Article

Widespread contamination of recreational seawaters, rivers and lakes with Shiga toxigenic *Escherichia coli*

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Abstract

Shiga toxigenic *Escherichia coli* (STEC) has the potential to cause serious gastrointestinal illness with approximately 10–15% of patients developing Haemolytic Uraemic Syndrome (HUS). Ireland consistently reports the highest incidence of human infection with STEC in Europe. In this study, seawater (n = 84) and freshwater (river and lake) samples (n = 27) were collected from locations around Ireland over a three-year period (2016-2019). There were two phases to the investigation. Initially, samples were collected between May and September in 2016 and 2017 (Phase 1). Based on the results obtained during this first phase a more extensive investigation was undertaken between December 2018 and December 2019 (Phase 2). Samples were tested using a two-step multiplex real-time PCR protocol. The first step was to screen for the presence of *eae, stx1* and *stx2*. Samples giving positive signals for *eae* and at least one toxin gene target were analyzed for the presence of gene targets associated with serogroups O157, O26, O103, O104, O111 and O145. Overall, STEC was detected in 21/27 (78%) of the lake and river samples tested and in 48/84 (57%) of all seawater samples tested. These findings indicate widespread contamination of recreational waters with STEC which may act as an important and under-recognized transmission route to humans.

Keywords Shiga toxigenic *Escherichia coli*; recreational waters; real-time PCR; contamination; transmission.

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1 Introduction

Shiga toxigenic *Escherichia coli* (STEC) are pathogenic *E.coli* which produce Shiga Toxin 1 (*stx1*) and /or Shiga Toxin 2 (*stx2*). These organisms can cause very severe intestinal infectious disease in humans and represent a major threat to public health. STEC are particularly hazardous due to their low infectious dose and their ability to survive in the environment (Mc Dowell et al., 2008). The spectrum of human illness associated

with STEC is wide. Some people remain asymptomatic, some develop self-limiting gastroenteritis and some develop bloody diarrhoea and painful abdominal cramps. The most serious complication is haemolytic uraemic syndrome (HUS) which is associated with fragmentation of red blood cells, renal failure and risk of death. Approximately 10% of STEC cases develop HUS, with those most at risk being children under 5 years of age (HPSC, 2012). Those who survive may require long term renal dialysis or kidney transplant. The risk posed to public health by STEC was demonstrated in 2011 by the outbreak which centred in Northern Germany and caused 3821 cases and 47 deaths (Beutin and Martin, 2012). Ireland consistently reports the highest incidence of human infection with STEC in the EU. Data for 2017 (HPSC, 2017) showed that the incidence rate in Ireland was 16.6/100,000 compared with the EU average of 1.6/100,000.

STEC can be detected in the gastrointestinal tract of a variety of animal species (Murphy et al., 2008; Lenehan et al., 2006; Thomas et al., 2006; Prendergast et al., 2011).Transmission of STEC to humans occurs via contact with animals, exposure to STEC-contaminated material such as soil or water, consumption of STEC-contaminated food or water, or as result of close contact with an infected person. Available data suggests the dominant transmission routes for STEC infection in Ireland are person-to-person and waterborne transmission. However, each year the transmission route for up to 50% of STEC cases is reported as unknown or not specified (HPSC, 2017). STEC is a statutory notifiable disease in Ireland and is subject to public health management. While previous international studies have reported STEC outbreaks due to swimming in contaminated waters (Keene et al., 1994; Rangel et al., 2005), to our knowledge, no investigations have focused on recreational water in Ireland as a potential reservoir of the pathogen. Currently there is no requirement under EU bathing water regulations to monitor recreational waters in Europe for the presence of STEC, and waters are typically only sampled in an outbreak situation and in these instances, typically only one, small volume sample (up to 1 L) is collected. Therefore, the extent of contamination of waters with STEC in Ireland and elsewhere is largely unknown.

The aim of this study was to examine recreational waters (both freshwaters and seawaters) for the presence of STEC.

2 Materials and Methods

2.1 Study sites

In total 111 samples were collected from 50 locations in the east, south and west of the country. The study was divided into two phases. Phase 1 consisted of sampling seawaters at five locations on the west coast of Ireland (Galway City and County Council Local Authority Areas) over a five-month period between May and September in 2016 and 2017. Location A and B only were sampled in 2016 and locations C, D and E were sampled in 2017. In Phase 2, samples were collected from seawaters (62), rivers (17) and lakes (10) in the east (Fingal Local Authority area), south (Cork Local Authority area) and west (Galway Local Authority areas) of Ireland (Fig. 1) between December 2018 and December 2019.

2.2 Collection and processing of water samples

Samples (30 L) were collected from each location in sterile containers and returned to the lab for processing within 4 hours in line with current bathing water quality regulations (S.I. 79 2008). Samples were processed using the CapE method (Morris et al., 2016). Briefly, the samples were filtered through a 0.45 μm filter which was then incubated overnight at 42°C in Buffered Peptone Water.

2.3 Processing of enrichment samples

Following overnight incubation, 1 mL of enrichment broth was removed and centrifuged at 22,000 g for 10 minutes. The supernatant was removed and the pellet was re-suspended in 200 μL of sterile molecular grade

water. The samples were then heated to 95°C for 10 minutes and centrifuged at 22,000 g for a further 10 minutes. The supernatant was removed to a clean tube and stored at -20°C for further use.

Fig. 1 Location in Ireland of the local authority areas of Galway, Cork and Fingal where samples were collected for this study.

2.4 Real-time PCR

Three multiplex real-time PCR assays were used to test for the presence of STEC gene targets (Møller and Thorup, 2013; Perelle, 2004, 2005). In Phase 1, the first assay targeted the *stx1/stx2* and *eae* genes. Samples found to be *eae* and *stx1* and/or *stx2* positive were subsequently tested for O157, O26. Similarly in Phase 2, samples were screened for the *stx1/stx2* and *eae* genes and those samples found to be *eae* and *stx1* and/or *stx* positive were screened for O157, O26 and additionally O103, O111, O145 and O104 in line with ISO 13136 (ISO/TS 13136:2012). For all assays a 20 µL reaction mix consisting of primers at a final concentration of 0.5 µM, probes at a final concentration of 0.2 µM, 12.5 µL TaqMan™ Fast Universal PCR Master Mix Applied Biosystems, 2.5 µL IPC Master Mix (Applied Biosystems), 0.5 µL IPC DNA (Applied Biosystems) and 3.2 µL of molecular grade water. To the reaction mix 5 µL of sample DNA from the lysed enrichment broth (2.3.1) was added. The samples were cycled on an ABI 7500 Fast system (Applied Biosystems) through one cycle of 50°C for 2 mins and 40 cycles of 95°C for 20 sec and 95×3 sec / 60×30 sec. This was followed by one cycle of cooling at 50°C.

3 Results and Discussion

3.1 Results from Phase 1 testing

During Phase 1, twenty two samples of seawater were collected from five locations (A-E) in the West of Ireland (Galway Local Authority Areas) and screened for the presence of *eae*, *stx1*, *stx2* and targets for serogroups O157 and O26 only. Results are summarized in Table 1. For the purpose of interpreting the results the definition of STEC used was that set out in ISO/TS 13136 (ISO/TS 13136:2012).

In total, fifteen of the twenty two samples collected over the two seasons were STEC positive. Five samples were collected at each of two locations A and B in the bathing season of 2016 and STEC serogroups O26 and O157 were detected in both locations in consecutive months (June – September). In the bathing season of 2017, at location C, serogroups O26 and O157 were detected samples from June to September. When location D was sampled in 2017, O26 and O157 serogroups were detected in August and September samples. O26 was detected in the sample taken in June and no STEC was detected in the July sample. Location E was sampled once in July of 2017 and was found to be positive only for the *eae* target and therefore not STEC.

Table 1 Results obtained when seawater samples from locations on the west coast of Ireland were tested in Phase 1 of this study for the presence of STEC gene targets.

Region	Location	STEC*	Date Collected
West	A	N	May 2016
West	A	Y	June 2016
West	A	Y	July 2016
West	A	Y	August 2016
West	A	Y	September 2016
West	B	N	May 2016
West	B	Y	June 2016
West	B	Y	July 2016
West	B	Y	August 2016
West	B	Y	September 2016
West	C	N	May 2017
West	C	Y	June 2017
West	C	Y	July 2017
West	C	Y	August 2017
West	C	Y	September 2017
West	D	N	May 2017
West	D	Y	June 2017
West	D	N	July 2017
West	D	N	July 2017
West	D	Y	August 2017
West	D	Y	August 2017
West	E	N	July 2017

*Positive PCR result for *eae* plus *stx1* and/or *stx2* and at least one serogroup target.

3.2 Results from Phase 2 testing

3.2.1 Seawater samples

During this second phase, thirty-three seawater samples from twelve locations in the West of Ireland (Galway Local Authority Areas), fifteen samples from eight locations in the East (Fingal Local Authority Area) of the country and fourteen samples from seven locations in the South (Cork Local Authority Area) were tested.

Of the sixty-two samples tested, thirty three (53%) were found to be STEC positive (Table 2). Forty-nine samples were found to be positive for the *eae* gene, twelve were positive for the *stx1* gene and thirty-two for the *stx2* gene. Further testing was carried out for the presence of genes associated with serogroups O26, O157, O103, O104, O111 and O145. All samples were found to contain multiple serogroup gene targets with O103

being the most common. Twenty-six samples were positive for O26, twenty-three for O157, twenty nine for O103, nineteen for O104, twenty-five for O111 and twenty four for O145 (Fig. 2). Table 2 also shows the rainfall data for the days on which the samples were collected which was included in an attempt to identify any link between higher levels of rainfall and STEC status. The data indicates that there is no correlation (P = 0.176 > 0.05) between high rainfall and detection of STEC, as samples were found to be positive on days when there was relatively low or no rainfall and also on days when there was rainfall of up to 20mm of rain. However overall, higher rainfall was observed in the West of the country where 60% of samples were found to be STEC positive compared to 47% in the East and 36% in the South.

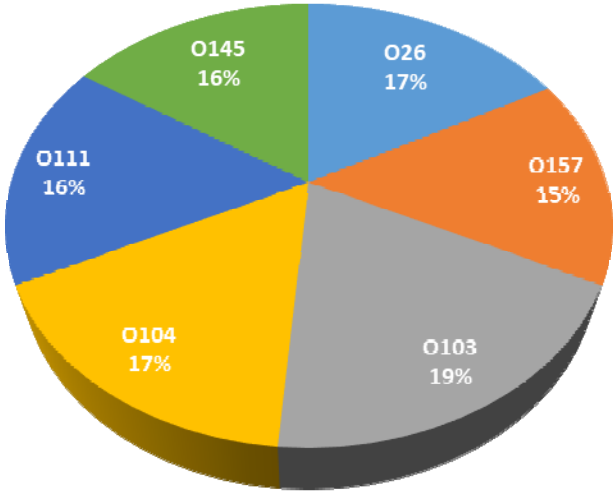

Fig. 2 Breakdown of STEC serogroups detected in Phase 2 seawater samples tested in this study.

Table 2 STEC status of seawater samples collected from Irish coastal locations in Phase 2.

Region	Location	STEC*	Rainfall (mm)
West	1	Y	0.40
West	2	Y	2.80
West	3	Y	5.3
West	3	N	4.2
West	3	N	2.3
West	3	N	0.2
West	3	N	2.6
West	3	Y	1.2
West	3	Y	1.2
West	3	Y	1.2
West	4	Y	5.3
West	4	Y	20.7
West	4	Y	20.7
West	5	Y	0.1
West	6	N	2
West	6	N	9
West	6	Y	1.2

West	6	Y	4.6
West	6	Y	7.2
West	7	Y	1.5
West	8	Y	1.2
West	8	Y	3.3
West	8	Y	1.5
West	8	N	1.5
West	9	Y	4.4
West	9	Y	4.4
West	9	Y	4.4
West	10	N	4
West	10	N	1.5
West	10	N	1.5
West	11	N	4
West	11	Y	4.4
West	12	N	4
East	1	N	0.5
East	1	Y	0.2
East	1	N	0.2
East	2	N	0.5
East	3	Y	0.2
East	4	N	0.5
East	5	N	0.5
East	5	Y	0.2
East	5	Y	0.2
East	5	Y	0.2
East	6	N	0.5
East	6	Y	0.6
East	6	Y	0.6
East	7	N	0.1
East	8	N	0.5
South	1	Y	0.1
South	2	N	0
South	3	N	3.8
South	4	N	3.8
South	5	N	0
South	5	N	0
South	6	N	0
South	6	N	0
South	3	N	7
South	3	N	7
South	2	Y	8.8
South	2	Y	8.8
South	7	Y	0.4
South	7	Y	0.4

*Positive PCR result for *eae* plus *stx1* and/or *stx2* and at least one serogroup target.

3.2.2 Lake samples

In total, seven lake water samples were collected from three locations in the West and three samples were collected from one location in the South of Ireland. Six of the samples were STEC positive (Table 3). Of all the samples tested, eight were positive for the presence of the *eae* gene, three were positive for *stx1* and six were positive for *stx2*. One sample which was positive for *stx2* only was included for further serogroup testing out of interest but for the purpose of reporting results this sample was not defined as STEC. Six samples were found to be positive for multiple serogroups with the most common serotype found being O103 which was found in 6/7 samples. With the seawater samples, rainfall data was analyzed on the days on which the samples were collected with no correlation observed ($P = 0.128 > 0.05$).

Table 3 STEC status of lake water samples collected in Phase 2 of this study.

Region	Location	STEC*	Rainfall
West	1	N	0.2
West	1	N**	7.8
West	2	Y	1
West	2	Y	1
West	2	Y	1
West	3	Y	0.5
West	3	Y	0.2
South	1	Y	0
South	1	N	1.8
South	1	N	1.8

*Positive PCR result for *eae* plus *stx1* and/or *stx2* and at least one serogroup target. **Sample positive for *stx2* only and serogroup O103.

3.2.3 River samples

Ten river water samples were collected from ten locations in the West, five samples from three locations in the East and two samples from one location in the south of the country. Fifteen samples were positive for STEC (Table 4). From all the samples collected in the West, ten were *eae* positive, eight *stx1* positive and eight were *stx2* positive. The samples from the East showed four *eae*, one *stx1* and four *stx2* positives. Two samples from the south were positive for *eae* and one sample was positive for *eae* and *stx2*. As with the other water sources, multiple serotypes were present in each sample. The most common serotypes across samples were O157 (16/17) and O103 (15/17). Rainfall data was analyzed for the days on which the samples were taken with no correlation observed ($P = 0.426 > 0.05$).

Table 4 STEC status of river waters tested in Phase 2 of this study.

Region	Location	STEC*	Rainfall
West	1	Y	2.8
West	2	Y	2.8
West	3	Y	20.7
West	4	Y	20.7
West	5	Y	2.4
West	6	Y	0.5
West	7	Y	2.8
West	8	Y	0.5
West	9	Y	2.3
West	10	Y	7.3
East	1	Y	0.2
East	1	N	0.2
East	2	Y	0.6
East	2	Y	0.6
East	3	Y	0.2
South	1	Y	0.2
South	1	N	0.2

*Positive PCR result for *eae* plus *stx1* and/or *stx2* and at least one serogroup target.

4 Discussion

According to the most recent data (HPSC, 2017), Ireland has the highest notification rate for confirmed cases of STEC across Europe (16.6/100,000 population), 10 times the European average incidence of 1.6/100,000 population. This was followed by Switzerland (8.2/100,000 population) and Norway (7.3/100,000 population) respectively. The data shows that O157, along with O26, was the most common serogroup associated with HUS in Ireland in 2017 with seven HUS cases associated with O145 compared with only ten cases in the previous ten years. While the most common transmission routes reported are person to person contact and waterborne transmission (Garvey et al., 2010; O'Sullivan et al., 2010; Óhaiseadha et al., 2017), other transmission routes have been reported including food and contact with infected animals (EFSA, 2017; Griffin, 2010). Animals are an important reservoir of STEC due to the fact that STEC can be a commensal in the gastrointestinal tract of cattle, sheep and goats (Murphy et al, 2016). While water has been well studied as a route of transmission, investigations to date have mainly focused on drinking water (Ding et al., 2018). Limited studies have demonstrated the presence of STEC in the broader aquatic environment including, lakes (McCarthy et al., 2001) wastewater (Blanch et al., 2003), and rivers (Johnson et al., 2014). These studies looked at a variety of sample types including lake sediment, drinking water, lake water, ice and sewage and a variety of methods were used to investigate the presence of STEC in these samples including culture, colony hybridization and PCR. A common issue among the studies is the difficulty associated with culturing STEC from the sample of interest. The inclusion of immunomagentic separation along with the use of more sensitive molecular detection methods such as real-time PCR along with frequent water sampling was recommended as a route to improving STEC monitoring and detection in these potential reservoirs. An additional complexity in terms of STEC detection in the aquatic environment also exists due to the fact that phage carrying *stx* genes are distributed widely in environments polluted with fecal material. These phage particles have a role in the transduction of *stx* genes to susceptible bacteria (Lejla et al., 2010) and may be a potential reason for the difficulty in isolating STEC organisms from samples of interest. In order to overcome this potential issue in

this study, detection of STEC serogroup gene targets was included in addition to assays for *stx* gene targets thus ensuring the presence of DNA from STEC organisms in the samples tested. Whilst there are over 200 serogroups of STEC, a relatively small number of serogroups are associated with human infection, including O157 O26, O103, O104, O111 and O145. Methods of detection of STEC in human clinical and food samples have evolved to accommodate the detection of strains which are clinically relevant. Early detection methods relied on culture specifically for detection of serogroup O157. Subsequently, culture combined with PCR was introduced for detection of the top six serotypes, O157 O26, O103, O104, O111 and O145 (ISO/TS 13136 2012). This method involves screening by PCR for particular markers for the various serogroups followed by confirmation that these markers are present in a cultured STEC isolate. In this work, we have utilized real-time PCR to detect the various STEC gene targets. Molecular detection is a good indication of contamination of waters with STEC (Smith et al., 2009) and is widely used for STEC detection in the clinical setting including here in Ireland where positive cases have been reported by the Health Protection Surveillance Centre based on PCR only positives (HSE, 2017).

Over the three year period in which this investigation was carried out a total of fifty urban and rural sites were sampled. STEC was detected in 78%of the lake and river samples tested and in 57% of all seawater samples tested. These findings indicate widespread contamination of recreational waters with STEC which may act as an important and under-recognized transmission route to humans. As an island nation, use of water for recreational purposes in Ireland is high. Participants in most water-based recreational activities come into close contact with the water, with 12% of adults swimming at least once during the year; 7% of adults engaging in angling activity; 5% engaging in boating and sailing activities, and smaller proportions participating in other water-based sports (EPA, 2017). Because of this and our findings on the widespread contamination of various water sources it is important to consider recreational water as a potential route for transmission of STEC.

From our study it is notable that serogroup O103 was the most prevalent serogroup present in both seawater and lake samples tested. Results from samples tested showed that 88% were positive for O103 compared with 94% O157 positive. STEC O103:H2 was first described as a causative agent of HUS in 1992 and can occur as shigatoxin-negative aEPEC (Atypical Enteropathogenic *E. coli*) as well as toxin-positive *E. coli* carrying only *stx1* or *stx2* or both variants, with *stx 1* variants being the most common cause of human STEC illness (Mariani-Kurkdjian et al., 1993). A number of studies have demonstrated serogroup O103 prevalence in sheep and cattle (Sekse, 2013; Moore et al., 2015; Karama et al., 2008) which is a cause for concern given the potential for animal faecal material to cause contamination of recreational water sources particularly in countries like Ireland where farming is a primary activity. A number of reports have also linked this serogroup to different food sources including cheese, milk and beef (Schimmer et al., 2008; Mylius et al., 2018; Onyeka et al., 2020) highlighting the link to consumption of raw or undercooked food and the serious nature of the infection. To date there has been no specific report linking serogroup O103 to waterborne transmission from recreational sources but it is worth noting that all the samples found to be O103 positive in this study were either *stx1* and/or *stx2* positive. From the most recent report available 923 STEC cases reported in Ireland in 2017, 35 (4%) were associated with serogroup O103 (HPSC, 2017).

In Ireland between 2004 and 2012 there were a total of 355 STEC notifications of which 55 waterborne outbreaks with 234 people reported ill (Garvey et al., 2016). Most outbreaks were associated with private wells (n = 46). There were no outbreaks linked to public water supplies and no reported incidences from use of recreational water. The most recent version of the VTEC Enhanced Surveillance Form (2018) in use in Ireland by public health authorities does include questions on recreational activities including swimming and water use which is important information to consider in light of the results of this study. It is worth noting that all

the seawater locations in which STEC was detected in this study were consistently reported as of either good or excellent quality based on current EU bathing water monitoring criteria (S.I. 79 2008). This points to potential limitations of using the number of colony forming units (CFU) of *E. coli* per 100 mL as an indicator for bathing water quality (Edberg et al., 2000), as it does not reflect the pathogenicity of some variants of *E. coli*, such as STEC for which the infectious dose is very low (<10 CFU/mL) (Croxen et al., 2013).

5 Conclusion

The findings of this study highlight the need for recreational water to be examined more closely for its role in transmission of STEC in order to inform public health policy and bathing water monitoring criteria, to better understand the scale and potential sources of STEC contamination and to develop mitigation measures.

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