: Community outbreaks, epidemiology, Haemolytic Uraemic Syndrome, public health, verotoxin-producing *Escherichia Coli* – VTEC.

INTRODUCTION

Ireland has the highest notification rate of confirmed verotoxin-producing *Escherichia Coli* (VTEC) in Europe [1]. The four counties of the midlands region have some of the highest rates of VTEC notifications within Ireland [2]. VTEC is a gram-negative bacterium, which can cause serious gastroenteritis and approximately 10–15% of patients with VTEC develop Haemolytic Uraemic Syndrome (HUS). HUS is an acute kidney injury characterised by renal failure, haemolytic anaemia and thrombocytopenia [3]. HUS is the leading cause of acute kidney injury in childhood, and VTEC predominates as the leading cause of HUS in childhood. HUS has a significant mortality rate associated with it (up to 5%) and a 25% risk of developing chronic kidney disease [4]. It is critically important that children with VTEC are identified and that parents are informed of the need for vigilance and understand the signs and symptoms of deterioration and development of HUS, such that they can present for clinical care appropriately. We also need to prevent the onwards spread of infection. In the under-six population VTEC

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infection is most commonly caused by person-to-person spread [5]. The bacterium is shed in stools and maintaining optimal hygiene amongst this age group can be challenging, particularly in busy childcare facilities (CCF). VTEC is a statutory notifiable disease in Ireland and is subject to public health management under the Infectious Diseases legislation [5]. As per national guidelines, children under six years suspected to have VTEC infection are excluded from CCF until proven to be microbiologically free of infection, classified as having two consecutive stool samples, taken 48 h apart, which test negative for VTEC [5]. This exclusion can be lengthy and very difficult for families to manage on a practical basis. In this study we analyse data from the Midlands's, Ireland on VTEC clearance times for the under-six population and perform subanalysis of data where possible and appropriate. The results of our analysis are then discussed in context of the international literature available on the time taken for children to microbiologically clear VTEC infection.

METHODS

All culture-positive laboratory-confirmed cases of VTEC from 1 July 2005 until 30 June 2015, who were under the age of 6 years at diagnosis, were eligible for inclusion into the study. Cases were identified through the information system CIDR (Computerised Infectious Diseases Reporting) developed and used in Ireland, and case notification records held locally. Cases were required to be resident in the four counties of the Midlands – Laois, Offaly, Longford or Westmeath. At least one negative sample was required to be included in the study. A database was established in Microsoft Excel and populated. The data were cleaned and imported to STATA version 10 for analysis. All data collection, analysis and reporting occurred within an ethical framework and followed strict data protection guidance [6].

Statistical analysis

Kaplan–Meier survival analysis was undertaken to investigate the length of time children under the age of 6 years take to clear VTEC infection in the Midlands. The final dataset for analysis consisted of 188 cases.

All data were analysed, with the onset date being used as time point zero, and the date of the second negative sample being used as the exit point from the analysis. A symptomatic date of onset was identified for 134 samples. The remaining 54 samples had no symptomatic onset data and the date of first positive sample was used for these cases. In 13 samples there was no second negative sample provided. These cases were not, or were no longer, attending CCF and therefore did not require full microbiological clearance. The initial analysis performed did not include these samples with no second clearance date. This analysis was to ascertain whether inclusion of the samples would change the output of the results. As no significant difference was identified all subsequent analyses were performed including these 13 samples, utilising the date of the first negative sample as the date of clearance. The data were stratified by gender, symptoms, age, organism serotype, verotoxic (VT) genes and diagnosis of HUS. Log-rank scores are presented for relevant analyses.

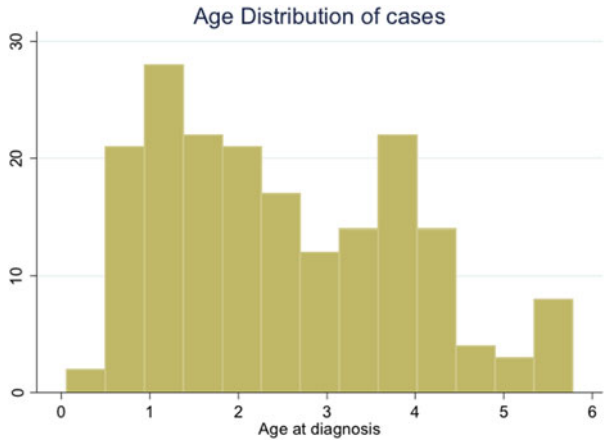

Fig. 1. Age distribution of VTEC cases.

RESULTS

Description of VTEC cases

The mean age of VTEC diagnosis was 2 years 6 months, range 7 months to 5 years 7 months (Fig. 1). Males accounted for 53% of cases and females 47% of cases. In 75% of cases children were symptomatic at diagnosis, the remaining 25% were asymptomatic and identified through contact screening. Ten organism serotypes were identified. *E. coli* O157 and O26 accounted for 85% of infections (41% and 44%, respectively). VT 1&2 predominated (43%) over VT1 only (20%) or VT2 only (37%).

Kaplan–Meier time to clearance analysis

The median time taken to achieve microbiological clearance for children under 6 years, in the Midlands

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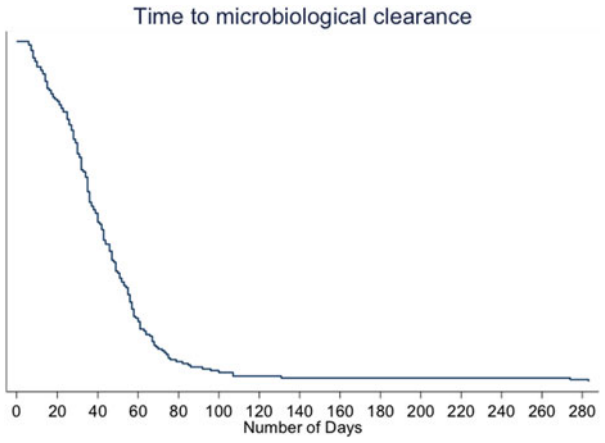

Fig. 2. Time to microbiological clearance – all samples.

was 39 days (range 1–283, interquartile range (IQR) 27–56 days) (Fig. 2). At 70 days, 90% of cases were microbiologically clear of VTEC.

Different time points were explored further (Table 1).

In three samples a very prolonged period of time was required to achieve mirobiological clearance (131, 274 and 283 days). Without these three very prolonged shedders, the median time to clearance was 38 days (IQR 27–55). Of the three very prolonged shedders identified two were male, one female and they were aged 11, 14 and 23 months. All were symptomatic. Two had *E. coli* O157, one *E. coli* O145. All were VT2 positive. One developed HUS.

Data stratification

Gender

Males accounted for 53% cases with a median clearance time of 38·5 days (IQR 25–56). Females accounted for 47% cases, median clearance time of 39·5 days (IQR 28–56). The log-rank test was not significant ($P = 0{\cdot}46$).

Symptomatic

Symptomatic cases had a median clearance time of 43 days (IQR 31–58) whilst asymptomatic cases had a median clearance time of 25 days (IQR 13–43). The log-rank test was significant ($P = 0{\cdot}0000$) (Fig. 3).

Age

Twenty-seven cases were <1 year of age at diagnosis and had a median clearance time of 44 days (IQR 25–68). There were 92 cases between the ages of 1 and 3 years, with a median clearance time of 37·5 days (IQR 26–56). Sixty-nine cases were between the ages of 3 and 6 years, with a median clearance time of 37 days (IQR 27–51). The log-rank test was not statistically significant between the clearance rates over time ($P = 0{\cdot}051$).

The data were sub-stratified by symptomatic cases only (44 asymptomatic cases were removed) and analysed by those under 1 year at diagnosis, and those 1 year and older at diagnosis. Symptomatic children under 1 year had a median clearance time of 56 days (IQR 35–74) whereas symptomatic children over 1 year had a median clearance time of 42 days (IQR 31–57). The log rank test was significant ($P = 0{\cdot}049$) (Fig. 4).

Organism serotypes

E. coli O157 accounted for 77 cases with a median time to clearance of 47 days (IQR 32–60). *E. coli* O26 accounted for 83 cases with a median time to clearance of 36 days (IQR 22–50). The log-rank test was not significant ($P = 0{\cdot}057$) (Fig. 5).

VT genes

VT1 only was present in 38 cases. The median time to clearance for VT1 was 36·5 days (IQR 28–57). VT2 was present in 69 cases. This included the three very prolonged shedders. The median time to microbiological clearance for VT2 was 40 days (IQR 31–56) and 39·5 days (IQR 30–55) with these three prolonged shedders removed. VT 1&2 were present in 80 samples with a median time to clearance of 39·5 days (IQR 23–55·5). The log-rank test was not statistically significant between the clearance rates over time ($P = 0{\cdot}67$).

When analysing *E. coli* O157 by VT2 and VT 1&2 or *E. coli* O26 by VT1, VT2 and VT 1&2 there were no significant difference in clearance times identified.

HUS

Twenty-four cases of HUS were identified over the 10-year period, equating to 10·6% of VTEC cases in children under the age of 6 years in the Midlands. *E. coli* O157 and O26 accounted for 84% of HUS cases and of these 42% were VT2 only and 42% were VT 1&2. The remaining HUS cases were non O157 and O26 strains. No HUS cases were VT1 only positive. The median clearance time for HUS cases was 40 days (IQR 26–61), as compared with 39 days (IQR 27–56) for those who did not develop HUS.

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Table 1. *Percentage of children clearing VTEC at specific time points*

Duration of shedding (days)	Number cleared VTEC	% Cleared VTEC infection	Number still positive for VTEC	% Still positive for VTEC
30	62	33	126	67
40	95	50	93	50
50	128	68	60	32
60	155	82	33	18
70	170	90	18	10
80	177	94	11	6
90	180	96	8	4
120	185	98	3	2
180	186	99	2	1
300	188	100	0	0

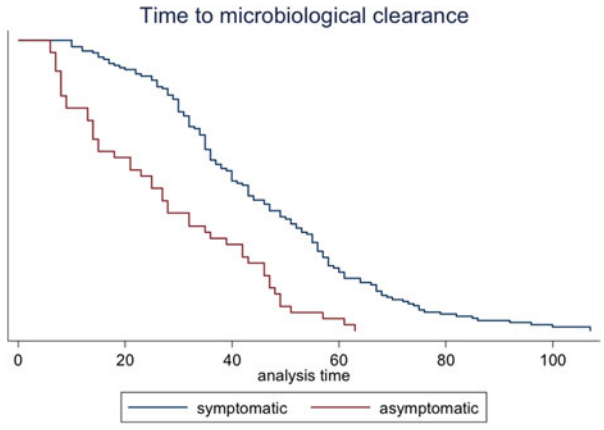

Fig. 3. Symptoms: Time to microbiological clearance, very prolonged shedders removed.

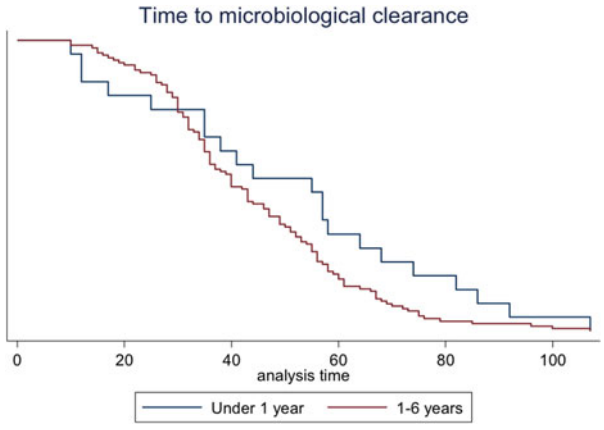

Fig. 4. Age: Time to microbiological clearance, very prolonged shedders removed.

SUMMARY OF KEY RESULTS

The median VTEC clearance time for the Midlands under-six population was 39 days (IQR 27–56).

At 40 days post-symptom onset, only 50% of children had cleared VTEC.

At 70 days post-infection, 90% of children had cleared VTEC.

Asymptomatic cases cleared infection significantly faster than symptomatic cases (median time 25 and 43 days, respectively).

Symptomatic children over 1 year cleared infection significantly faster than symptomatic children under 1 year (median time 42 and 56 days, respectively).

The three very prolonged shedders (two male, one female) ranged in age from 11 to 23 months. They were all VT2 positive. Two were O157 positive, one was O145 positive. One individual developed HUS.

No significant difference in clearance time between the two predominant organisms (O157 and O26) was identified. No significant difference in clearance times was identified between VT genes.

The median clearance times for those who developed HUS was not significantly different to those who did not develop HUS (median time 40 and 39 days, respectively).

DISCUSSIONS

This report has identified data on the length of time children, under the age of 6 years, take to microbiologically clear VTEC infection. International data on this was searched from both population level studies and from outbreak reports where clearance times were reported and extracted. This data is presented in Table 2. The median time taken to achieve microbiological clearance from the two large international population-level studies in Sweden and England were 20 and 31 days, respectively. The maximum period of time to achieve microbiological clearance from these

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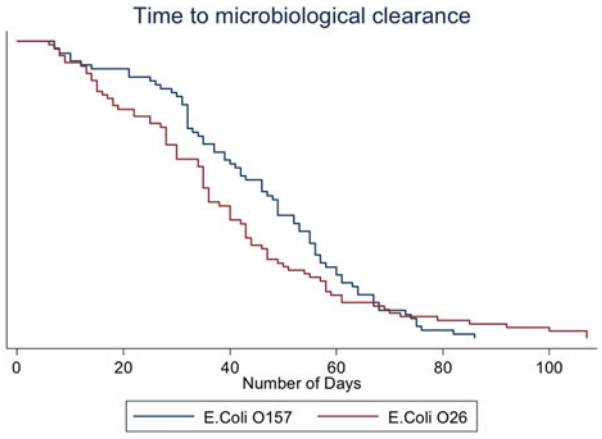

Fig. 5. *E. coli* O157 and O26: Time to microbiological clearance, very prolonged shedders removed.

cohorts was 256 and 135 days, respectively [7, 8]. Ten years of Midlands data were analysed and identified an overall median time to microbiological clearance of 39 days, with a maximum time period to clearance of 283 days. Our data identifies slightly longer median clearance times, but is consistent with the international evidence.

The data from Sweden and England both support the Midlands data that the upper limits of the time taken to microbiologically clear VTEC can be very prolonged at 256, 135 and 283 days, respectively. The exact upper limit of clearance time may well be subject to sampling frequency. Initially families are asked to submit three samples per week and so these families will have been submitting multiple stool samples for many months at this stage. This is both unpleasant and inconvenient. The frequency with which these samples are submitted ultimately decreases, to around one sample per week often, and therefore it is possible that a week or nearly 2 weeks might pass, during which time clearance may have been achieved but a sample has not been submitted for testing. This was not a concern identified for the majority of samples, as the frequency of submission and motivation to achieve clearance was very high. However for those excreting VTEC for such a prolonged period, alternate childcare arrangements have likely been made and therefore the necessity for such frequent stool specimens might be slightly diminished from a parental perspective.

The level of financial hardship that exclusion may impose on families, and, the social and behavioural impact that exclusion from stable childcare might have on children has not been widely described in the literature. This analysis does not add to this qualitatively beyond the knowledge that severe hardship was encountered by some families in managing these restrictions. Whilst a comprehensive literature review has not been performed on this topic, it appears there is a gap in international literature on the qualitative impacts that VTEC exclusion has on young children and families.

The data analysis on VTEC clearance times performed in the Midlands includes the largest cohort of children under 6 years reported in the literature, as far as the authors are aware, and several clear findings are elicited. The first clear finding was a bimodal distribution of VTEC cases. The first peak can be seen around 1 year of age, the second around 4 years of age. The first peak may be a reflection of the significant number of nappy changes which are required in the infant population and the high risk of cross infection that this provides, especially if staff hygiene practices are not optimal. The second peak may be a reflection of those children who appear confident, and assumed to be competent in their own toileting ability and thus less likely to be supervised, and this lack of supervision could have led to a greater degree of cross infection.

The second clear finding was that *E. coli* O157 and O26 (41% and 44%, respectively) predominate as causal organisms, in line with that identified through national data [2]. In England the proportion of *E. coli* O157 was much higher amongst the cases seen (97%) [7] whereas in Sweden *E. coli* O157 comprised just 19% of cases, with *E. coli* O26 comprising 16% of cases [8]. The third clear finding was that asymptomatic cases clear VTEC infection significantly faster than symptomatic cases. The age of the child when infected with VTEC is also important. The fourth clear finding was that symptomatic children older than 1 year clear VTEC infection significantly faster than symptomatic children <1 year. The fifth clear finding was that children who develop HUS require the same period of time to clear VTEC infection as children who do not go on to develop HUS. Our finding of 10·6% of children developing HUS is consistent with international literature [4].

Our data identified a shorter length of time to achieve microbiological clearance amongst those infected with *E. coli* O26 rather than *E. coli* O157, although this was not a significant finding. The clearance times of VTEC were not affected by the presence of particular VT genes.

The international literature and the Midlands data analysis can help inform local practice on discussions with parents as to VTEC clearance times and

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Table 2. *International data on time to microbiological clearance*

Year	Country of study	Age/population identified	Number included in study	Median time (days)	Range of time (days)
2003–2013	Sweden [8]	Under 10 years	165	20	1–256
2010–2011	England [7]	Under 6 years	151	31	135*
2011	USA [9]	CCF outbreak	18	30·5	14–52
2009	USA [10]	CCF outbreak	31	22	2–48
2009	Norway [11]	CCF outbreak	16	20	0–71
2004	USA [12]	CCF outbreak	11	?	All clear at 3 weeks
2002–2005	Argentina [13]	CCF outbreak	7	19	15–37
1998	Ireland [14]	CCF outbreak	10	5	2–105
1995	USA [15]	CCF outbreak	12	29	11–57
1988–1993	Germany [16]	Samples from paediatric centres (median age 3·6 years)	28 (Diarrhoea patients) 25 (HUS patients)	13 21	2–62 5–124

*Author approximation from graph provided.

exclusions. This will help assist both parents and staff with formulating management plans. Our information indicated clearly that the median time for children under the age of 6 years to achieve microbiological clearance from VTEC was 39 days. We know that we can expect to still see approximately 32% of children positive for VTEC at 50 days after the initial infection. This is a significant number of children who will be excluded for a period of time that would be very difficult for most working families to manage without formal childcare. Finally, we can be confident, that whilst unusual, it is possible for some children to require a very extended period of time to clear VTEC. Over the 10-year period, three children were still positive for VTEC at 120 days (4 months post-infection).

Certain themes have also been elucidated which could help further inform our guidance to parents. A symptomatic infant who is *E coli* O157 positive is likely to require a longer period of time to achieve microbiological clearance, than, for example, an older asymptomatic child who is O26 positive.

Our study has some limitations. To assess sufficient numbers the study examined data from the Midlands over a 10-year period. Subtle changes naturally occurred during this time in case definitions, guidelines and laboratory testing procedures and will have impacted on the number of cases identified. However, it is worth noting that these were not large or substantial changes. Importantly, the trends within the data identified should still be robust and independent of these subtle changes.

CONCLUSION

This report has identified the time taken to achieve microbiological clearance for a large cohort of children under the age of 6 years from the Midlands, Ireland. Our study identifies that the time taken to achieve microbiological clearance in this population can be very prolonged. A median time of 39 days was calculated from this study, with a maximum time to clearance of 283 days. At 40 days post-infection 50% of all cases were still positive; at 70 days post-infection 90% of all cases were cleared. Asymptomatic cases clear VTEC infection significantly faster than symptomatic cases. Symptomatic children over 1 year clear VTEC infection significantly faster than symptomatic infants.

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DECLARATION OF INTEREST

None.

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