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The epidemiology, microbiology and clinical impact of Shiga toxin-producing *Escherichia coli* in England, 2009–2012

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SUMMARY

Between 1 January 2009 and 31 December 2012 in England, a total of 3717 cases were reported with evidence of Shiga toxin-producing *E. coli* (STEC) infection, and the crude incidence of STEC infection was 1·80/100 000 person-years. Incidence was highest in children aged 1–4 years (7·63/100 000 person-years). Females had a higher incidence of STEC than males [rate ratio (RR) 1·24, $P < 0·001$], and white ethnic groups had a higher incidence than non-white ethnic groups (RR 1·43, $P < 0·001$). Progression to haemolytic uraemic syndrome (HUS) was more frequent in females and children. Non-O157 STEC strains were associated with higher hospitalization and HUS rates than O157 STEC strains. In STEC O157 cases, phage type (PT) 21/28, predominantly indigenously acquired, was also associated with more severe disease than other PTs, as were strains encoding *stx2* genes. Incidence of STEC was over four times higher in people residing in rural areas than urban areas (RR 4·39, $P < 0·001$). Exposure to livestock and/or their faeces was reported twice as often in cases living in rural areas than urban areas ($P < 0·001$). Environmental/animal contact remains an important risk factor for STEC transmission and is a significant driver in the burden of sporadic STEC infection. The most commonly detected STEC serogroup in England was O157. However, a bias in testing methods results in an unquantifiable under-ascertainment of non-O157 STEC infections. Implementation of PCR-based diagnostic methods designed to detect all STEC, to address this diagnostic deficit, is therefore important.

Key words: *Escherichia coli*, foodborne zoonoses, gastrointestinal infections, infectious disease epidemiology, zoonotic foodborne diseases.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are a group of bacteria associated with human disease and are defined by the presence of one or both phage-encoded Shiga toxin genes; *stx1* and *stx2*. While in England around 900 cases of STEC are reported annually compared to around 10 000 *Salmonella* and 60 000 *Campylobacter* cases, STEC are of significant public health concern due to the severity of disease. Symptoms can range from mild gastroenteritis through severe bloody diarrhoea, to haemolytic uraemic syndrome (HUS). HUS is most commonly seen in children aged <5 years, is recognized as the most common cause of acute kidney failure in children in the UK and can be fatal, particularly in infants, young children and the elderly [1].

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Healthy cattle are the main reservoir of STEC although they are also carried by sheep and other animals [2, 3]. The infectious dose for human infection has been estimated to be <100 bacteria [4]. Transmission to humans occurs through either consumption of contaminated food or water, or exposure to a contaminated environment involving direct or indirect contact with animals or their faeces [2, 3]. The low infectious dose of STEC means that once in the population person-to-person spread is common [4–6]. STEC cause both sporadic and epidemic infections. While small outbreaks due to person-to-person spread are reported in closed settings, particularly childcare facilities [7, 8], large outbreaks are often associated with foodborne transmission [9, 10], and contact with ruminants, such as in open farms [11, 12].

The O157 STEC serogroup is most commonly associated with human disease in the UK. The majority of STEC O157 do not ferment sorbitol. Frontline diagnostic laboratories in England use standard methods, involving cefixine tellurite sorbitol MacConkey (CT-SMAC) agar [13, 14], to exploit this characteristic to preferentially detect STEC O157 from other faecal *E. coli*.

There are no simple, generally applicable culture-based tests for the detection of STEC other than serogroup O157 (non-O157 STEC) in faecal specimens, because of phenotypic diversity. However, over 400 serogroups of *E. coli* have been shown to produce Shiga toxins [15], and many non-O157 STEC have been associated with outbreaks [16–20]. The development of HUS has been shown to be associated with more than 100 serotypes of STEC [15]. In summer 2011, the largest outbreak of HUS ever recorded occurred in Germany and was found to be caused by an emergent strain of STEC, serotype O104:H4, not previously considered a significant pathotype to humans [18, 21–23].

While national laboratory report surveillance of STEC has been conducted in England for over 30 years [24, 25], on 1 January 2009, Public Health England [PHE; formerly the Health Protection Agency (HPA)] introduced a national enhanced surveillance scheme for STEC (NESSS) in England. Standardized microbiological, demographic, clinical and exposure data are collected and collated. These data are used to improve outbreak detection and elucidate the epidemiology of STEC in England. In this study we describe the microbiological characteristics and clinical impact of STEC infections, the demographic and geographical distribution of STEC infection and examine the associations between exposure to environmental factors and incidence of STEC infection by analysing data drawn from the first 4 years of enhanced surveillance (2009–2012). This is the first detailed report reconciling microbiological, epidemiological and clinical data for STEC cases in England.

METHODS

Microbiological investigations

Stool specimens from patients with suspected gastrointestinal infection were sent to local hospital laboratories where they were cultured for the presence of *E. coli* O157, following the UK Standards for Microbiology Investigations (http://www.hpa.org.uk/ProductsServices/MicrobiologyPathology/UKStandardsForMicrobiologyInvestigations/). Isolates identified locally as presumptive STEC O157, defined as non-sorbitol-fermenting *E. coli* agglutinating with *E. coli* O157 antisera, were sloped on nutrient agar and sent for confirmation and typing at the PHE Gastrointestinal Bacteria Reference Unit (GBRU) which provides the national reference service for STEC in England, Wales, and Northern Ireland. If STEC O157 was not isolated from a patient at the frontline laboratory, with clinical symptoms indicative of STEC (e.g. a child with HUS), faecal specimens were referred to GBRU and re-tested for the presence of both O157 and non-O157 STEC [26]. In cases where faecal specimens are unavailable, serum samples are referred for detection of antibodies to the lipopolysaccharide of *E. coli* O157 [27].

At GBRU, confirmation of STEC was performed using real-time PCR for the detection of *stx1, stx2, eae* (encoding intimin, associated with intimate attachment of the bacteria to the host gut mucosa) and *rfbE* O157 genes [26] on the sloped culture, which was then plated out onto sorbitol MacConkey (SMAC) agar, MacConkey agar and blood agar. Colonies from the plate found to be positive for either or both of the *stx* genes were identified as *E. coli* and serotyped using biochemical and serological tests [26]. Strains belonging to serogroup O157 were further differentiated by phage-typing [28].

Microbiological case definitions

Confirmed. STEC isolated from stool, serotyped and presence of *stx* genes confirmed.

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Probable. Serum tested and antibodies to *E. coli* O157 or a limited range of other serotypes identified.

Negative. Specimen sent to GRBU but no evidence of STEC infection found

NESSS

Local laboratories reported presumptive isolates of STEC directly to PHE centres (PHEC), responsible for health protection. Each PHEC arranged for the STEC Enhanced Surveillance Questionnaire (ESQ) to be administered to patients. The ESQ collects data in the following categories: demographic details; risk status; clinical condition (including progression to HUS); household or other close contact details; exposures including travel, food and water consumption, contact with animals, and environmental exposures; case classification; outbreak status. Completed questionnaires were forwarded for inclusion in NESSS which is managed by the PHE Department of Gastrointestinal, Emerging and Zoonotic Infections (GEZI).

Epidemiological case definitions

Primary case. A symptomatic case with no history of close contact with a confirmed case in the 7 days prior to onset of illness.

Secondary case. Case with a date of onset >4 days after the primary case or where transmission is believed to be through exposure to a primary case.

Asymptomatic case. A person identified through contact screening procedures, with no symptoms consistent with STEC infection.

Unsure: It is not possible to determine whether the case is primary or secondary with the information available. This may be because the patient was lost to follow-up, is asymptomatic or in an outbreak where it is not possible to identify the primary case(s).

Travel-related case. Case who has reported any travel outside of the UK in the 7 days prior to their date of onset of illness.

Data handling

Data from laboratory referral forms and microbiological results were entered and stored in an electronic laboratory database. Data from each final report generated by GBRU was exported into NESSS and reconciled with data from ESQs based on patient identifiable information.

Data were exported into an Access database for coding and analysis. Incidence rates were calculated using the Office for National Statistics (ONS) 2010 mid-year population estimates as the denominator [29]. For incidence by ethnicity the 2009 population estimates were used for the denominator as the latest figures available [30]. Cases were assigned to rural, town/fringe or urban using case home postcodes. The Department for Environment, Food and Rural Affairs rurality classifications (http://www.defra.gov.uk/rural/ruralstats/rural-defn/rural-urban-method.pdf) were aggregated from six categories into three: urban, town & fringe, and rural. Incidence rates and rurality were visualized in ArcGIS at the middle super output level boundaries defined by ONS. Ethnic groups collected in five categories (white, Asian/Asian British, black/black British, mixed, Chinese) were recoded as white or non-white for analyses. Data were extracted from the laboratory database to compare age and gender composition of cases of STEC with cases of *Campylobacter* and *Salmonella*.

Incidence rate ratios (RR) were calculated in Stata v. 12·0 (Stata Corporation, USA) to compare incidence in different groups. Comparisons were made by broad geographical regions based on former HPA regions. Environmental exposures were examined for primary indigenous cases only. Rurality was treated as an ordered categorical variable and the χ^2 test for a linear trend used to assess the association between rurality and reported exposures. Comparisons were made between microbiological subtypes and disease severity using Fisher's exact test to test statistical significance ($P < 0{\cdot}05$).

RESULTS

Cases reported to NESSS

Between 1 January 2009 and 31 December 2012, 4792 suspected STEC cases were reported to NESSS (Fig. 1). This included 3939 (82·2%) patients for whom both an ESQ and one or more laboratory reports were received. Laboratory reports were received for 674 individuals for whom no questionnaire was received; most ($n = 561$, 83·2%) were STEC negative and 113 (3·0%) questionnaires for STEC-infected individuals were lost to follow-up. The number of questionnaires lost to follow-up was greatest ($n = 88$) in 2009, but declined to a total of 15 cases throughout the following 3 years.

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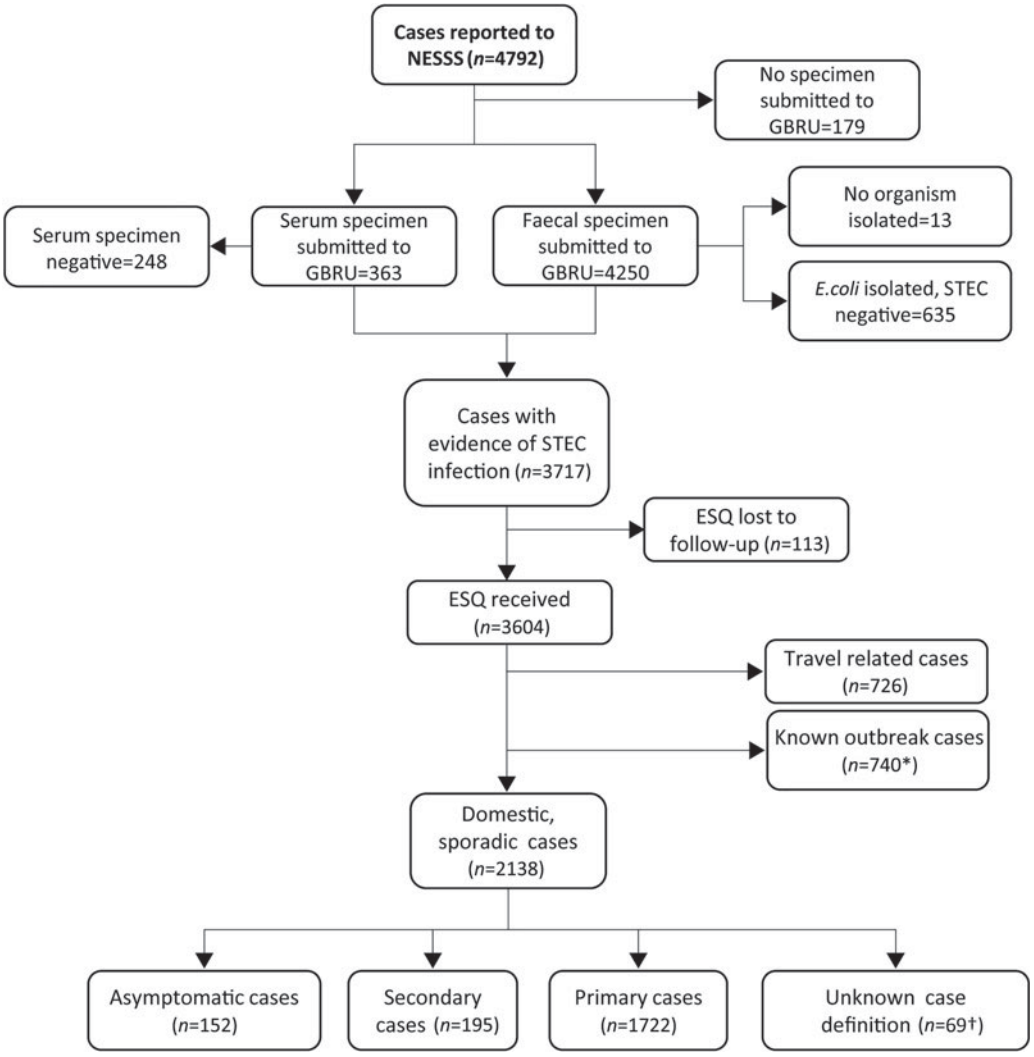

Fig. 1. Flowchart of all cases reported to the national enhanced surveillance scheme for STEC (NESSS), 2009–2012. GBRU, Gastrointestinal Bacteria Reference Unit; STEC, Shiga toxin-producing *E. coli*; ESQ, enhanced surveillance questionnaire. * Thirty-four cases were attributed to both travel and outbreaks. † It was not possible to determine an epidemiological case definition with the information available.

There was evidence of STEC infection for 3717 (80·6%) patients tested (Fig. 1) including 3602 patients with confirmed and 115 probable cases (patients with serological evidence of STEC infection). ESQs were received for 3604 confirmed (3512) and probable (92) cases. Of those, 740 were designated to known outbreaks and 726 were reported as travel cases; 34 of whom were attributed to travel-associated outbreaks. The most frequently visited countries for travel cases were Turkey ($n = 153$), followed by Egypt ($n = 101$) and Spain ($n = 91$).

A total of 2138 domestic, sporadic cases were reported; primary cases accounted for 80·5%, secondary 9·1% and 7·1% were asymptomatic. For 69 (3·3%) cases it was not possible to determine an epidemiological case definition with the available information.

Microbiological subtypes

The most commonly detected serogroup was O157, accounting for 3558 (98·8%) of cases (Table 1). Nineteen different non-O157 serogroups between 44 cases were detected. Foreign travel was reported significantly more frequently in non-O157 cases compared to O157 cases (52·3% *vs.* 20·6%, $P = 0·021$), while a similar proportion of O157 cases (21·1%) and non-O157 cases (20·5%) were associated with outbreaks. Serogroup O26 was the most common non-O157 serogroup with 15 cases (seven in 2012), five of whom were travel related. This was followed by O104 with six cases all linked to a large outbreak in Germany in summer 2011 [9, 18, 21]. Three cases

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Table 1. *Serogroups of confirmed cases of STEC by travel status* in England, 2009–2012*

STEC serogroup	Travel status			Total
	Non-travel	Travel	Not known*	
O157	2738	734	86	3558
O26	8	5	2	15
O104	1	5	0	6
O145	2	1	0	3
O unidentifiable	1	0	1	2
O113	2	0	0	2
O146	2	0	0	2
O27	0	1	0	1
O103	0	1	0	1
O111AC	0	1	0	1
O118	0	1	0	1
O156	1	0	0	1
O161	1	0	0	1
O165	1	0	0	1
O172	1	0	0	1
O186	0	1	0	1
O49	1	0	0	1
O71	0	1	0	1
O92	1	0	0	1
O117	1	0	0	1
O159	0	0	1	1
All serogroups	2761	751	90	3602

STEC, Shiga toxin-producing *E. coli*.
* Cases where no enhanced surveillance questionnaire (ESQ) was provided and travel status was therefore unknown.

of O145 were reported, including one case with STEC O26 also isolated from their specimen.

Most STEC strains carried *stx2* genes; 2390 (66·3%) carried *stx2* genes only, 1194 (33·2%) encoded both *stx1* and *stx2*. Just 20 (0·5%) cases were infected with strains carrying *stx1* genes only. A higher proportion of non-O157 strains carried *stx1* genes only than O157 strains (15·9% *vs.* 0·36%, $P < 0·001$). All O157 STEC strains encoded intimin, whereas almost half (21/44) non-O157 STEC strains encoded intimin. No O157 strains were sorbitol fermenting, compared to 33 (75·0%) non-O157 strains.

Thirty-six different phage types (PTs) were isolated from cases with STEC O157 infection (Table 2). The most common overall were PT21/28 and PT8 with 1076 and 1069 cases, respectively. For each year of the study these two PTs contributed between 54·1% and 58·0% of STEC O157 cases except for 2011 (69·2%) when indigenous cases of PT8 were elevated due to 232 (25·4%) cases associated with a single national outbreak spanning December 2010 to July 2011 [31]. There was greater diversity in travel-related cases with 53·9% of cases being of a PT other than PT21/28 or PT8 compared to 36·0% of indigenous cases ($P < 0·0001$). In travel-related cases PT8 was the most common (41·6%) and PT21/28 comprised a very small number of infections (4·5%), whereas in indigenous cases it was the predominant PT (36·9%).

Faecal specimens were referred to GBRU for 646 STEC-negative cases. Presumptive STEC was isolated from specimens from 633 (98·3%) patients but were found to be negative by PCR for the presence of *stx* genes (i.e. *stx*-negative *E. coli* was isolated). In these isolates, 78 different *E. coli* serogroups were detected; O157 most frequently ($n = 150$), of which PT1 was the most common PT ($n = 71$). A higher proportion of *stx*-negative *E. coli* O157 strains fermented sorbitol than STEC O157 strains (14·8% *vs.* 0·25%, $P < 0·0001$). Other frequently detected ($n \geqslant 10$) serogroups were O145, O25, O148, O6, O2 and O49. Intimin was encoded by 146 (23·1%) *stx*-negative *E coli* strains including 74 serogroup O157 strains.

Incidence

The crude incidence of confirmed and probable STEC was 1·80/100 000 person-years [95% confidence interval (CI) 1·74–1·86]; however, this varied by age, gender, ethnicity and geography.

Age, gender and ethnicity

Incidence of confirmed and probable STEC increased from infants aged <1 year to its highest in those aged 1–4 years (7·63/100 000 person-years, 95% CI 7·11–8·18) (Fig. 2), it then declined with each subsequent age group to reach a trough in the 20–59 years (1·15/100 000 person-years, 95% CI 1·09–1·22) and ⩾60 years (1·16/100 000 person-years, 95% CI 1·07–1·86) age groups. Over 40% of reported STEC cases were children aged <15 years (Fig. 3*a*), compared to 7·0% for cases of campylobacteriosis (Fig. 3*b*) and 17·8% for cases of salmonellosis (Fig. 3*c*).

Females were overrepresented in STEC cases with 55·6% of cases being female and a higher incidence than males (RR 1·24, $P < 0·001$). The gender difference was most apparent in those aged 20–59 years (RR 1·73, $P < 0·001$) and ⩾60 years (RR 1·43, $P < 0·001$). This gender disparity was not observed for cases of campylobacteriosis (53·3% male) or salmonellosis (50·1% male).

Ethnicity was reported for 1430 (38·5%) cases and 1300 (90·9%) were of white ethnicity. Incidence in

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Table 2. *Phage types of confirmed STEC O157 cases in England by travel status: 2009–2012*

STEC O157 PT	Travel-related cases, *n* (%)	Indigenous cases, *n* (%)	All cases*, *n* (%)
PT21/28	33 (4·50)	1014 (37·03)	1047 (30·16)
PT8	305 (41·55)	751 (27·43)	1056 (30·41)
PT32	93 (12·67)	197 (7·20)	290 (8·35)
PT2	13 (1·77)	181 (6·61)	194 (5·59)
PT4	13 (1·77)	102 (3·73)	115 (3·31)
PT14	57 (7·77)	51 (1·86)	108 (3·11)
PT34	40 (5·45)	61 (2·23)	101 (2·91)
PT54	38 (5·18)	57 (2·08)	95 (2·74)
PT31	19 (2·59)	58 (2·12)	77 (2·22)
PT33	4 (0·54)	64 (2·34)	68 (1·96)
Other phage types	49 (6·68)	116 (4·24)	165 (4·75)
Uncharacterized†	70 (9·54)	86 (3·14)	156 (4·49)
Total	734 (100·00)	2738 (100·00)	3472 (100·00)

PT, Phage type; STEC, Shiga toxin-producing *E. coli*.
* Excludes 86 cases where no enhanced surveillance questionnaire (ESQ) was received and travel status was therefore unknown.
† Includes untypable isolates and PT results which did not conform to a known PT.

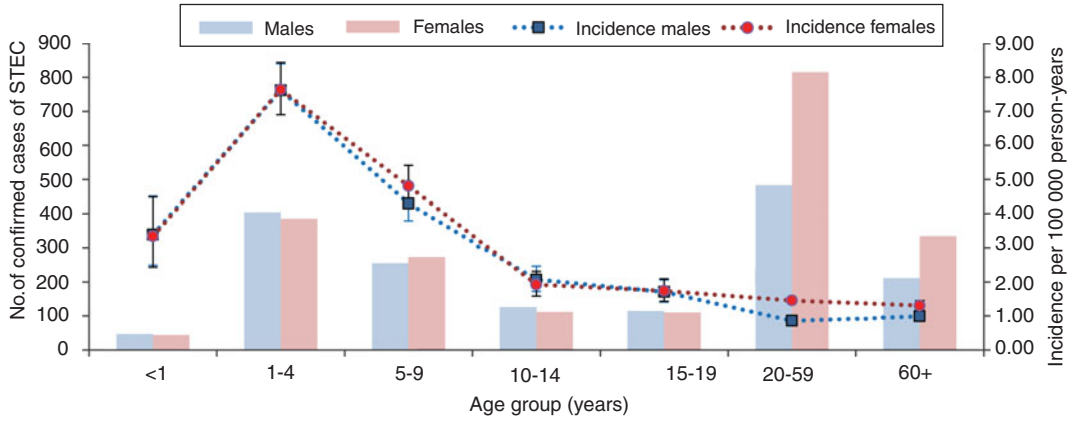

Fig. 2. No. confirmed and probable cases of Shiga toxin-producing *E. coli* (STEC) and incidence of STEC/100 000 person-years by age group and gender reported to the national enhanced surveillance scheme for STEC (NESSS), 2009–2012.

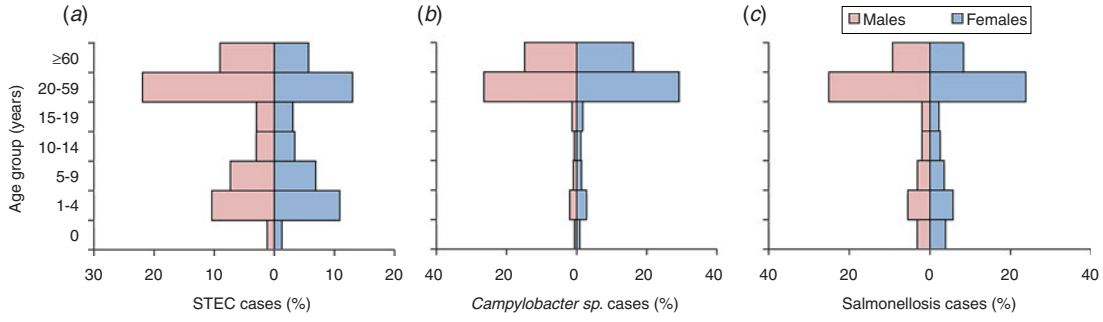

Fig. 3. Age and sex distributions for cases of (*a*) Shiga toxin-producing *E. coli* (STEC), (*b*) campylobacteriosis, and (*c*) salmonellosis and reported to national surveillance systems in England, 2009–2012.

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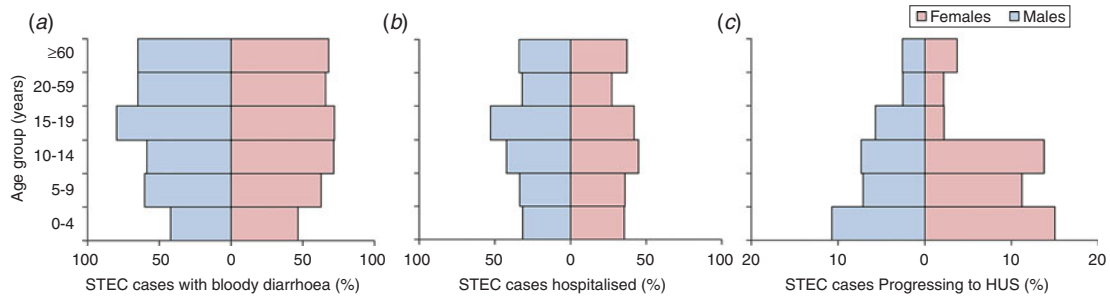

Fig. 4. Age and sex distribution for symptomatic confirmed and probable Shiga toxin-producing *E. coli* (STEC) cases in England reporting (*a*) bloody diarrhoea, (*b*) hospitalization and (*c*) progression to HUS, 2009–2012.

the white population was significantly higher than those of non-white ethnicity (RR 1·43, $P < 0·001$).

Clinical features

ESQs were available and symptomatic infection reported for 3267 (90·7%) confirmed and 92 (80·0%) probable cases of STEC. A history of diarrhoea was indicated for 92·8% (3117) cases, including bloody diarrhoea for 61·0% (2050). Abdominal pain was frequently reported (79·2%, 2662), while fever (32·5%, 1092), nausea (46·6%, 1564) and vomiting (37·3%, 1252) were reported less often. Half (1745) of cases were still ill when the ESQ was administered, the median duration of illness for the remaining cases was 6 days [interquartile range (IQR) 1–92]. Hospitalization was reported for 34·3% (1151) of cases and the median duration of hospitalization was 3 days (IQR 1–21). Cases were reported as STEC-HUS either on laboratory referral forms or ESQs for 6·4% (215) of cases. Thirteen deaths were reported, although cause of death was not provided. These included eight STEC O157 cases, one O26 case, and four fatalities with serological evidence of O157 infection only. Nine were female and all but one were adult cases with a mean age of 65 years (95% CI 52·7–77·3).

For most age groups, bloody diarrhoea and hospitalization in confirmed and probable STEC cases was similarly reported for both sexes (Fig. 4*a*, *b*). Both were reported most frequently in males aged 15–19 years; however, increased progression to HUS in this group was not observed (Fig. 4*c*). Three quarters of STEC-HUS cases were in children aged 0–14 years, with a mean age of 14·6 years (95% CI 11·7–17·5). STEC-HUS was more frequently reported in females than males in both children and adults aged ⩾60 years (Fig. 4*c*). The highest proportion of STEC-HUS cases was in females aged 1–4 years at 15·0%. Although serum specimens only were submitted for 92 probable STEC cases, a diarrhoeal prodrome was reported in most (80) cases, including bloody diarrhoea in over half (47). The majority (86) were hospitalized and over half (50) developed HUS.

Severity of illness also varied by microbiological subtype and specimen type (Table 3). In STEC O157 cases, vomiting was more frequently reported in cases of PT21/28 than PT8 (40·1% *vs.* 34·2%, $P = 0·0078$). Hospitalization rates were higher (41·7% *vs.* 29·3%, $P < 0·001$), as was progression to HUS (8·5% *vs.* 1·1%, $P < 0·001$). PT2 was also associated with more severe disease than PT8, including vomiting (43·8% *vs.* 34·2%, $P = 0·0153$), hospitalization (43·8%, $P = 0·0001$), and progression to HUS (7·6%, $P < 0·0001$).

There were 18 HUS cases with non-O157 STEC serogroups; ten cases of O26, two cases of O104 (related to the outbreak in Germany in 2011) and one case each of O113, O145, O159, O165, O172, and O186. Clinical features in non-O157 cases did not differ significantly by presence of intimin although numbers were small. Hospitalization rates were higher in non-O157 cases compared to O157 cases (Table 3, $P = 0·0121$). Progression to HUS was reported ten times more frequently in non-O157 cases than O157 cases ($P = 0·0001$). Two cases of non-O157 STEC-HUS, including one fatal O26 infection, were reported but no ESQ was received so information on clinical prodrome was not available. Isolates from all cases of confirmed STEC-HUS encoded *stx2*; 139 were *stx2* only, and 23 were *stx1* and *stx2*.

Clinical data were available for 302 cases infected with *stx*-negative *E. coli* [89 carried the intimin gene (*eae*)]. Of these cases, diarrhoea, vomiting, abdominal pain, fever, and nausea were reported by similar proportions as STEC cases. Bloody diarrhoea, however, was reported by only 60 (19·9%) and hospitalization by 49 (16·2%) of these *stx*-negative *E. coli* cases.

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Table 3. *Clinical features of confirmed STEC cases in England, by microbiological subtype, 2009–2012*

Clinical feature	Serotype O157					Non-O157 serotypes	All serotypes
	PT21/28	PT8	PT2	Other PTs	All O157		
No. of cases*	967	1002	185	1077	3231	36	3267
Diarrhoea	878 (90·8)	957 (95·5)	176 (95·1)	996 (92·5)	3007 (93·1)	30 (83·3)	3037 (93·0)
Bloody diarrhoea	665 (68·8)	667 (66·6)	137 (74·0)	513 (47·6)	1982 (61·3)	21 (58·3)	2003 (61·3)
Abdominal pain	742 (76·7)	857 (85·5)	155 (83·8)	822 (76·3)	2576 (79·7)	25 (69·4)	2601 (79·6)
Vomiting	388 (40·1)‡	343 (34·2)	81 (43·8)‡	356 (33·0)	1168 (36·2)	15 (41·7)	1183 (36·2)
Fever	297 (30·71)	340 (33·9)	64 (34·6)	696 (64·6)	1036 (32·1)	10 (27·8)	1046 (32·0)
Hospitalizations	403 (41·7)‡	294 (29·3)	81 (43·8)‡	268 (24·9)	1046 (32·4)	19 (52·8)§	1065 (32·6)
HUS†	82 (8·5)‡	11 (1·1)	14 (7·6)‡	35 (3·3)	142 (4·4)	16 (44·4)§	158 (4·8)
Deaths†	6 (0·6)	0 (0·0)	0 (0·0)	2 (0·2)	8 (0·3)	0 (0·0)	8 (0·24)

Values given are *n* (%).
STEC, Shiga toxin-producing *E. coli*; PT, phage type.
* Cases where an enhanced surveillance questionnaire (ESQ) was received and reported as symptomatic only (excludes 80 cases where ESQs were lost to follow-up and 245 cases reported as asymptomatic).
† Three additional haemolytic uraemic syndrome (HUS) cases (O159 *eae*-, O157 *eae*+, O26 *eae*+), including one death (O26 strain) were reported but no ESQ was received so are not included.
‡ Denotes statistically significant difference compared to PT8.
§ Denotes a statistically significant difference compared to all O157 cases.

There were no significant differences in reporting of symptoms between *stx*-negative *E. coli* depending on the presence of intimin (data not shown). However cases with *stx*- and *eae*-negative strains were significantly more likely to report hospitalization than those with intimin-positive strains (24·07% *vs.* 8·53, $P = 0·001$).

An additional 115 cases of HUS were reported to NESSS without evidence of STEC, including three who died: 87 cases had only submitted serum samples which were negative for antibodies to *E. coli* O157 and no specimen was received by GBRU for 13 cases. For 15 HUS cases *stx*-negative *E. coli* was isolated, including one unidentifiable serogroup and three O157 strains encoding intimin. A gender disparity was also observed in these HUS cases with 55·7% being female with a mean age of 25·5 years (95% CI 20·5–30·5), which was significantly higher than 14·6 years for STEC-HUS cases ($P = 0·0001$). Compared to STEC-HUS cases, a significantly higher proportion of cases were adults aged ⩾60 years (20·0% *vs.* 7·9%, $P = 0·007$).

Seasonality

Two-thirds of STEC cases were reported in the summer months of May–September, with the highest frequency of cases in August of each year (data not shown). Similar seasonal fluctuations were observed in both domestic and travel cases. While the majority (48/72, 66·7%) of outbreaks occurred in the summer months, outbreaks also occurred in the winter months. Between December 2010 and June 2011, the largest recorded national outbreak of STEC in the UK occurred with 167 associated cases in England [31].

Geography

There were regional variations in the incidence of STEC. Incidence was lowest in the London region (1·04/100 000 person-years) and highest in the Yorkshire & Humber region (2·48/100 000 person-years). Rates were similarly high in the North East, North West and South West regions. The age and gender distribution of cases was similar across all regions. Ethnicity, however, varied regionally, with the highest non-white British population comprising 40·5% of the population in London and the lowest in the North East (7·6%) [18].

The incidence of primary domestic sporadic STEC cases declined with decreasing rurality (Fig. 5). Compared to cases living in urban areas, those living in town and fringe areas had a significantly higher incidence rate (RR 1·27, $P < 0·001$), while the incidence of STEC was over four times higher in people living in rural areas than those from urban areas (RR 4·39, $P < 0·001$).Many areas had zero incidence (i.e. no cases) of STEC infection during the study period,

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Fig. 5. (*a*) Rurality and (*b*) geographical distribution of Shiga toxin-producing *E. coli* (STEC) incidence in England, 2009–2012.

and although the highest incidence was in rural areas, not all rural areas had high incidence and some had no STEC cases during the study period.

Geographically associated factors

Of 1722 primary, domestic, sporadic cases for whom an ESQ was received, one or more environmental exposures were reported for 681 (39·5%) (Table 4). These included direct or indirect contact with farm animals and/or their faeces (649, 37·7%), drinking water from private water supplies (74, 4·3%) and recreational exposure to open-freshwater sources, e.g. paddling, swimming or fishing in ponds, lakes, rivers or streams (63, 3·7%).

While contact with farm animals was reported in a farm setting for 301 (17·5%) cases, an additional 298 (17·3%) cases only reported contact with farm animals or their faeces in other settings, including at agricultural shows, livery yards and in paddocks or fields where animals were grazing. The species of farm animal was not specified for 298 (45·9%) cases who reported farm animal/faeces contact. Where one or more animal species was reported ($n = 351$), cattle were most frequently reported ($n = 173$), followed by horses ($n = 148$), sheep ($n = 133$), poultry ($n = 127$), goats ($n = 57$) and pigs ($n = 54$). Exposure to more than one species was reported for 187 cases.

Reporting of any environmental exposure increased with increasing rurality (Table 4) and was reported by twice the percentage of cases in rural areas compared to urban areas ($P < 0·001$). Reported consumption of water from private water supplies, open freshwater exposure, direct and indirect contact with farm animals/faeces were significantly higher in rural and town and fringe cases compared to urban cases ($P < 0·001$). Where farm settings were reported, the majority (84·5%) of urban cases reported visiting an open farm or holidaying on a farm, whereas the majority (58·9%) of rural cases reported living on, working at, or having access to a farm through relatives or neighbours (data not shown).

DISCUSSION

In England the highest burden of STEC infection is in children, consistent with previous reports from the UK and elsewhere [25, 32, 33]. Children with infectious intestinal disease are more likely than adults to present to primary care and have clinical specimens taken [34], meaning laboratory reporting emphasizes the impact of disease in children more than adults. However, the skew towards children in the age distribution of STEC cases is not observed for *Salmonella enterica* or *Campylobacter* sp. Transmission routes are largely environmental for STEC and less so for

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Table 4. *Reported environmental exposures of 1722 primary, domestic, sporadic STEC cases in England, 2009–2012, by urbanicity of residential postcode*

Exposure/urbanicity group	Exposed *n* (%)	χ^2 test for trend *P* value
Any environmental exposure*		
Urban	340 (29·9)	
Town and fringe	113 (53·6)	
Rural	228 (61·3)	<0·0001
All cases	681 (39·5)	
Private water supplies		
Urban	22 (1·9)	
Town and fringe	5 (2·4)	
Rural	47 (12·6)	<0·0001
All cases	74 (4·3)	
Open freshwater exposure†		
Urban	33 (2·9)	
Town and fringe	14 (6·6)	
Rural	16 (4·3)	<0·0001
All cases	63 (3·6)	
Direct/indirect contact with farm animals/faeces on farms‡		
Urban	168 (14·7)	
Town and fringe	51 (24·2)	
Rural	82 (22·0)	<0·0001
All cases	301 (17·5)	
Direct/indirect contact with farms animals/faeces in non-farm setting§		
Urban	156 (13·7)	
Town and fringe	57 (27·0)	
Rural	135 (36·3)	0·0002
All cases	348 (20·2)	

STEC, Shiga toxin-producing *E. coli.*

* Includes any reported exposures to private water supplies, open freshwater sources and any contact with farm animals and/or their faeces.

† Includes paddling, swimming, boating or fishing in a freshwater pond, stream, lake or river.

‡ Includes direct or indirect contact with farm animals including cattle, sheep, goats, horses, pigs and poultry at private or open farms.

§ Includes direct or indirect contact with farm animals including cattle, sheep, goats, horses, pigs and poultry *not* at farms but in other settings including walking in fields where those animals graze or exposure to manure.

S. enterica or *Campylobacter* sp., and as children have poorer hygiene practices than adults this may contribute to the higher incidence in children. The low infectious dose and propensity for person-to-person transmission of STEC in childcare facilities also increases risk in these age groups [4, 7, 8]. The nature of STEC as an environmentally acquired pathogen is reflected in the increased incidence from infants aged <1 year to those aged 1–4 years. While infants have more limited food consumption than older age groups, they are less mobile and therefore less exposed to contaminated environments.

A small number of reports have described a gender disparity in adults with higher infection rates reported in females [16, 18, 33, 35]. To our knowledge the reasons for this are not established. There may be host factors placing women at greater risk of developing severe symptoms. Alternatively, females might have a higher level of exposure to foodborne STEC as principal food handlers; however, a gender disparity was not apparent for *S. enterica* or *Campylobacter* sp. Females may have different consumption practices compared to males which increase their risk of STEC. Similarly, if adult females are more commonly primary carers within households, they may be expected to have higher levels of exposure to STEC excreted by primary cases, particularly children, increasing their risk of acquiring infection through person-to-person transmission. Additional questions have been added to the ESQ so that these factors can be explored in more detail. There are marked variations in the geographical distribution of ethnic minority populations in England which may in part explain the higher incidence rates in those of white ethnicity than non-white ethnicity. There may also be behavioural aspects reducing the risk of acquiring STEC infection in ethnic minorities, or differences in ethnic groups accessing primary care. Ethnicity was only reported for 38·5% of cases and ethnicity categories were very broad: collection of more detailed ethnicity data would enhance the ability to explore these differences in more detail.

The geographical distribution of reported STEC infection in the UK and Republic of Ireland is extremely heterogeneous. At the country level, Ireland and Scotland report rates of infection which are more than twice that of England (5·33 and 4·4 *vs.* 1·80 cases/100 000 population [5, 32, 36]. In England the highest observed rates of infection were reported in the Northern and Western regions, consistent with the finding that cases were more likely to live in rural areas than urban areas. While this observation has been reported in previous studies the risk ratio of rural to urban cases observed in England was over twice that reported from Grampian, Scotland [37]. This might be expected given the relative diversity of England's population, topography, land usage and climate. The increased risk associated with living in rural areas and the wider geographical variations in disease incidence observed across the UK and Ireland appear to be markers for environmental exposure to

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STEC in areas where ruminants are raised [32, 37]. Cases living in rural areas were more than twice as likely than those from urban areas to have had contact with livestock. Farm exposures differed in nature between urban and rural cases, with the former being exposed more often to commercial premises and rural cases to private farms. Open farms have the potential to cause outbreaks, [11, 12] and guidelines exist around control of infection [38, 39]. Our findings demonstrate that environmental/animal contact remains an important risk factor for disease transmission, and that non-commercial farm premises are a significant driver in the burden of sporadic STEC infection.

The morbidity burden of STEC in England remains high; overall a third of cases were hospitalized and progression to HUS was reported for 6·4% (215) STEC cases in England. This compares with 9·7% for Scotland and 7·8% for the USA [5, 40]. A relatively large number of HUS cases were microbiologically undiagnosed due to no available faecal specimens and this will have contributed to under-ascertainment of STEC-HUS cases. In addition, collection of HUS data on the laboratory referral form and ESQ may occur prior to a case developing HUS leading to further under-ascertainment. A high proportion of patients who submitted serum samples had HUS and no specimens were submitted for a small number of reported HUS patients, although, many reported a diarrhoeal prodrome including bloody diarrhoea. The operational guidelines for control of STEC infection in England recommend that serum and faecal specimens should be submitted to GBRU for further testing for all cases of HUS [41]. The microbiologically uncharacterized HUS cases were older than STEC-HUS cases, many in adults, a group not commonly recognized as being at risk of developing STEC-HUS and it may be that they are not being investigated and/or managed for STEC infection early in their course of illness. It is well documented that children, particularly those aged <5 years are at greater risk of developing STEC-HUS, and our findings are consistent with this [1, 40, 42, 43]. However, progression to HUS was greater in females and children and those aged $\geqslant$60 years. This may again be suggestive of a host factor placing females at greater risk of developing STEC-HUS, and further investigation is needed.

The low identification rate (1·2%) of non-O157 strains during the study period is consistent with previous surveillance reports in England (and Wales) [24, 25]. However, in other European countries, non-O157 STEC have been identified as a significant cause of disease [16, 18, 32] and reporting to Europe-wide surveillance in 2010 [36], indicates non-O157 STEC accounts for many more reported infections. This is unsurprising given local diagnostic laboratories in England do not routinely screen diarrhoeal stool specimens for non-O157 STEC. Higher detection rates of STEC O26, particularly in Scotland and the Republic of Ireland, further highlight the under-ascertainment of non-O157 STEC in England [32, 36]. Significantly higher hospitalization and HUS rates were reported for non-O157 cases. However, while some non-O157 strains can clearly cause severe disease, faecal samples from patients with severe disease suggestive of STEC infection are referred to GBRU for comprehensive testing using a combination of PCR and culture of faeces. Consequently, there is bias towards detection of non-O157 STEC and sorbitol-fermenting STEC O157 from patients with disease from the severe end of the clinical spectrum – which may contribute to the significantly higher proportion of HUS cases in non-O157 strains reported here. Thus the overall burden of non-O157 STEC in England is, largely, unobservable through current surveillance systems. It is important therefore to encourage the implementation and evaluation of PCR-based diagnostic methods designed to detect all STEC, to address this diagnostic deficit and to ultimately inform and improve the health protection response to these pathogens.

PT21/28 was the most frequently detected PT in STEC-HUS cases and PT21/28 and PT2 cases were significantly more likely to report vomiting, hospitalization and HUS. All STEC-HUS cases had strains encoding either Stx2 only or Stx1 and Stx2 toxins, consistent with previous studies indicating that both PT21/28 and PT2, and Stx2 toxin profiles are associated with progression to HUS [1, 42]. The most common PT in indigenous cases was PT21/28, while PT8 which caused less severe disease was largely travel acquired. Thus, the greatest burden in terms of both numbers and morbidity of STEC in England is due to domestic acquisition and is where interventions should be targeted.

Confirmatory tests conducted by GBRU on specimens from presumptive cases demonstrated that almost one fifth of cases reported to NESSS did not have STEC infection, but many were infected with *stx*-negative *E.coli* O157. Most were common serogroups of non-sorbitol-fermenting strains of *E. coli*, many of which exhibited cross-reactions with *E. coli* O157 antiserum (in-house data from GBRU). The

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initial screening processes used by firstline diagnostic laboratories are not designed to distinguish between STEC O157 and *stx*-negative *E. coli* O157. These isolates are thus considered as presumptive STEC O157 and cases are rapidly referred to local public health specialists for management including administration of the ESQ. The symptoms reported in cases of *stx*-negative *E. coli* in this dataset were similar to that of STEC cases, with the exception of bloody diarrhoea and HUS, indicating less severe disease was associated with *stx*-negative strains. *stx*-negative strains have been shown to be isolated during the course of STEC infection and may be progenies of STEC strains that have lost the *stx*-enconding phage [44]. However, the less severe symptoms and lower hospitalization rates in this group, compared to STEC cases, along with the absence of known pathogenicity factors in most strains, indicate they are more likely to be commensal strains mistakenly identified as STEC at the frontline laboratories and that some other microbiological or non-microbiological cause of gastrointestinal symptoms was present but not identified. Almost a third of *stx*-negative strains had the *eae* gene but the *eae*-positive strains were less likely to cause hospitalization or be associated with bloody diarrhoea than the *eae*-negative strains, supporting the assumption that the *stx*-negative strains are unlikely to be progenies of STEC strains that have lost the *stx*-encoding phage.

Continuation of NESSS will allow monitoring and analysis of longer term trends including emerging subtypes and risk factors. The addition of increased characterization of STEC strains through the application of molecular methods such as multi-locus variable number tandem repeat and whole genome sequencing will supplement enhanced surveillance data. Thus information from ESQs should work in synergy with microbiological characterization data to build a detailed picture of STEC strains, their pathogenicity and impact.

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DECLARATION OF INTEREST

None.

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