Epidemiology of Non-O157 Shiga toxin producing Escherichia coli (STEC)
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Introduction

There are over 400 known non-O157 Shiga Toxin Producing *E. Coli* (non-O157 STEC) serotypes with more than 100 of these known to cause gastrointestinal disease in humans.¹²³ Non-O157 STECs are increasingly recognised as significant causes of gastrointestinal infections including outbreaks⁴. STEC infections cause illness ranging from mild diarrhoea to haemorrhagic colitis and life-threatening haemolytic uraemic syndrome (HUS). In the USA and in most countries worldwide, the serotype most frequently isolated and most strongly associated with HUS, and responsible for most outbreaks is O157:H7. In 1995 an enzyme immunoassay (EIA) capable of detecting any STEC was approved by the U.S. Food and Drug Administration (FDA) and introduced for use in clinical laboratories⁵.

In the USA Non-O157 STEC infection became a nationally notifiable disease in 2000. In 2009, the Centers for Disease Control and Prevention (CDC) recommended routine culture for O157 STEC on selective and differential media, and testing for non-O157 STEC using a Shiga toxin EIA or a molecular assay that detects the Shiga toxin genes (*stx1* and *stx2*).⁶⁷ Increasing use of shiga toxin assays by clinical laboratories in recent years has resulted in increased detection of these infections⁸. In the USA in 2010, for the first time, non-O157 STEC infections detected though active sentinel surveillance exceeded O157 STEC infections. Implementation of this testing strategy in the United States has contributed to the increase in reported foodborne infections attributed to STEC from 100,000 illnesses yearly in 1999, with non-O157 STEC contributing approximately 30% of these infections⁹ to 175,000 illnesses in 2011, with an estimated 64% of these infections attributed to non-O157 STEC¹⁰. In Europe similar trends have been observed and following the large German outbreak of *Escherichia coli* O104:H4 in 2011¹¹ the European Food Standards Agency (EFSA) Panel on Biological Hazards (BIOHAZ) reviewed the evidence available from European members states on the robustness of STEC surveillance systems and their ability to detect non-O157 STEC with the potential to cause large outbreaks of severe disease¹².
Incidence and burden of disease of non-O157 STEC

The problem with estimating the true incidence and burden of disease associated with non-O157 STEC is the variation worldwide in the ability to test for these pathogens. They cannot be isolated by the standard techniques used for O157 isolation. The Republic of Ireland STEC surveillance system is well developed and has observed a significant increase in the incidence of STEC infections since 2012 coinciding with the implementation of PCR (polymerase chain reaction) screening of faecal samples by front line laboratories. Serogroup O26 is currently the most common serogroup followed by O157. In 2015 the overall incidence of STEC was estimated as 16.0 per 100000 (inclusive of PCR confirmed but culture negative results) or 13.7 per 100000 if confirmed by isolation. The incidence of STEC O157 was 3.2 per 100000 population. The ratio of non-O157 STECs to O157 STEC infections is estimated to be about 4.3:1.

In Norway from 2007 the majority of laboratories implemented techniques for detection of stx/Stx. The number of STEC cases increased from fewer than 20 annually to over 50 per year by 2012. The ratio of O157 STEC to non O157 STECs was estimated to be 1:3. The most common serogroups were O157 (34.6%), O103 (15.0 %), O26 (10.2 %), O145 (7.2 %), O91 (3.9 %), O117 (3.3 %), O121 (2.1 %), O113 (1.8 %), O146 (1.8 %), and O111 (1.2 %).

A nationwide multicentre study was performed in the Netherlands, over a 12 month period, using standardised real-time PCR assays. Patients were selected if their stool contained blood on macroscopic examination, or had a history of bloody diarrhoea, or were diagnosed with HUS, or were aged <6 years. During the 12 month period there were 68 positive PCR results and STEC strains were subsequently isolated from 25 (38%) PCR-positive faecal samples. The most common serogroups in this small selected sample were O157(20%), O103(12%), O8(12%), O26(8%), O91(8%) and O174(8%). The results are consistent with those from other studies and suggests that the ratio of O157 STEC to non-O157 STEC infections in this population is at least 1:5.
Researchers in Japan used a different approach to estimate the extent of STEC carriage in the population\textsuperscript{17}. In Japan, STEC infection is a notifiable disease, and there are about 3,500-4,500 cases annually (2.7–3.5/100,000 pop). STEC O157 comprises 58.6% of the total. Screening samples are routinely collected from contacts. A total of 2,774,824 faecal samples were collected from 472,734 healthy adults throughout Japan during April 2010–March 2012, 0.08\% (398) were positive for STEC. The estimated incidence rate of asymptomatic carriers among healthy adults was 84.2/100,000 population, indicating that asymptomatic STEC infections are highly prevalent among healthy adults who were in contact with symptomatic STEC cases. These estimates must be treated cautiously because they are not representative of the general population.

The annual report of The OzFoodNet Working Group\textsuperscript{18} summarising the incidence of diseases potentially transmitted by food in Australia reported that notified cases of STEC infection were strongly influenced by state practices in the screening of faecal specimens\textsuperscript{19}. For example, South Australia public health laboratories routinely test all bloody stools with a PCR assay specific for shiga toxin genes, and rates for this state are the highest in the country. In 2011, South Australia had the highest rate of STEC reports with 3 cases per 100,000 population (n=49) followed by the Australian Capital Territory with 1.4 cases per 100,000 (n=5). The increase in the notification rate for the Australian Capital Territory relates to the commencement of an STEC screening study in October 2011 based in a local laboratory. The incidence rate for the remaining states was ten fold lower at 0.3 per 100\,000. In 2011, serogroup information was available for 61\% of STEC cases (58/95). The most common serogroups identified were: O157 (38\%, 22); O111 (17\%, 10); O26 (12\%, 7); and O128 (7\%, 4). Serotype information was obtained by serotyping cultured isolates or by PCR targeting serotype-specific genes. The remaining 37 isolates either could not be serotyped or were Shiga toxin positive by PCR only. In 2010, O157 accounted for 59\% (30/51) and O111 10\% (5/51) of serogrouped specimens.

Two large prospective, population-based studies of infectious intestinal disease (IID) incidence and aetiology have been conducted in the UK in 1993–1996 (IID1) and in 2008–2009 (IID2)\textsuperscript{20}. Culture based techniques were used for STEC O157 and PCR-based procedures for the detection of stx genes were also employed. In the
IID2 study STEC O157 was detected by culture in one out of 866 patients and non-O157 STEC in seven out of 866 patients with diarrhoea, an estimated ratio of non-O157 to O157 STEC infections of 7:121. In the USA the number of reported non-O157 STEC infections increased from an incidence of 0.12 per 100,000 population in 2000 to 0.95 per 100,000 in 2010 while the rate of O157 STEC infections decreased from 2.17 to 0.95 per 100,000, the ratio was 1:16. As a consequence of increased detection of non-O157 STECs through implementation of stx detection strategies the ratio of non-O157 to O157 STECs is currently estimated to be about 2:110.

In a prospective cohort study conducted over a 3 yr period in the South East of England an area in which many of the front line laboratories screen all faecal samples for stx DNA by PCR there were 169 non-O157 isolates and 43 O157 isolates. The ratio of culture confirmed non-O157 to O157 STEC infections was 3.9:1 (Carroll K unpublished data) for results from these laboratories.

In the study population, of over 1.8 million. The rate of all STEC infections confirmed by PCR at reference laboratory was estimated at 9.2 per 100000. The rate of culture confirmed non-O157 STEC and O157 infections was 4.4 and 1.2 per 100000 respectively. Of the non-O157 isolates 56 (33%) belonged to HUS associated serogroups (O26, O55, O91, O103, O104, O111, O121, O145). STEC O157, O26, O146 and O91 were the most common serogroups (20.3%, 11.5%, 9.2% and 8.3% respectively).

A similar increase in the isolation of non-O157 STECs has been reported by the Gastrointestinal Bacteria Reference Unit (GBRU), Public Health England (PHE) since 2012 reflecting the increased detection of these organisms by front line laboratories implementing stx DNA PCR testing2. This analysis has recently been updated for the period 2012-2016 and the increase in non-O157 STEC isolates has continued (Carroll K unpublished data). There was a twenty fold increase from fewer than 18 non-O157 STEC isolated in 2011 rising to over 370 isolated in 2016. The most common serogroups, accounting for 68% of all isolates were O26, O146, O55, O91, O103, O128, O145, O104, O111, O121, O80. The most frequently isolated serogroups were O26(21.7%), O146(12.4%) and O55(9.0%). Serogroups O26 and O55 were isolated from 73.1% of HUS cases.
Non-O157 STEC isolates: Data from Gastrointestinal Bacteria Reference Unit. Jan 2009 – Dec 2016

Key summary points
Internationally there is variation in the reporting of non-O157 STEC infections. Where PCR is routinely used to screen faecal samples for stx DNA the incidence of non-O157 STEC infections exceeds that of O157 STEC and ratios between 2:1 to 7:1 have been reported.

In England since 2012 the most common non-O157 serogroups, accounting for 68% of all isolates were O26, O146, O55, O91, O103, O128, O145, O104, O111, O121, O80. The most frequently isolated serogroups were O26(21.7%), O146(12.4%) and O55(9.0%). Serogroups O26 and O55 were isolated and confirmed from 73.1% of HUS cases.
Non-O157 STEC illness severity relative to *E. coli* O157:H7

Although in general, *E. coli* O157:H7 causes severe illness more frequently than non-O157 STEC, pathogenic non O157 STEC have been shown to cause the same range of symptoms as *E. coli* O157:H7, ranging from mild non-bloody diarrheoa to more significant health outcomes, including HUS and death, especially in the young, the elderly or immunocompromised individuals\textsuperscript{22,23}. An analysis performed by the US Foodborne Diseases Active Surveillance Network (FoodNet)\textsuperscript{24} found that non-O157 STEC infections were more common among children and Hispanics. Infections were less severe than those caused by O157 STEC, for example 85% of O157 associated cases reported bloody diarrheoa versus 55% of cases associated with all non-O157 STEC and 11% of O157 cases were diagnosed with HUS versus 1% of non-O157 cases, but this varied by serogroup. Fewer non-O157 STEC infections were associated with outbreaks (7% versus 20% for O157), while more were associated with international travel (14% versus 3% for O157). Another study from Minnesota in the USA used surveillance data from a sentinel site surveillance system\textsuperscript{23}. The Sentinel sites included an urban health maintenance organization laboratory and a hospital laboratory serving a small city and rural area. The investigators received Sorbitol-MacConkey agar plates from every stool culture performed at both sites during 2000–2006. Two hundred and six STEC isolates were identified: 108 (52%) belonged to non-O157 serotypes, and 98 (48%) were O157. Of non-O157 cases, 54% involved bloody diarrhea, and 8% involved hospitalization. O157 cases were more likely than non-O157 cases to report bloody diarrhea (78% vs 54%; $P<0.001$), hospitalization (34% vs 8%; $P<0.001$), and HUS (7% vs 0%; $P=0.005$). The urban site yielded 28 O157 and 54 non-O157 STEC isolates, whereas the rural site yielded 70 O157 and 54 non-O157 STEC isolates. As observed in other studies from the USA 74% of the non-O157 isolates were from five serogroups: O26(27%), O103(21%), O111(19%), O145(5%), and O45(4%).

The national STEC surveillance system in Japan reported that in 2011 the most frequent serogroups were O157(59%), O26(21%) and O145(5.7%). It was also reported that 41.3% of STEC O157 isolates were associated with bloody diarrheoa and 3.2% with HUS. This compared with 21.3% and 5.5% of non-O157 isolates reporting bloody diarrheoa and HUS respectively\textsuperscript{25}. 


Good quality information about clinical features of non-O157 STEC infections is lacking for most of Europe. In the period 2007-2010\textsuperscript{26}, 13,545 confirmed STEC infections were reported to ECDC. For the majority of these cases, the clinical outcome was not reported: the case fatality was not reported for 52\% of these cases, hospitalisation was not reported for 90\% and HUS status was unknown for 41\%. Data on hospitalisation have only been collected for the last two years (2009 and 2010). The clinical features (expressed as bloody diarrhoea, diarrhoea or asymptomatic) were not reported for 47\% of cases.

The UK GBRU\textsuperscript{2} study reported that for both O157 and non-O157 STEC just over half of cases were female, a lower proportion of cases were children under 15 yrs (35.7\%) than observed with STEC O157 infections (42.5\%). Diarrhoea was reported in 89.2 \% cases, bloody diarrhoea in 47.2 \% cases. Abdominal pain and vomiting were reported by 63.5 \% and 39.2 \% cases, respectively. These are comparable to symptoms reported by STEC O157 cases with the exception of bloody diarrhoea and abdominal pain, which are reported by a higher proportion of STEC O157 cases (61.0 and 79.2\%, respectively). 37.8\% of non-O157 STEC cases were hospitalized and 24.3\% developed HUS. Hospitalization rates were similar (34.3\%) amongst STEC O157 cases, but HUS was reported in approximately 5\% of STEC O157 infections\textsuperscript{27}. Two-thirds of HUS cases were children aged under 15. There was no significant difference in development of HUS by gender. One caveat attached to this analysis is that during most of the period covered by this analysis there was limited capacity to identify non-O157 STEC infections in front-line laboratories. It was standard practice for front-line laboratories to refer samples to GBRU if symptoms indicated STEC infection (HUS and/or bloody diarrhoea) and stool culture was negative for \textit{E. coli} O157 and other likely causative pathogens. This selection process together with the almost complete referral of O157 STEC isolates to GBRU may explain the very high HUS and bloody diarrhoea rates associated with non-O157 STECs in this study rather than indicating that non-O157 STECs cause more severe illnesses.

A preliminary analysis of GBRU surveillance data on 410 non-O157 STEC isolates with completed surveillance information reported during 2009-2016 from England
has been performed (Carroll K unpublished data). There was an eight-fold increase in non-O157 STEC isolates reported between 2012 and 2016 due to several frontline laboratories implementing stx DNA detection by PCR. Bloody diarrhoea was reported in 33.5% and HUS was reported in 8.8% of the cases, 63% were children under 11 yrs of age. 63% of HUS cases reported a history of bloody diarrhoea. At the present time there is no comparable data for O157 for the same period.

A cohort study from SE England of 212 cases with STEC isolates (169 non-O157 and 43 E. coli O157) observed a slight female excess in both non-O157 and O157 STEC infections and about 12% were under the age of 11 yrs in both groups. Bloody diarrhoea was reported in 51% of O157 cases compared with 13% of non-O157 STEC. There was only 1 case of HUS in a child of 6 yrs infected with a non-O157 STEC.

**Key summary points**

A number of international studies consistently report that although both O157 and non-O157 STECs cause a similar range of illness the rates of bloody diarrhoea and HUS are generally significantly higher in O157 associated cases.

STEC O26 strains are the commonest cause of non-O157 associated HUS and bloody diarrhoea in many European countries.
New diagnostics for detecting non-O157 STEC infections

Current CDC guidance from 2009\textsuperscript{6} for STEC detection and diagnosis recommends that human fecal samples suspect for STEC are first plated on O157 STEC selective and differential medium. Any phenotypically suspect colonies are then tested by latex agglutination for O157 antigen. If no phenotypically suspect colonies are detected, then the fecal sample is plated on less-selective medium and colonies from that plate are tested for Stx using appropriate methods, including but not limited to stx gene PCR. If the stx gene PCR, or comparable test, is confirmed positive for Stx then the sample is further characterized using PCR for stx1 and stx2 genes and at this point it is also tested for serogroups O26, O45, O103, O111, O121, O145, or O157. This series of tests take a total of 2–3 days if all steps are carried out.

Following a meeting in 2013 the EFSA Panel on Biological Hazards (BIOHAZ)\textsuperscript{12} recommended that for public health investigation of STEC infection, clinical and/or food samples should be screened by PCR for the presence of the stx genes. If positive, all efforts should be made to isolate and characterise the causative organism.

During the last decade there has been a rapid increase in the number of commercially available PCR methods targeting DNA variants specific to different serotypes. These are often combined with PCR assays detecting genes coding for Shiga toxins or other virulence markers. Some assays target a gene associated with the O antigen such as the \textit{wzx} gene in serotype O26 and in serotype O103, while others target genes associated with both the O and H antigens as in methods described for O111:H8 and O26:H11\textsuperscript{36,38}. More recent multiplex methods detect specific genes present in O-antigen gene clusters of four or five different O groups\textsuperscript{29,30}. These procedures can identify serogroups isolated from foods and fecal samples and in various evaluations they have proved to be sensitive and specific. The most recent PCR multiplex systems\textsuperscript{31,32,33} are designed to detect the most important STEC associated serogroups (O26, O45, O103, O104, O111, O121, O145, O157) and the three principal virulence factors (\textit{stx1, stx2, eae}).

PCR assays for \textit{stx} genes are generally designed for testing isolated cells from media or bacteria growing in enrichment broths rather than bacterial DNA extracted
directly from foods or fecal specimens. PCR procedures have been developed and evaluated for identification of STEC from human faecal samples, cattle faeces, and foods. Some PCR multiplex assays are designed to screen for different types of diarrheagenic E. coli targeting virulence genes found in enterohemorrhagic, enteroinvasive, enteropathogenic, enterotoxigenic, and enteroaggregative strains. An evaluation of the GeneDisc assay a multiplex assay targeting genes for stx, intimin, and DNA sequences characteristic of O26, O103, O111, and O157, found that it was very sensitive and capable of detecting 2 to 3 STEC colonies in a lawn of 50,000 bacteria.

In England a number of front-line laboratories have implemented PCR screening of faecal specimens for a selection of gastrointestinal pathogens including the stx genes as a proxy for STEC infections. Two systems in common use by NHS laboratories are, EntericBio PCR Multiplex or BDMax enteric bacterial panel PCR assay.

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<td>Recommendations were made by CDC in 2009 to test clinical and food samples for STEC and the EFSA followed up with similar recommendations in 2013 following the large O104:H4 outbreak. A number of PCR multiplex systems are now available that can screen faecal samples for pathogens including stx genes as a proxy for STEC.</td>
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Commercial kits are also available and in development to detect the presence of the eae gene and to identify the most important serogroups/serotypes. The kits are of two types (1) a one stage procedure screening for serogroup specific genes together with stx genes or (2) as a second stage to test samples that are positive for stx genes on a generic PCR multiplex. These tests are becoming widely used in USA and Europe.
Outbreak potential of non-O157 STEC

A review\textsuperscript{36} of the importance of non-O157 STEC identified 80 outbreaks reported in the literature or government websites between 1984 and 2009 in the U.S., Europe, Australia, and Japan. It is very likely that many other outbreaks have occurred but were not recognized because of the difficulties in identifying and characterizing non-O157:H7 STEC serotypes. The most frequently reported non-O157 serogroups associated with the outbreaks were O26(37.5%), O111(33.8%) and O121(6.3%), O103(4%) and O145(2.5%), Luna-Gierke et al \textsuperscript{37} summarized the epidemiology of non-O157 STEC outbreaks up until 2010 in the USA. Single aetiology outbreaks were defined as >2 epidemiologically linked culture-confirmed non-O157 STEC infections; multiple-aetiology outbreaks also had laboratory evidence of >2 infections caused by another enteric pathogen. 46 outbreaks were reported from twenty-six states with 1727 illnesses and 144 hospitalizations. A greater percentage of persons infected by \textit{stx}2 positive strains had HUS compared with persons infected by strains positive for \textit{stx}1 only (7% vs. 0.8%) in single aetiology outbreaks. Although non-O157 STEC are estimated to cause nearly twice as many infections as STEC O157 in the USA\textsuperscript{10} the number of detected outbreaks caused by non-O157 STEC is substantially less. This is probably due to the relatively less severe illness associated with the broad group of non-O157 STEC infections. The STEC serotype O26:H11 has been responsible for a number of outbreaks of diarrhoeal illness associated with HUS worldwide and is the commonest non-O157 STEC reported from many countries. In Italy and Rumania such outbreaks now exceed those caused by STEC serotype O157:H7\textsuperscript{38,39} and the vehicles of transmission are often dairy products such as unpastuerised cheese. In 2011 the \textit{E. coli} hybrid serotype O104:H4\textsuperscript{71} emerged for the first time and caused a large outbreak in Germany with a total of 3816 cases (including 54 deaths), 845 of which (22%) involved HUS.

**Key summary points**

The most frequently reported non-O157 serogroups associated with outbreaks internationally are O26, O111, O121, O103 and O145. The hybrid strain O104:H4 emerged in Europe in 2011 and caused one of the largest outbreaks with a high rate of HUS in adults. Recently outbreaks caused by STEC O55:H7 and O80:H2 and associated with high rates of HUS and other severe manifestations have been reported.
The significance of non-O157 serotypes

CDC data indicates that 75-80% of reported and serogrouped non-O157 STEC isolates from humans with severe symptoms (including bloody diarrhea and HUS) are from serogroups O26, O45, O103, O111, O121, and O145. The FoodNet study also found that these serogroups were most commonly reported in the proportions: O26 (26%), O103 (22%), O111 (19%), O121 (6%), O45 (5%), and O145 (4%). In 2003, Karmali et al proposed a seropathotype classification for STEC serotypes based on the reported frequency in human illness, and their known associations with outbreaks and severe outcomes including HUS and hemorrhagic colitis (see Table 1).

Karmali concluded that the ability to produce Shiga toxin does not by itself render E. coli pathogenic. The presence and expression of additional virulence factor genes are required to cause human illness. STEC virulence factors are associated with bacteriophages, plasmids and pathogenicity islands (PAIs) eg locus of enterocyte effacement (LEE), and O island (OI-122). Karmali et al reported that several genes located on OI-122 were present in 60 to 100% of STEC strains of serotypes highly associated with severe disease and outbreaks, while the same genes were detected in upto 15% of strains of serotypes not associated with severe disease or outbreaks. Researchers have demonstrated that several combinations of virulence factor genes combined with shiga toxin genes are associated with severe human illness. In addition to the German outbreak in 2011 caused by the virulent E. coli serotype O104:H4 there have been several other emergent serotypes. In France, during 2005–2014, a total of 54 patients were infected with EHEC O80:H2 and 91% had HUS. Two patients had invasive infections, and 2 died. All strains carried stx2 (variants stx2a, 2c, or 2d) and eae and at least 4 genes characteristic of pS88, a

<table>
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<th>Seropathotype</th>
<th>Relative incidence</th>
<th>Frequency of involvement in outbreaks</th>
<th>Association with severe disease</th>
<th>Serotypes</th>
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<tr>
<td>B</td>
<td>Moderate</td>
<td>Uncommon</td>
<td>Yes</td>
<td>O91:H21, O104:H21, O113:H21, others</td>
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<tr>
<td>C</td>
<td>Low</td>
<td>Rare</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>D</td>
<td>Low</td>
<td>Rare</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>E</td>
<td>Nonhuman only</td>
<td>NA</td>
<td>Multiple</td>
<td>Multiple</td>
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*HUS or hemorrhagic colitis.

*NA, not applicable.
plasmid associated with extraintestinal-virulence pathogenic *E. coli* (ExPEC) which the EHEC strain had acquired at some time. In England there have been several clusters in SW and SE England of illness caused by serotype O55:H7 which possessed both *stx2a* and *eae* genotypes and has been associated with a high incidence of HUS with neurological involvement.

**Key summary points**

In the USA 75-80% of reported and serogrouped non-O157 STEC isolates from humans with severe symptoms (including bloody diarrhea and HUS) are from strains belonging to serogroups O26, O45, O103, O111, O121, and O145.
Shiga toxin

First discovered in 1977, verocytotoxin was found to be biologically and structurally similar to Shiga toxin produced by Shigella dysenteriae Type 1. There are antigenically distinct cytotoxins found in different E. coli serotypes. STEC/VTEC strains are characterized by their ability to produce either one or both of these cytotoxins, referred to as stx1 or VT1 and stx2 or VT2. The cytotoxin production is usually bacteriophage-mediated. The shiga toxins are divided into two branches, stx1 (almost identical to Stx from S. dysenteriae Type 1) and stx2. There are a number of stx1 and stx2 subtypes that are clinically relevant. Stx2a (with or without stx2c) is strongly associated with HUS. STEC strains carrying stx2d usually predict a milder disease, however strains that produce elastase-activatable stx2d appear to predict a severe clinical outcome, particularly if the strains are negative for the eae gene and infecting adults. Other variants, such as stx2e and stx2f, are associated with animals and are rarely isolated from STEC strains infecting humans. In vitro studies using Vero monkey kidney cells have shown that stx2a, stx2d and elastase-activatable stx2d are the most potent toxins followed by stx2c. The least potent toxin subtypes were stx2b and stx1. Epidemiological studies have also suggested that STEC strains possessing both stx1a and stx2a genes are less likely to cause HUS than strains possessing Stx2a alone. Russo et al. used a mouse model to demonstrate that orally delivered purified fluorescent labelled shigatoxin Stx1a and Stx2a was less toxic than Stx2a alone. Both toxins crossed the intestinal barrier at the same rate and accumulated in the kidneys. Co-intoxication resulted in increased survival and an extended mean time to death, compared with intoxication with Stx2a only. The mechanism by which Stx1a appears to reduce the toxicity of Stx2a on the kidney tubules is being investigated. The combination of stx2a and (E. coli attachment and effacement) eae gene in particular has been associated with the development of HUS and bloody diarrhea.
The Foodnet study\(^\text{24}\) analysed STEC data for 2007–2010 and reported the Shiga toxin types for 74\% (941/1266) non-O157 STEC isolates and 61\% (1213/1973) of O157 STEC isolates. Of the non-O157 STEC isolates, 74\% had Stx1 only, 17\% had Stx2 only, and 9\% had Stx1 and Stx2. Of the O157 STEC isolates, 2\% had Stx1 only, 47\% had Stx2 only, and 51\% had Stx1 and Stx2. Patients with isolates that had Stx2 were more likely to be hospitalized than those that had isolates with Stx1 only (38\% versus 12\%, \(p < 0.001\)). All cases of HUS occurred in patients infected with STEC isolates that produced Stx2.

An analysis of 272 EHEC O26 strains isolated between 1996 and 2012\(^\text{64}\) in 7 European countries found that of the 272 STEC O26 isolates 107 (39.3\%) were \(stx1\)a, 139 (51.1\%) were \(stx2\)a and 26 (9.6\%) possessed both genes. Strains harboring \(stx2\)a only were significantly associated with HUS (OR, 14.2; 95\% confidence interval, 7.9–25.6; \(p < 0.001\)) compared to other \(stx\) genotypes. Strains harbouring \(stx1\)a were strongly associated with diarrhoeal illness only. 50\% of all \(stx2\)a-harboring strains found in 6 of the 7 countries corresponded by plasmid genes to a new virulent clone of EHEC O26:H11/H-\(^\text{65}\) that emerged in Germany in the 1990s.

Data from the European Surveillance System (TESSy data), on virulence characteristics of reported confirmed STEC cases in 2007–2010, was broken down by all cases, hospitalized cases only, and HUS cases only. Most HUS cases (89.2\%), for which information was reported on virulence factors, were either \(eae\) \(stx2\) positive, or \(eae\) \(stx1/stx2\) positive. An additional 5.9\% were either \(stx2\) positive or \(stx1/stx2\) positive but without the \(eae\) gene. Only 2.3\% were positive for \(stx1\)
(1.6% eae, stx1 and 0.7% stx1).\textsuperscript{12} None of the 124 reported infections due to serotype O103:H2 caused HUS, all but one were eae and stx1 positive.\textsuperscript{12} A study looking at clinical data on Danish patients with HUS due to STEC specifically with the virulence profile eae and stx1 positive found that other factors may have significantly contributed to the severity of disease such as antibiotics given during the acute infection and underlying co-morbidities\textsuperscript{51} and that the eae and stx1 positive combination is rarely associated with HUS.

Luna-Gierke et al\textsuperscript{37} investigating outbreaks of non-O157 STEC in the USA found that shiga toxin information was available for 35/38 single-aetiology outbreak strains and for 11 strains from 7/8 multiple-aetiology outbreaks. All but one strain in each group, for which information was available, were eae positive and all were ehxA positive. Outbreaks with stx1/stx2 or stx2 positive strains were significantly more likely to have HUS cases reported than outbreaks with stx1 positive strains (32% vs. 4% of outbreaks, P=0.02). In an analysis of single-aetiology outbreaks, 7% of patients in outbreaks with stx1/stx2 or stx2 positive strains developed HUS compared with 0.8% of patients in outbreaks with stx1 positive strains (P<0.001).

Byrne et al\textsuperscript{2} found that the development of HUS was significantly associated with non-O157 STEC strains possessing eae (odds ratio [OR] 5.845, P=0.0235) and/or stx2a (OR 9.56, P=0.0034) subtypes, when compared to all other strains. All 18 HUS cases (24 isolates) were infected with at least one strain of non-O157 STEC carrying stx2, and most strains (N=19, 86.4%) possessed the stx2a subtype and eae genotype. Two cases that developed HUS had multiple non-O157 STEC serotypes detected. The first had STEC serotypes O26, O45 and O91. Although it is not possible to determine whether one strain was more significant in the progression to HUS for this case, both the STEC O26 and O91 harboured stx2a and eae whereas the STEC O45 strain was stx2a and eae negative. The other case had STEC O26 and STEC O145 both were positive for eae and stx2a.

An analysis of the factors associated with the development of bloody diarrhea and HUS among 343 patients with STEC registered in Denmark\textsuperscript{66} during a 6-year period found bloody diarrhea developed in 36.4% and HUS in 6.1%. Multivariate analysis found that risk factors for HUS were the presence of the stx2 (OR 18.9) and eae (OR undefined) genes, being a child under 8 yrs of age, and having bloody diarrhea.
Serogroup O157, although associated with HUS in univariate analysis (OR 4.0), was not associated in the multivariate analysis (OR 1.1). This finding indicates that rather than the O group, the combined presence of the eae and stx2 genes is the important predictor of HUS.

In another study from the Danish STEC cohort of 255 stx2 positive strains, all 20 HUS cases were associated with subtype stx2a and/or stx2c, i.e. 19 HUS cases were associated with subtype stx2a either alone (11 cases) or in combination with variant stx2c (8 cases). One strain associated with HUS was found to contain variant stx2c. Subtypes stx2a and stx2c accounted for the majority of cases (83%) associated with bloody diarrhoea. O157 was by far the most common O group, accounting for 109 (42.7%) of 255 stx2-positive strains, and was associated with 12 of the HUS cases. All O157 strains from patients with HUS were stx1 negative and eae positive. stx2a (OR 43.1, 95%CI 4.9-380), stx2c (OR 3.3, 95%CI 1.04-10.5), and age under 8 yrs (OR 10.4, 95%CI 2.7-50) were strongly associated with HUS.

In a Norwegian study STEC strains were isolated from 138 (1.09%) of 12,651 patients tested between 1996-2010. Strains of serogroups O26, O103, O121, O145, and O157 were the most frequent. These serogroups were also the most frequent among the 11 patients (all <5 years old) who developed HUS. All of the HUS associated strains were positive for eae and carried stx2a. Analysis of the presence or absence of virulence genes in this study revealed that eae and stx2a were significantly more frequent in HUS-associated than non-HUS-associated strains, whereas STEC strains containing stx1 were exclusively associated with non-HUS infection. The authors concluded that stx2a and intimin are important virulence factors in STEC strains that have been associated with severe disease. Another study from Germany screened 5487 faecal samples for STEC by PCR. The STEC isolation rates from patients with HUS, bloody diarrhea, and watery diarrhea were 52.5%(268/510), 20.9%(14/67), and 5.1%(248/4910), respectively. PCR was used for stx genotyping and detection of the eae gene. 66.4% of isolates from HUS cases were serogroup O157 the remaining isolates comprised O26(13%), O145(7.1%), O103(3.4%), O111(3.0%) and others (7.1%). The investigators found that HUS was most strongly associated with isolates possessing subtype stx2a (54.9%), followed by stx2a+stx2c(25.4%), stx1+stx2c(9.7%), stx1(3.7%) and stx2c(3.7%). Isolates from cases with diarrhoea (bloody and non-bloody) commonly expressed stx1(42.4%).
189 isolates with complete shiga subtyping and eae results were studied regardless of HUS history. All stx2a and stx2c producing O157 strains were positive for eae compared with 95.4% of stx2a producing non-O157 strains and 33.3% of stx2c producing non-O157 strains.

**Key summary points**

The combination of eae gene and stx2a is strongly associated with the development of HUS and bloody diarrhoea in a number of observational studies. Observational studies have also demonstrated an association with stx2d and stx2c shiga toxin subtypes, although not as strong as the association with stx2a.

*In vitro studies have shown that stx subtypes stx2a, stx2d (in particular elastase-activatable stx2d) are very potent toxins. Stx1, stx2b, stx2e, stx2f are the least potent. Stx2c has intermediate potency but is 25 times less potent than stx2a.*

*Epidemiological studies suggest that STEC infections caused by strains possessing both stx1a and stx2a are less likely to cause HUS than strains with stx2a only. In vivo studies using mice have provided an explanation at the molecular level. Stx1a interferes with the expression of Stx2a toxicity in the kidneys possibly by competing for common receptors.*
Other virulence factors

Enterohemolysin, a plasmid-encoded toxin expressed by the ehxA gene causes the hemolysis of washed sheep erythrocytes and liberates hemoglobin from the red blood cells during infection, has been linked to severe disease symptoms. The production of EHEC Hly (an enterohemolysin) is a characteristic of E. coli of serogroup O157 and is closely associated with STEC belonging to other serogroups68,69.

The LEE pathogenicity island encodes a type III secretion system involved in the formation of attaching and effacing lesions on the colonic mucosa. The most important protein is intimin encoded by the gene eae, which is responsible for both the adhesion of bacteria to the intestinal epithelium and the attaching and effacing lesion. The combination of the LEE-encoded eae gene for intimin and stx2 is significantly more frequent in isolates from serotypes found in humans and is most strongly associated with disease in humans, particularly with severe disease. Conversely stx1, is found more frequently in serotypes not usually isolated from humans.

The LEE appears to confer enhanced virulence, since 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 outbreaks than are LEE-negative serotypes. On the other hand, serotype O157:H7 is associated with outbreaks and HUS much more commonly than other LEE-positive serotypes, and some LEE-positive serotypes isolated from cattle have never been associated with human disease. This suggests that STEC O157 strains have additional factors that enhance infectivity and pathogenicity in susceptible patients.

A second PAI, OI-122 contains at least six genes with significant homology to known virulence genes: pagC of Salmonella enterica serovar Typhimurium; sen of Shigella flexneri; two non-LEE effector (nle) genes nleB and nleE of Citrobacter rodentium; and the EHEC factor for adherence gene cluster efa1 and efa2 found in STEC O157:H7. The different virulence profiles of the non-O157 serotypes indicate that the complete OI-122 is more frequently present in isolates associated with HUS and that
the individual genes \textit{stx2}, \textit{eae}, \textit{espP}, as well as the OI-122-associated genes \textit{sen}, \textit{nleB}, \textit{nleE}, and the \textit{efa/lifA} gene cluster are significantly more often present in non-O157 STEC associated with HUS, and that the combined virulence profile \textit{stx2-nleE-efa/lifA} showed the strongest association with HUS\textsuperscript{70}.

Recently, strains of \textit{stx}-producing EAEC that do not have the \textit{eae} gene but carry a plasmid encoding \textit{aggR}, usually associated with the enteroaggregative \textit{E. coli} group have also been associated with large outbreaks with a high proportion of HUS, the most significant was the 2011 German STEC outbreak that was caused by a highly virulent strain of O104:H4 which contained \textit{aaiC} and \textit{aggR} as well as \textit{stx2a} genes\textsuperscript{71}. \textit{Stx}-producing EAEC do not possess the LEE locus common to most pathogenic STEC and have a genetic background typically present in the classical EAEC. Two genes are typically present in the EAEC strains isolated from human disease: \textit{aaiC}, encoding a secreted protein of EAEC and a plasmid associated gene encoding the \textit{AggR} activator, both are implicated in pathogenesis of EAEC\textsuperscript{72}.

These observations suggest that, in addition to LEE, OI-122 and EAEC plasmid associated genes, other unknown factors, possibly PAIs, may also enhance the virulence potential of STEC strains. There are also other potential factors that have been identified that regulate the production of shiga toxin\textsuperscript{51}.

The EFSA Panel on Biological Hazards (BIOHAZ)\textsuperscript{12} recommended a molecular approach to the classification of STEC which is described in the table below that has been taken from the report.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Group} & \textbf{Gene\textsuperscript{(b)}} & \textbf{Serogroups} & \textbf{Potential risk\textsuperscript{(d)}} & \\
\hline
I & \textit{eae}-positive or (\textit{aaiC} and \textit{aggR})-positive & O157, O26, O103, O145, O111, O104 & \text{High} & \text{High} \\
\hline
II & \textit{eae}-positive or (\textit{aaiC} and \textit{aggR})-positive & Any other & \text{High} & \text{Unknown} \\
\hline
III & \textit{eae}-negative and (\textit{aaiC} plus \textit{aggR})-negative & Any other & \text{Unknown} & \text{Unknown} \\
\hline
\end{tabular}
\caption{Proposed\textsuperscript{(a)} molecular approach for the categorisation of VTEC (\textit{vtx} present)}
\end{table}

\textsuperscript{(a)} As yet this proposed molecular approach must be regarded as provisional. This is because screening VTEC for the presence of \textit{eae}, \textit{aaiC} and \textit{aggR} genes is not routinely undertaken by all laboratories reporting data to TESSy.

\textsuperscript{(b)} Additional to the presence of \textit{vtx} genes, \textit{eae} = intimin-coding gene, \textit{aaiC} = chromosomally-encoded gene encoding secreted protein of EAEC, \textit{aggR} = plasmid-encoded regulator gene.

\textsuperscript{(c)} Needs epidemiological studies for confirmation.

\textsuperscript{(d)} HUS = haemolytic uraemic syndrome, HC = haemorrhagic colitis.
The panel concluded that pathogenicity can neither be excluded nor confirmed for a given STEC serogroup or serotype based on the seropathotype concept or analysis of the public health surveillance data and they proposed a molecular classification scheme, and that any ready to eat (RTE) product contaminated with an isolate of one of the STEC serogroups in group 1 (O157, O26, O103, O145, O111, O104) in combination with stx and [1] eae or [2] aaiC and aggR genes should be considered as presenting a potentially high risk for diarrhoea and HUS. For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS. In the absence of these genes, current available data do not allow any inference regarding potential risks.

Scheutz\textsuperscript{51}, based on the experience of managing non-O157 STEC in Denmark, and the well-documented association between Stx2 and eae or ability to colonize and persist in the gut, such as the EAEC hybrid strains (eg O104:H4), has gone further than the BIOHAZ panel and has proposed a primary definition of HUS-associated \textit{E. coli} (HUSEC) for first-line public health action. These are \textit{E. coli} with Stx2 and eae or aggR positive genotypes. If second-line subtyping of stx genes is available in a timely manner eg as a result of whole genome sequencing (WGS) then the definition of HUSEC can be refined to include only stx2a and stx2d harbouring strains which have the strongest association with HUS. All other STEC strains are considered “low-risk” STEC.

This pragmatic approach has been generally well accepted by public health services in Denmark. This approach is practical and easy to use in an operational environment, which is expected to quickly (i) evaluate the risk of progression of the disease in individuals, (ii) minimize transmission of HUSEC, (iii) rehabilitate individuals to prevent the worsening of an individual’s health, and (iv) reduce the socioeconomic impact on their families through in-appropriate public health interventions such as screening and exclusion of contacts.
**Key summary points**

European Food Standards Agency BIOHAZ panel in 2013 adopted a precautionary approach and recommended that STEC in serogroups O157, O26, O103, O145, O111, O104 in combination with stx and eae or aaiC and aggR genes should be considered as presenting a potentially high risk for diarrhoea and HUS. For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS.

Other researchers have recommended that public health action should be prioritised to E. coli strains with Stx2 and eae or aggR positive genotypes, particularly those with the stx2a and stx2d genotypes. They refer to these strains as HUSEC.
Reservoirs of non-O157 STEC

Understanding the epidemiology of non-O157:H7 STEC serotypes requires a knowledge of where they live and grow in nature (their reservoir) and how humans come into contact with them. Ruminants have been identified as the major reservoir of E. coli O157 and also appear to be the main reservoir of non-O157 STEC strains.\textsuperscript{36,73,74,75} STEC have been isolated from cattle, sheep, goats, and deer. In a novel ecological study from Germany\textsuperscript{76} a positive association was found for the incidence of 5 HUS-relevant STEC serogroups in pediatric patients (O26, O103, O111, O145, O157) and cattle density in the geographical area of residence. STEC have also been isolated from other wild and domestic animals. It is believed that, in many cases, they are transiently colonised and the STEC acquired from foods or water contaminated by fecal material from ruminants. Nevertheless, some of these transient hosts may be vehicles of infection for humans and have been associated with outbreaks.

Non-O157 STEC have been detected in numerous species of animals including psittacine birds (cockatiels, budgerigars etc) housed as pets\textsuperscript{77}. Two non-O157 STEC outbreaks in Australia were traced to contact with non-ruminants: a 2002 outbreak at a petting zoo with pigs and alpacas infected with STEC serotype O26\textsuperscript{78} and an outbreak in 2007 at an animal sanctuary was probably caused by koalas and/or kangaroos infected with serotype O55:H80\textsuperscript{79}. A child with diarrhoea, and domestic pigeons in Germany were found to harbor STEC serotype, O128:H2\textsuperscript{80}, and another child in Germany and her cat were found to be shedding identical strains of STEC O145:H\textsuperscript{81}. A survey of wildlife meat in Germany found a number of non-O157 STEC serotypes present in deer, wild boar, and wild rabbit meats\textsuperscript{82}. Of the 140 STEC strains examined in the study 80 (57.1\%) belonged to 18 serotypes previously associated with human pathogenicity. Genes linked to high virulence for humans (stx2a, stx2d, and eae) were present in 46 (32.8\%) STEC strains from game. In experiments some strains of the non-O157 STEC serotypes O26 and O111 have been reported to survive in untreated well water for over 56 days at 10\(^\circ\)C. Bacteria died more quickly at 22\(^\circ\)C but did persist in significant numbers for four weeks\textsuperscript{36}. 
**Key summary points**

Non O157 STEC strains with serotypes and virulence profiles commonly causing human disease have been isolated from a variety of ruminant species in different surveys from around the world.

Ruminants are the principal reservoir for both O157 and non-O157 STEC bacteria. Other species including birds may be transiently colonised and can act as vehicles for human infections.
Transmission pathways for non-O157 STEC infections

Most large outbreaks associated with non-O157 STEC have been associated with contaminated food or water as the main vehicle of infection. Luna-Gierke et al.\(^3^7\) in their analyses of outbreaks in the USA found that of 38 single-aetiology outbreaks, 66% were caused by non-O157 84% and transmitted through food (n=17) or person-to-person spread (n=15). The authors found that food vehicles included dairy products, vegetable produce, and meats. Childcare centres were the most common setting for person-to-person spread. Outbreaks caused by multiple pathogens including STEC were more frequently transmitted through water or animal contact. In Italy and Rumania there have been several outbreaks of STEC O26:H11 attributed to contaminated dairy products such as unpasteurised cheese\(^3^8^,\)^\(^3^9\).

In a review of food safety and non-O157 STEC infections\(^3^6\) the authors reviewed the evidence in the international literature and found a number of reports of outbreaks in children associated with direct contact with cattle. They also identified a number of reports of outbreaks probably caused by person-to-person spread of non-O157 STEC in day-care, schools and senior care facilities (see Table 2), and it appears that this form of spread might be a more common route for non-O157 STEC infections.

**Table 2. Comparison of the relative importance of vehicles associated with outbreaks of non-O157:H7 STEC and *E. coli* O157:H7.**

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>non-O157:H7 STEC</th>
<th><em>E. coli</em> O157:H7(64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal contact</td>
<td>6.2%</td>
<td>9.7%</td>
</tr>
<tr>
<td>Water</td>
<td>10.0%</td>
<td>25.6%</td>
</tr>
<tr>
<td>Person–person contact</td>
<td>28.8%</td>
<td>6.8%</td>
</tr>
<tr>
<td>Dairy</td>
<td>10.0%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Meat</td>
<td>11.2%</td>
<td>24.6%</td>
</tr>
<tr>
<td>Produce</td>
<td>6.2%</td>
<td>9.2%</td>
</tr>
<tr>
<td>Other food</td>
<td>8.8%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Unknown</td>
<td>18.8%</td>
<td>5.8%</td>
</tr>
</tbody>
</table>

Source: 36

Contaminated food is a recognised source of STEC O157 infections, and often causes large outbreaks. Non-O157 STEC outbreaks have been associated with contaminated dairy products, salad vegetables, bean sprouts and fenugreek seeds.
There were fewer reports of non-O157 STEC outbreaks caused by meat products. There were also fewer reports of water associated outbreaks caused by non-O157 STEC serogroups than for STEC O157.83,84 Several outbreaks were reported involving children playing or swimming in pool water, infected children may have been the source of bacteria for these cases. Other outbreaks were traced to water consumed at summer camps and faecal material from domestic and/or wild ruminant animals may have contaminated lakes, rivers, and some drinking water sources. Outbreaks involving food handlers are unusual. One outbreak associated with a prison and caused by STEC serogroup O45 (stx1) has been reported85 and the investigators concluded that an infected food handler was the source. There were 341 probable cases during a large restaurant associated outbreak caused by STEC O111:NM in Oklahoma. and epidemiological evidence suggested that the outbreak resulted from cross-contamination of restaurant food from food preparation equipment or surfaces, or from an unidentified infected food handler.86 During the O104:H4 outbreak in Germany an outbreak of 23 cases in a family party associated with a restaurant is postulated to have been caused by a food handler contaminating several food items from which the organism was isolated.87

A meta-analysis was performed on transmission risk factors for sporadic STEC infections. The authors selected reports with STEC infections confirmed by traditional as well as PCR based microbiological techniques88. The reports finally selected were predominantly derived from routinely gathered surveillance data. 31 studies were included, of which 25 were case-control or case-case studies. 62.5% studies found consumption of undercooked/raw meat was associated with sporadic STEC infection, 70.4% studies found contact with animals or their environment a risk factor and 57.1% of the studies found person-to-person transmission was a risk factor for sporadic cases of STEC infection. The meta-analysis indicated that infections from undercooked or raw meat occur more often with O157 strains while non-O157 STEC are more often associated with animal contact but only 6/31 studies contained sufficient information to enable this comparison to be done.
Key summary points
From the currently available evidence which comes mainly from observational studies using outbreak or routine surveillance data the main routes of transmission are similar for all STEC that commonly cause illness in humans. Person-to-person transmission may be relatively more common in non-O157 STEC infections and water associated transmission may be more common in O157 infections.

A variety of contaminated foods have been implicated in the transmission of non-O157 STEC. Food handlers have been reported to be possible sources in several outbreaks caused by a non-O157 STEC but the evidence has been epidemiological.
Transmissibility and duration of shedding of non-O157 STEC

Most of our understanding about the infectious dose, transmissibility and duration of shedding of STEC by human cases has been derived from investigations conducted in response to cases of STEC O157. There is consensus that young children pose a significant risk in terms of onward transmission and disease severity. In contrast to STEC O157 there is much less information regarding transmission and duration of shedding associated with non-O157 STEC infections, which is further complicated by their heterogeneity.

A study was reported from the United Kingdom\(^8\) of 225 children aged <6 years with microbiologically confirmed STEC. Serogroup O157 was the causative organism in 99% of cases and 201 childcare facilities were attended. The median duration of shedding was 31 days and shedding was longest in children under 12 months of age. In this study about half the children for whom this information was available had attended their childcare facility while infectious for a median of 2 days. Secondary cases were reported in 7% of facilities attended by infectious primary cases, but not all of these were considered to be due to transmission within the childcare facility itself. In a large STEC O157 outbreak associated with animal contact at a petting farm in Southern England\(^9\) there were 65 primary cases and 28 secondary cases detected through screening of household contacts. Of these, 13 were asymptomatic cases. The secondary attack rate in household members including asymptomatic cases was estimated to be 21%. An analysis of a sample of 17 primary cases (K Carroll unpublished data) with a median age of 3 yrs found that the median duration of shedding was 28 days (range 4-53 days). The primary cases belonged to 15 households but the secondary cases were found in only 4 households. In one household there were 4 secondary infections and a child of 1 yr continued to shed for 62 days. An investigation of 22 cases associated with a primary school outbreak in London\(^1\) associated with STEC O157 found that the mean and median duration of shedding were 34 days in children less than five years of age. The shortest duration of shedding was seen in the older age group of 10-15 years age (mean 13.6 days, median 12.9 days). The investigations suggested that this was a point source outbreak associated with school dinners followed by some person to person spread.
but no transmission rate was presented in the paper. In the studies described from the United Kingdom clearance was assessed by culture and isolation.

Researchers from Argentina\textsuperscript{92} investigated the duration of fecal shedding of STEC O157 and non-O157 in symptomatic and asymptomatic cases during four events in which transmission occurred among children in day-care centers. In each event, the cases were identified among children, family contacts and staff members of the Institution. The isolates were characterized by pheno-genotyping and subtyping methods. The STEC fecal shedding was prolonged and intermittent. Strains O157:H7 (1st event); O26:H11 (2nd event); O26:H11 (3rd event) and O145:NM (4th event) were shed for 23-30, 37, 31 and 19 days, respectively.

Worldwide STEC serogroup is O26 is the most frequently reported non-O157 and several investigations have examined transmission and duration of shedding. In an outbreak investigation of diarrhoeal illness in an Oregon pre-school\textsuperscript{93} caused by a strain belonging to serogroup O26 expressing stx1 and eae. Sixty-one (69%) of 89 persons in the center were screened for stx, including all 13 staff, all 41 attendees in the 4 preschool classrooms, and all 7 siblings of preschool attendees who attended school-age classrooms. Including the 2 initially reported cases, 9 (19%) of 48 children and 1 of 13 staff tested positive for STEC. All isolates were serogroup O26, tested positive for stx1 only and shared indistinguishable PFGE patterns. All O26-positive children attended 3 of the 4 preschool classrooms. Median age of infected children was 1 year (range: 6 months–5 years); 45% were female. Four children (40%) reported recent diarrhea. All children recovered within 2 weeks; none were hospitalized or suffered HUS. None of the siblings in the older age groups or any of the household members tested were positive for stx, and none reported gastrointestinal symptoms. Duration of shedding was studied in all nine O26-infected children by testing for stx using PCR, until two consecutive samples were negative. Six were followed until 2 consecutive stool samples tested negative. Duration of stx-shedding for these 6 children was 15–46 (median, 29) days. The authors did not observe any secondary transmission in households and they did not describe how the outbreak might have occurred.
In another outbreak in a childcare facility in Colorado\textsuperscript{94} caused by a strain belonging to serotype O26:H11 expressing \textit{stx1} (\textit{eae} not reported). The outbreak was probably started by an infected child introducing it into the facility followed by person-to-person spread. The spectrum of illness in this outbreak was also mild; the majority of cases did not visit a doctor for their illnesses, only 4 had bloody diarrhea, and there were no hospitalizations or HUS cases. There were 18 confirmed and 27 suspect cases detected. The illness rate was 60% for children and employees. The risk of being a case in children <36 months was twice the risk among children of 36 to 47 months (risk ratio: 2.10; 95% confidence interval: 1.00, 4.42). Duration of shedding in symptomatic confirmed cases was defined as the interval between the onset of illness and the date of the first negative \textit{stx} PCR test. The median duration of shedding among symptomatic confirmed cases (all under 4 yrs of age) was 30.5 days (range: 14–52 days). Four (22%) confirmed cases were asymptomatic and 3 (17%) shed intermittently. Nearly half (49%) of the household contacts of confirmed cases developed a diarrheal illness but were not investigated microbiologically.

In a nursery outbreak of STEC in Norway caused by serotype O103:H2 (\textit{stx1a} and \textit{eae}) cases were diagnosed by presence of \textit{stx} DNA by PCR and the same testing was used to demonstrate microbiological clearance. All cases were under 6 yrs of age and the median duration of shedding was 48 days for confirmed cases (range 30-98 days).\textsuperscript{95} A study undertaken in Germany during the large STEC O104:H4 outbreak following infected patients\textsuperscript{96} found that for all cases, patients with HUS had a median shedding duration of 13-14 days compared with 33-34 days for those with non-HUS illness. Children age ≤15 years had longer shedding durations than adults (median, 35–41 vs 14–15 days). However these results may reflect differences in antibiotic usage. The use of antibiotics was high and significantly reduced shedding duration. A sub group analysis found no difference in shedding durations between HUS and non-HUS cases when cases receiving antibiotics were excluded.\textsuperscript{97,98}

There were also very few young children in the study population. In another investigation during the same outbreak\textsuperscript{99} attempts were made to identify secondary cases. The authors report that in their opinion among cases linked to the outbreak, 7% were secondary cases (8/81 STEC gastroenteritis cases and 1/55 HUS cases). The secondary cases included six household transmissions, two nosocomial and one laboratory transmission. In the study from Hesse the proportion of secondary
cases is lower than that estimated for STEC O157 in the studies described above. However this may be because the outbreak involved very few children under the age of 6 yrs or that STEC O104:H4 is less easily transmissible person to person.

During the investigation of another Norwegian day-care centre outbreak caused by STEC O145:H28\(^{100}\) (stx1 and eae-positive), all 61 children, 14 staff and 74 close contacts submitted faecal samples. The outbreak strain was isolated from 16 cases, 9 were symptomatic with diarrhoea. The duration of faecal shedding was estimated as time interval between the first and last positive sample. The median duration of shedding was 20 days (range 0-71 days). Of the 74 household contacts screened stx1 was detected by PCR from a sample belonging to a 6 yr old sibling of one case and from the mother of another case. The attack rate was significantly higher among children aged 1-3 years compared to children aged 4-5 years. The secondary transmission rate was 2.7% in household contacts. The authors could not identify the source for this outbreak but considered person-to-person transmission to be unlikely.

The Children in the day-care centre were exposed to faecal contamination from a sheep herd, and although STEC O145 was not detected in the sheep they considered sheep faeces to be a more likely source.

Several international studies have analysed data from routine surveillance systems. A study from The Republic of Ireland\(^{101}\) analysed duration of shedding in 188 cases of STEC under 6 yrs of age reported over a 10 yr period. *E. coli* O157 and O26 accounted for 85% of infections (41% and 44%, respectively). Strains positive for both stx1 and stx2 predominated (43%) over strains possessing stx1 only (20%) or stx2 only (37%). Overall, median duration of shedding was 39 days (range 1–283). At 40 and 70 days, 50% and 90% of cases respectively had stopped shedding STEC. There was no difference in shedding duration between HUS and non HUS cases. Symptomatic children over 1 year cleared infection significantly faster than symptomatic children under 1 year (median time 42 and 56 days, respectively). There was no significant difference in clearance time between the two predominant serogroups (O157 and O26). However over the ten year period changes were made to diagnostic testing procedures which are not discussed in the paper.
A similar study evaluated a 10-year STEC screening regimen using PCR testing in a county of Sweden. All routine stool culture specimens from patients below 10 years of age were included. Patients were divided into three groups, one group where testing for STEC was requested by the clinician (n = 2366), a screening group (n = 7976) and a third group of contacts of the index cases (n = 202). 191 samples were positive for stx genes. Data on stx shedding duration was available for 165/191 (86%) children with positive stx results. The overall median duration of shedding was 20 days (1–256 days). There were no significant differences in median durations of shedding for cases with bloody diarrhoea, uncomplicated diarrhoea or HUS. The median duration of shedding were 29 days (8–107 days), 20 days (1–256 days) and 23 days (18–105 days) respectively. There was no difference in mean duration of stx shedding between stx sub types. STEC were isolated from 88 samples and 19% belonged to serogroup O157. The authors did not analyse shedding durations by serotype or by age sub-groups. They estimated that the proportion of contacts with positive stx results was 14% (29/202) which represented transmission from index cases.

A Japanese study examined factors contributing to secondary household transmission in outbreaks of associated with child care day centres during a one year period retrospectively. The authors restricted the analysis to outbreaks with >=10 cases and to families with one case attending the day centre ie transmission rates from a single infectious source only were analysed in this study. They identified 16 outbreaks in Japan, 11 associated with serogroup O26 stx1 and 5 associated with serogroup O157(stx1+stx2). The secondary household transmission rates in 16 outbreaks ranged from 0% to 34·4% (median 4·4%). The overall transmission rate was 9.2%(69/751) in household members. The transmission rates were similar for households with symptomatic and asymptomatic child cases. The rates of transmission to family members were 21·3% in brothers, 18·3% in sisters, and 10·0% in mothers. The rates were much lower for other family members. The highest rate of transmission in households were seen in siblings <10yrs of age (21%). There was also an inverse relationship between age and rates of transmission. Transmission in the younger index cases aged 1-2yrs was14% with an unexplained 25.6% transmission rate associated with cases aged 6 yrs of age.
The authors also examined potential factors affecting transmission in a multivariate analyses. They found that transmission of the O157 STEC strains was more frequent than the O26 STEC strains controlling for confounding factors. Transmission was also higher if there was a delay in providing information to care givers. Transmission rates were lower in households where information was provided face-to-face rather than by telephone or in written form.

**Key summary points**

Most information regarding trasmissability and duration of shedding of STEC has come from investigations of serogroup O157 related outbreaks or case reports. There is consensus that young children can shed for about 30-40 days but the range is broad and there is a inverse relationship between age and duration of shedding. There does not appear to be any differences related to sex, severity of disease, or stx subtype in the median duration of shedding.

There is a lack of information about shedding related to non-O157 STEC infections, complicated by the heterogeneity of serotypes. The observational studies that have been reported suggest that non-O157 STEC serogroups behave in a similar way to O157 and the factors affecting duration of shedding are likely to be similar. Investigations following the STEC O104:H4 outbreak in Germany have shown that antibiotics can reduce duration of shedding.

Secondary case rates between 7 and 21% for STEC O157 associated outbreaks have been reported. Similar transmission rates have been seen in outbreaks associated with non-O157 serogroups. In households the highest rates of transmission are generally seen when the sources are young children. The youngest children are are also those at greatest risk of acquiring the infection in households.
Public Health responses to stx DNA positive results and non-O157 STEC infections: experiences from Health Protection Teams

PCR Multiplex systems to screen faecal samples for pathogens including STEC were first implemented during 2012 in several London frontline laboratories and over the course of the following years PCR Multiplex systems have been introduced into frontline laboratories in the South East of England and the Midlands. Most laboratories have adopted one of two systems the Senbio EntericBio PCR Multiplex or BDMax BD max enteric bacterial panel PCR assay. Local Health Protection Teams (HPTs) of PHE began to receive either stx DNA PCR positive results with no local culture isolation of STEC O157 from frontline laboratories or reports of confirmed non-O157 STEC from GBRU. The numbers of results generally exceeded the reports of O157 from frontline laboratories and in the absence of any epidemiological information relevant to England concerning non-O157 STEC infections there was no clear guidance on Public Health Management, other than the PHE VTEC Operational Manual produced in 2010. The evidence for the guidance was based on STEC O157 which was the most common STEC at that time and was unequivocally associated with severe illness and a large number of outbreaks worldwide.

Following a presentation given at the annual five nations conference in Edinburgh in 2014 an informal group formed to share experiences of responding to positive results for stx by PCR subsequently confirmed to be non-O157 STEC infections. The group shared experiences which were collated and updated in 2016, in anticipation of formal guidance coming from PHE for local HPTs. The guidance was expected to address the risk assessment and public health responses to stx PCR results and non-O157 STEC infections, about which little was known in the United Kingdom at the time.

In August 2016 a number of HPTs provided updates of their experiences with PCR testing. The table summarises information about laboratories that have implemented PCR multiplex testing as of August 2016. It is clear that those HPTs faced with managing stx positive results have found it very challenging. The principal issues have been:
30-40% of locally positive PCR results are not subsequently confirmed by reference laboratories.

The public health significance of most non-O157 STEC serogroups is not known in the UK setting.

The unavoidable time taken to complete confirmatory tests on samples positive by PCR screening at frontline laboratories creates dilemmas for local HPTs on the appropriate level of public health response, even to infections with confirmed STEC strains known to be potentially virulent.

The current STEC guidance recommends an all or nothing type response based on evidence related to STEC O157. In particular several HPTs have faced difficulties following the exclusion of individuals in risk groups with confirmed non-O157 STECs belonging to serotypes not usually associated with severe disease. Similarly screening of contacts in risk groups has proved problematic.

The experience has been that HPTs with frontline laboratories testing for stx by PCR can expect huge increase in workload related to possible STEC infections which may be unsustainable.

Since April 2016 GBRU has been forced to charge frontline laboratories for the confirmatory testing of stx positive faecal samples. Although the service is still heavily subsidised there are reports of several frontline laboratories no longer sending the majority of PCR positive samples to GBRU. If this becomes widespread practice it will make the risk assessment of these cases by HPTs even more difficult.

HPTs have developed various strategies to risk assess the PCR positive results. These have varied from a full response as recommended in the VTEC operational manual to the other extreme in which nothing is done until the serotype is confirmed by GBRU and the result fed back to the HPT the case is then risk assessed. The majority of teams have evolved approaches between these extremes. A common approach is to manage cases with a history of HUS or bloody diarrhoea (usually derived from information on the request form sometimes supplemented by contacting the requesting clinician) as recommened by the VTEC operational manual. Information and hygiene advice may be sent to all other cases while awaiting reference laboratory confirmation. Several teams have incorporated reference
laboratory information into the risk assessment and prioritise public health actions to cases with HUS and bloody diarrhoea and to other cases in which STEC strains have been isolated with particular virulence/serogroup profiles. GBRU has assisted by standardising the reporting of results on the web based GDW that HPTs have access to and extending the features reported to include serotype, stx subtypes, eae profile and SNP addresses.

HPTs have experienced difficulties in deciding the appropriate level of public health response to positive stx PCR results and although the number of non-O157 STEC isolates has exceeded the number of STEC O157 almost all of these are sporadic infections. None of the affected HPTs have reported an increase in outbreaks attributable to non-O157 STEC serotypes. The recent emergence of non-O157 STEC O55:H7 in the South West of England where frontline hospital laboratories were not using PCR for screening of faecal samples was detected as a result of sentinel cases of HUS. In North West Surrey there was also a cluster of HUS cases attributable to STEC O55:H7 which as a consequence of whole genome sequencing was confirmed not to be linked to the outbreak in the South West. Screening of family members by PCR demonstrated that several household members who had mild symptoms were shedding the bacteria.

During the national outbreak of STEC O157 PT34 linked to green leafy salad leaves in Summer 2016 PCR was used extensively in Surrey and Sussex to screen faecal samples obtained from contacts and suspected cases. The main advantage was that the test enabled rapid screening of people in risk groups. The inherent sensitivity of the PCR test enabled the usual period of exclusion to be shortened in those with negative results minimising disruption to their lives and to a care home that was involved. The use of PCR also shortened clearance regimes in those individuals who rapidly cleared the pathogen. In this strategy samples that were PCR positive were cultured and if STEC was not isolated a pragmatic decision was taken that the organism was no longer viable or was present in such low numbers that the risk of transmission was very low.
### Public Health responses to non-O157 STEC infections: experiences from HPTs updated August 2016

<table>
<thead>
<tr>
<th>HP team</th>
<th>Labs using PCR (date)</th>
<th>Kit</th>
<th>No: of results</th>
<th>% False positive</th>
<th>PH actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHE South East</strong></td>
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</tbody>
</table>
| KSS Horsham (Kevin Carroll, Lisa Harvey-Vince) | Brighton (BSUH) (4.11.2013) | Senbio | 124 | 29% | SS algorithm  
PCR positive with HUS and/or bloody diarrhoea, follow VTEC Ops Manual.  
ESQ only for cases requiring public health action  
Public health actions prioritised to VT2+ eae positive profile and selected High risk serogroups  
All others are sent letter and leaflet |
| | East Sussex Hospitals (Sept 2014) | BDMax | 62 | 40% | |
| | Wrexham Park (Dec 2013) | BDMax | 1 | 0% | |
| | Surrey and Sussex Healthcare (Crawley) (Imminent) | Senbio | 1 | 0% | |
| | Maidstone and Tunbridge Wells (1.7.2015) | Senbio | 0 | 0% | |
| KSS Ashford (Karthik Paranthaman, Anita Turley) | Maidstone and Tunbridge Wells (1.7.2015) | Senbio | 2-4 PCR / week (prev 5-10 culture / yr) | | PCR positive with HUS and/or bloody diarrhoea, follow VTEC Ops Manual.  
ESQ for all PCR positives |
| Thames Valley (Clare Humphreys, Kate McPhedran) | Wrexham Park (Dec 2013) | BDMax | 58 (17 in 2014) – 55 sent to ref lab  
Additional 16 from ref lab (source unknown) | 20% including additional 16 from ref lab | All follow VTEC Ops Manual.  
Now looking to follow KSS Horsham model  
Issue with O146 (VT2+, EAE-) contact being excluded from nursery for 6 months |
| HIOW (Noeleen McFarland) | Hampshire Hospitals (Sept 2014) | ? | 68 (Sept 14 to March 15) | 50% | Since July 15 practice splits cases into 3 groups  
- Children (aged 5yo or under)  
- Adults with bloody diarrhoea  
- Adults with no bloody diarrhoea |
<table>
<thead>
<tr>
<th>Area</th>
<th>Laboratory/Pathology</th>
<th>Tests</th>
<th>Case Numbers</th>
<th>Risk Assessment</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHE South West</td>
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</tr>
<tr>
<td>Avon, Gloucestershire and Wiltshire (Shaun Adams)</td>
<td>None</td>
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<tr>
<td>Devon Cornwall and Somerset</td>
<td>None</td>
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<tr>
<td>PHE East Midlands</td>
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<tr>
<td>(Neil Ansty, Samia Latif)</td>
<td>Northampton General Hospital</td>
<td>BDMX</td>
<td>50</td>
<td>(1 July 2014 to 30 June 2015 – pre Northamptonshire) 118 (1 July 2015 to 30 June 2016 – post Northamptonshire) 25%</td>
<td>Risk assessment to determine risk group, bloody D/HUS, obvious sources. Then CCDC decision on PH actions. If in risk group and BD then PH actions, if not no further actions until ref lab results known</td>
</tr>
<tr>
<td>PHE East of England</td>
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<tr>
<td>South Midlands &amp; Hertfordshire (Emma White)</td>
<td>Northampton General Hospital</td>
<td>BDMX</td>
<td>41 (Feb 2014 to Feb 2015) 31.7%</td>
<td>Wait for culture. If culture negative (ie non O157) then rapid assessment and CCDC decision on exclusion / screening of cases / contacts. Most cases follow VTEC Ops Manual Issue with O74 case (VT2+ EAE-) food handler excluded (limited sick pay) since March 2015, case ongoing. No non O157 outbreaks since Jan 2010</td>
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<tr>
<td>Anglia (Bernadette Nazareth)</td>
<td>None (but one is planning to)</td>
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<tr>
<td>Essex (Amelia Cummins, Deborah Barker)</td>
<td>None (but Addenbrookes lab, Cambridge is planning)</td>
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<td>PHE London</td>
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<tr>
<td>London NENC (Asha Abrahams)</td>
<td>Homerton Hospital</td>
<td>BDMX</td>
<td>TBC</td>
<td>NENC/NWL algorithm • PCR+ and local culture positive managed as</td>
<td></td>
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<tr>
<td>Location</td>
<td>Laboratory</td>
<td>Confirmed VTEC</td>
<td>PCR+ and local culture negative, short questionnaire sent to GP (symptoms, occupation, risk group, epi link)</td>
<td>PCR+ and local culture negative with bloody diarrhoea, epi link and/or HUS managed as probable VTEC</td>
<td>PCR+ and local culture negative with no epi link etc., are not reported to HP team and picked up via GDW as ref lab results come in</td>
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</tr>
<tr>
<td>Royal Free Hospital North Middlesex Hospital</td>
<td>EntericBio</td>
<td>6 (2014) 2 (2015)</td>
<td>0%</td>
<td>Follow NENC/NWL algorithm</td>
<td>No outbreaks of Non-O157 since Jan 2010</td>
</tr>
<tr>
<td>London NW (Sara Atkin)</td>
<td>None But Royal Free Hospital occasionally notify a PCR result for case living in NWL area</td>
<td>-</td>
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<tr>
<td>London South (Emma Crawley-Boevey, Mike King)</td>
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<tr>
<td>HP Scotland</td>
<td>Scottish E.coli Ref Lab (SERL)</td>
<td>Multiplex 81 (July 2014 to July 2015)</td>
<td>N/A as ref lab samples</td>
<td>50% of samples were non-O157</td>
<td></td>
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<tr>
<td>Grampian (Okpo Emmanuel and Fiona Browning)</td>
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<tr>
<td>Rest of Scotland</td>
<td>One lab is trialling</td>
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<tr>
<td>Region</td>
<td>Hospital</td>
<td>Department</td>
<td>Lesions confirmed</td>
<td>N. Lesions</td>
<td>% Lesions</td>
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<tr>
<td>West Midlands West</td>
<td>Queen Elizabeth Hospital</td>
<td>Entericbio</td>
<td>16</td>
<td>18.75%</td>
<td>SOP developed</td>
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<td>Nov 2015 to June 2016</td>
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<td>Queen Elizabeth Hospital</td>
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<tr>
<td></td>
<td>(Lorraine Lighton)</td>
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<tr>
<td></td>
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<td></td>
<td>Shah)</td>
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<td>Region</td>
<td>Description</td>
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<td>PHE Yorkshire and Humber</td>
<td>North Yorkshire and Humber</td>
<td>West Yorkshire</td>
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<tr>
<td>PHE North East</td>
<td>(Jonathan Lawler, Deborah Wilson)</td>
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<tr>
<td>South Yorkshire</td>
<td>(Suzanna Matthews)</td>
<td>None</td>
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</tbody>
</table>
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