E. coli O157: Investigation of space-time association between human cases and positive cattle farms

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Abstract

Escherichia coli O157 is by far the most common serogroup isolated from patients suffering from gastrointestinal illness due to verocytotoxigenic *E. coli* (VTEC) infection in the UK, with rates of isolation consistently higher in Scotland than in England, Wales and Northern Ireland. Much research has focused on cattle and the food chain as the major source and means of transmission respectively. More recent studies have identified increased risk for human infection from the environment in areas where contact with livestock or their faeces is more likely.

Space-time referenced data were available on 384 confirmed cases of human infection with *E. coli* O157 in Scotland between 01/01/2002 and 31/12/2003. During the same period, a cross-sectional study of 481 Scottish farms rearing beef cattle was undertaken to investigate the epidemiology and evolution of *Enterobacteriaceae* infections in humans and domestic animals. Employing database and GIS software, it was found that there were only 21 instances of human cases occurring within 1 month of a farm being identified as positive for *E. coli* O157 of the same phage type (21/28) in a postcode sector cut by a 10 km buffer around that farm. Phage type 21/28 is the commonest identified in both cattle and people in Scotland and, given the prevalence in both populations, the degree of association identified appears to be no more than would be expected by chance. There was no evidence for increased risk of confirmed human infection close in space-time to the known positive farms in this study.

Introduction

Human illness, ranging in severity from diarrhoea through haemorrhagic colitis to haemolytic uraemic syndrome (HUS) and death, caused by infection with verocytotoxigenic *Escherichia coli* (VTEC) has become a major cause of public health concern throughout the world (Borczyk *et al*. 1987; Gansheroff and O'Brien 2000; Tarr *et al*. 2005). In North America and in Europe, the dominant VTEC serogroup associated with human illness has been *E. coli* O157, for which cattle have been shown to be the primary reservoir (Chapman 1995; Gansheroff and O'Brien 2000). Since the mid 1990s, some of the most serious outbreaks worldwide of human illness associated with *E. coli* O157 infection have been seen in Scotland (Roberts *et* al. 2000; Cowden *et* al. 2001; Howie *et al*. 2003). Furthermore, human incidence rates are consistently higher in Scotland than in many other European counties, including neighbouring England & Wales (Locking *et al*. 2005).

Investigations of early outbreaks of the disease throughout the world often identified foodborne infection as the most likely route of transmission (Scottish Executive Health Department/Food Standards Agency (Scotland) 2001). The most commonly implicated foods have been those of bovine origin, particularly undercooked minced beef burgers and dairy products (Bryant *et al*. 1989; Coia *et al*. 2001). However, outbreaks have also been associated with water and with other produce, including fruit and vegetables, in some of which contamination with cattle manure used as

fertiliser has been implicated (Abdul-Raouf *et al*. 1993; Licence *et al*. 2001; Sivapalasingam *et al*. 2004). More recently, the importance of the environmental route of transmission has been highlighted (Michel *et al*. 1999; Valcour *et al*. 2002; Howie *et al*. 2003). A case-control study in Scotland identified contact with livestock faeces as the risk factor most strongly associated with sporadic human *E. coli* O157 infections (Locking *et al*. 2001). Spatial and temporal analyses of cases from this study revealed a positive association with increasing cattle population density (Innocent *et al*. 2005). The joint Scottish Executive/Food Standards Agency (Scotland) Task Force on *E. coli* O157 reported that, whilst the potential for foodborne outbreaks remained important, environmental and livestock exposures were likely to be a more common source of infection, particularly for sporadic cases (Scottish Executive Health Department/Food Standards Agency (Scotland) 2001).

Considering the prevalence of *E. coli* O157 in cattle faeces and the growing evidence of the importance of the environmental route of transmission, the current study aimed to test the hypothesis that microbiologically confirmed human infection would be close in space and time to cattle farms in Scotland where *E. coli* O157 was isolated, and that proximity to these positive farms would constitute increased risk of infection.

Material and Methods

As part of a large-scale investigation of the epidemiology and evolution of *Enterobacteriaceae* infections in humans and domestic animals in Scotland, visits were made to Scottish farms rearing beef cattle for human consumption, on a regionally and seasonally representative basis between 01/01/02 and 31/12/03. Faecal pat samples were collected from groups of cattle closest to slaughter, and a questionnaire on cattle management practices was administered. Farm locations were recorded using 6-figure Ordnance Survey grid references or a hand-held global position system (GPS) device in instances in which cattle were kept at a distance from the home farm steading. The number of pats sampled on each farm in this study was designed to ensure a 90% probability of identifying the sampled group as positive if at least one animal in the group was shedding. A number of these farms were subsequently revisited, as part of a food chain based component of the study, and individual faecal samples were obtained from groups of animals immediately prior to departure for slaughter. Faecal samples were examined for *E. coli* O157 strains using immunomagnetic separation (IMS) (Chapman *et al*. 1994), but using buffered peptone water (BPW) without added antibiotics during enrichment to increase the analytical sensitivity (Foster *et al*. 2003). Presence of verocytotoxin VT1 and VT2 sequences were detected in positive *E.* coli isolates using polymerase chain reaction (PCR) (Pollard *et al*. 1990). The *E. coli* attaching and effacing (*eaeA*) gene was detected using a second PCR test, with specific primers to detect *eaeA* sequences of *E. coli* (Louie *et al*., 1994). Phage typing was carried out following standard procedure described by Khakhria *et al*. (1990). Thus, a dataset of study results was available holding information on the date, location and epidemiological characteristics of cattle isolates.

There were 384 confirmed cases of human infection with *E. coli* O157 reported to Health Protection Scotland (HPS) between 01/01/02 and 31/12/03. Case reports in 2003 were unusually low in number at 153 cases (148 culture and 5 seropositive cases) (Locking *et al*. 2004), compared to a yearly average of 273 culture positive cases 1994-2003. Children under 16 years of age accounted for 48% of all cases in 2002-2003, and 44% required hospital admission. Haemolytic uraemic syndrome, a potentially fatal complication characterised by acute renal failure (Tarr *et al*. 2005), was reported in 41/384 patients (11%), all but 7 of whom were under 16. Isolation and typing methods used by the Scottish *E. coli* O157 Reference Laboratory (SERL) for isolates from human cases were identical to those described above; seven of the cases (for whom stools were culture negative or unavailable) were identified by SERL from detection of antibodies to *E. coli* O157 in serum. The HPS enhanced surveillance system (Locking *et al*. 2005) obtains epidemiological and

clinical details for all confirmed cases; and compiles the national dataset by combining these data with phage and toxin types reported by SERL. Cases linked to general outbreaks (i.e. incidents involving the members of more than one household) are differentiated from sporadic cases (i.e. incidents of isolated infection, or infection linked only to other cases within the same household). Of the 384 cases, 59 were excluded from this study because their travel, exposure and clinical histories indicated infection was acquired outwith Scotland, and a further 9 cases were excluded because no location data were available for them. For reasons of confidentiality, precise address data were not available for the remaining 316 cases and they were located to the centroid of the postcode sector in which they resided, or for outbreak cases, in which the outbreak was located (UK postcode geography is a hierarchical system, dividing the land area [Scotland = 77,500 km^2] on the basis of population into units within sectors within districts within areas. At the time of this study, there were 859 postcode sectors in Scotland with a median area of 13 km^2 [range 1 – 2,800 km^2]). Date of infection was identified by onset of symptoms or, for asymptomatic cases, date of first positive specimen. Thus, a second dataset was available holding information on the date, proxy location and epidemiological characteristics of human cases.

Both datasets were geocoded using MapInfo v. 5.5 (MapInfo Corporation) geographical information system (GIS) software. The potential for spatial relation between human cases and positive farms was deemed to be plausible where a 10 km circular buffer (created using standard GIS tools) around a positive farm cut or enclosed a postcode sector in which a human case was located. The querying tools of the GIS were used to create a dataset of plausible spatially related human case/positive farm pairs. Temporal relation between human cases and positive farms was deemed to be plausible in situations when date of infection for a human case was within a month before or after a positive isolate from a farm. Although it is generally accepted that the incubation period for human infection can potentially be as much as two weeks, it was considered that isolation of *E. coli* O157 on a farm on a single date could confidently be expected to represent the status of an average farm for at least the two weeks previous and subsequent to that date. Further querying of the spatially related dataset was used to produce a final dataset of human case/positive farm pairs related in both space and time from which *E. coli* O157 of the same phage and VT type had been isolated.

Results

E. coli O157 was isolated from 98 (91 from the original sampling and a further 7 in the subsequent food chain sampling) of 481 farms visited during the various components of the study. The 316 human cases involved 196 sporadic incidents accounting for 241 cases and 19 outbreak incidents accounting for 75 cases.

There were 620 spatially related case/positive farm pairs involving 84 (86%) farms and 240 (76%) cases (151 sporadic incidents accounting for 189 cases and 13 outbreak incidents accounting for 51 cases). However, there were only 54 case/positive farm pairs related in both space and time involving 27 (28%) farms and 43 (14%) cases (29 sporadic incidents accounting for 35 cases and 2 outbreak incidents accounting for 8 cases). Table 1 shows the phage type (PT) and verocytotoxin gene (VT) types isolated from these farms and incidents.

Only 21 case/positive farm pairs related in both space and time had matching PT and VT, involving 11 (11%) farms and 23 (7%) cases (associated with 14 sporadic incidents). In every instance, this involved phage/VT type 21/28 VT2.

Table 1 Phage and VT types of *E. coli* O157 isolated from case/positive farm pairs related in both space and time

Phage and VT type	Farms (n=27)[†]	Human 'incidents' (n=31)[‡]
21/28 VT2	13	25
32 VT2	9	0
8 VT1&2	4	2
2 VT2	1	3
33 VT2	1	0
34 VT2	1	0
RDNC*	1	1
Unknown	0	1[+]

[†]Two different phage types were isolated on three farms so the column sums to 30
[‡]The unknown isolate came from an outbreak in which the other isolates were all PT21/28 VT2, thus the column sums to 32 even though there were only 31 incidents
*RDNC = Phage Reacted but Did Not Conform to any known pattern
[+]The phage and VT type for this isolate were not available from the database

Discussion

In this study only 14 of 196 (7%) reported sporadic incidents of human infection with *E. coli* O157 in Scotland over a two year period could be associated in space and time with the same phage/VT type isolated from cattle on a known positive farm within the parameters used here. None of the outbreak incidents could be associated. No human cases with the same phage/VT type were found to be associated in space and time with almost 90% of known positive farms.

However, these findings need to be interpreted with caution. First, inevitably, there is likely to be an unknown degree of under-ascertainment of human cases. Secondly, the sampling of cattle farms only included approximately 3% of farms rearing beef cattle for human consumption in Scotland, and cattle farms not producing beef (e.g. some dairy farms) were not included in the sampling frame. Therefore, although the farms sampled are believed to be representative of most cattle farms throughout Scotland, they cannot be considered to provide a comprehensive geographical pattern of *E. coli* O157 throughout the Scottish environment. In order to compensate for this, a relatively large buffer zone was chosen around each farm (far beyond the farm boundary) to allow it to be considered as an indicator of local contamination, although there is little evidence to support or refute this assumption. Thirdly, there is some evidence that would suggest that farms from which *E. coli* O157 is isolated can remain positive for several months at a time (Maule 2000) and thus it could be considered that the temporal 'window' used in this study was too short. However, it was felt that, with only one observation per farm, it would be unsafe to assume anything beyond the temporal limits stated. At this stage, there are no plans to extend this study to explore the effects on the outcome of using different sized spatial and temporal windows.

It was interesting to observe no human/positive farm association with any phage/VT type other than PT21/28 VT2. As the data suggest, in Scotland PT21/28 VT2 is by far the most common type isolated from both cattle and people. In this study, in the space time related dataset, PT21/28 VT2 was isolated from 13 of 27 farms (48%) and 25 of 31 human incidents (81%). This suggests that 40% (0.48 × 0.81) of the incident/positive farms pairs, 12 and 11 respectively, would be expected to both be PT21/28 VT2 by chance alone. Thus, the finding of 11 farms and 14 incidents with PT21/28 VT2 in the current study shows very little more association than would have been expected.

These findings should be set in the context of wider enhanced surveillance data on cases reported 2002-2003. Microbiological evidence linked nine cases in the current study to a livestock-related

waterborne outbreak in 2002 (unpublished outbreak report 2002). Indistinguishable pulsed-field gel electrophoresis (PFGE) profiles were observed in isolates from human cases, from the private water supply they consumed, and from mud, surface water and cattle faeces surrounding the water source. These cases were not amongst the matched human/positive farm pairs in this study, as the outbreak location was not part of the IPRAVE study. This was the only outbreak reported to HPS in 2002-2003 in which environmental sampling was undertaken. Sampling in other Scottish outbreaks has provided microbiological evidence linking human and livestock, environmental or water isolates, including sheep and goats, in most years since 1997 (HPS unpublished data; SCIEH 2000; Licence *et al*. 2001; Howie *et al*. 2003). Although environmental, animal or water sampling is not routine, sporadic human infections were microbiologically linked to horses, a farm cat and other livestock exposures during 2002-2003 (Locking *et al*. 2004) and in other years since 1999 (HPS unpublished data, Locking *et al*. 2005).

It is probable that many farms not included in the current study were positive during the study period. Also, full activity details during the incubation period were not available for most 2002 and 2003 cases. Whether they were exposed to farmland or livestock markets or to faecal contamination of roads, vehicles or debris where *E. coli* O157 can survive (Maule 2000) was unknown. Fuller details were obtained for all cases in 2004, when livestock-related exposures were reported in 55% of cases; some infections were microbiologically linked e.g. to contaminated water supplies on farmland (Locking *et al*. 2005).

Using the absence of matching phage types to rule out associations between farms and cases in this study may also have influenced the findings. Multiple phage types have been identified in outbreaks (Health Protection Agency 2005, HPS unpublished data) and within sporadic family groups in Scotland on a number of occasions (HPS unpublished data). The availability of additional laboratory resources (e.g. allowing detailed investigation of faecal samples and multiple rather than single picks from culture positive plates, which is currently the standard practice) might facilitate identification of multiple phage types in more human cases. In this context, it is noteworthy that multiple phage types were isolated 3 of the 27 farms in the final dataset.

Case control, space/time, and outbreak studies in Scotland and other countries have identified strong associations between human *E. coli* O157 infection and livestock, environmental and water contamination (Michel *et al*. 1999; Licence *et al*. 2001; Locking *et al*. 2001; O'Brien *et al*. 2001; Howie *et al*. 2003; Innocent *et al*. 2005). Some of these studies originated from difficulties in identifying specific sources of infection, and from serious questions about how to minimise risk of illness and serious outcomes, particularly when sporadic infection predominates. This study may shed more light on some of those difficulties.

Conclusion

Although it is accepted that *E. coli* O157 is likely to be present in the Scottish rural environment where cattle are farmed, and previous studies found evidence of human illness associated with livestock exposures, it is difficult to link human infections microbiologically to specific farm sources. In accordance with previous work (Mellor *et al*. 2003, Scottish Executive 2003, SEHD 2005), providing farm-based prevention, sensible personal hygiene and child supervision recommendations are heeded, the risks of Scottish countryside exposures from the point of view of *E. coli* O157 infection can be managed and minimised.

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