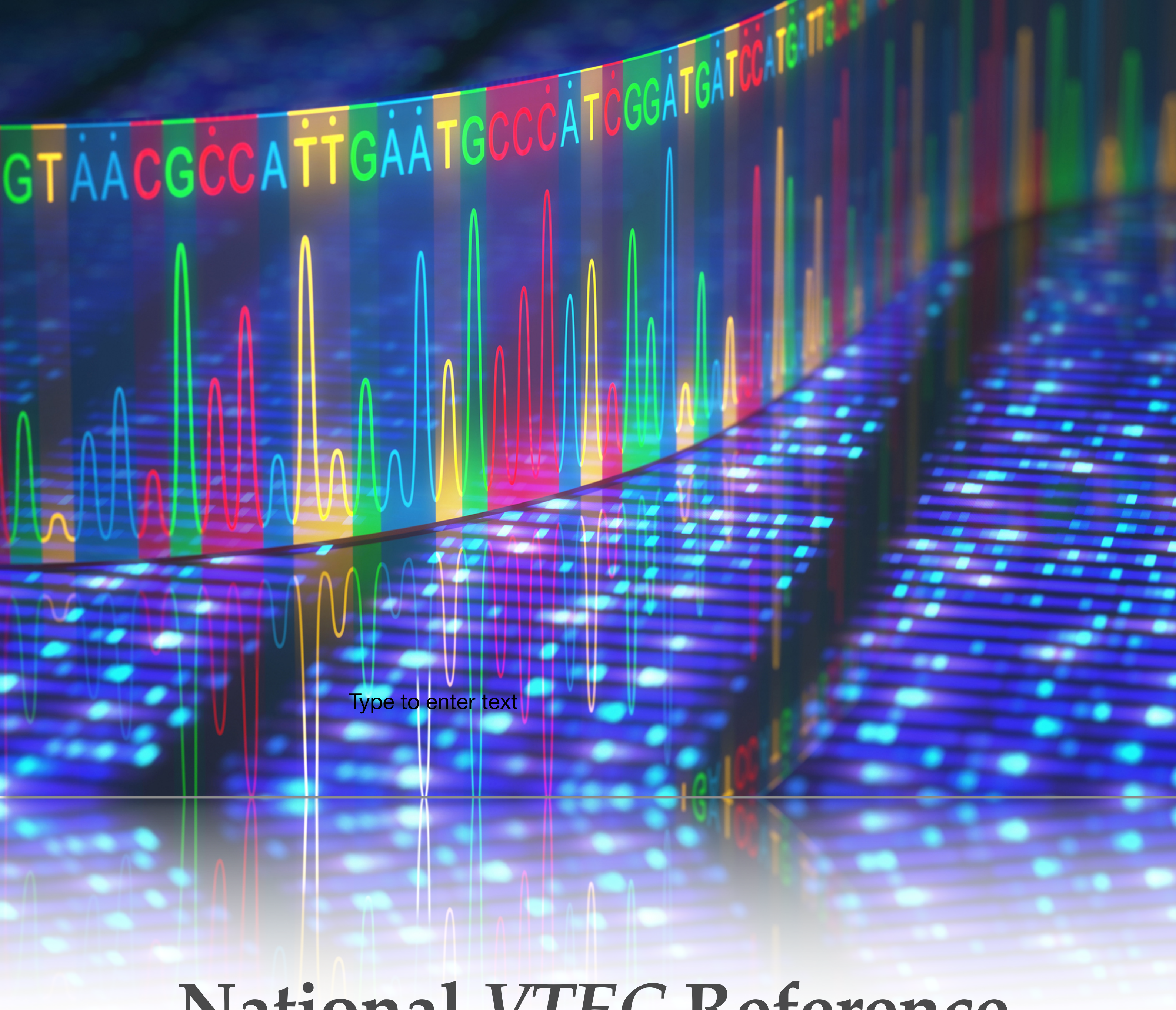

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National *VTEC* Reference Laboratory Annual Report 2023 & 2024

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

The Public Health Laboratory,
Health Services Executive,
Cherry Orchard Hospital,
Ballyfermot,
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Key points

2023

- 3728 specimens received for VTEC analysis
- 1779 VTEC positive specimens from 925 cases
- 6 cases were co-infected with more than 1 VTEC strain
- 76 serotypes identified
- VTEC O26:H11 most common serotype
- *stx1/stx2* most common toxin genotype

2024

- 3464 specimens received for VTEC analysis
- 1288 VTEC positive specimens from 804 cases
- 8 cases were co-infected with more than 1 VTEC strain
- 89 serotypes identified
- VTEC O26:H11 most common serotype
- *stx1/stx2* most common toxin genotype

Introduction

Verotoxigenic *Escherichia coli* (VTEC) is an enteric pathogen of considerable public health concern worldwide. VTEC causes a range of symptoms from diarrhoea to bloody diarrhoea to the more severe haemolytic-uremic syndrome (HUS) which is characterised by kidney failure, thrombocytopenia, and microangiopathic haemolytic anaemia. HUS can be fatal, particularly in young children. VTEC pathogenicity is expedited by verotoxins (vtx), of which there are 2 forms, stx1 and stx2; both are encoded on a lambdoid lysogenic bacteriophage.

The main reservoir of VTEC is grass-feeding animals, in particular cattle. Transmission is frequently related to the consumption of undercooked beef, unpasteurised milk and dairy products, salad vegetables, or inadequately managed private drinking water supplies. VTEC may also be spread person-to-person. Person to person spread is common in childcare facilities in Ireland.

The Public Health Lab-Dublin (incorporating the NRL-VTEC) has been providing reference services for VTEC since 1998 and has been receiving isolates from all Human clinical cases of VTEC in Ireland since 2002. The PHL is located in the grounds of Cherry Orchard Hospital, and is administered by the HSE within Integrated Health Area Dublin South and Wicklow.

The NRL-VTEC is committed to providing a high quality and timely service and is accredited to both ISO 15189 and ISO 17025 by INAB, for culture and PCR. WGS was introduced in 2018, one isolate from all VTEC cases has characterised by WGS since. For Full scope of accreditation see the link.

Sample Processing

Stool specimens are inoculated into 10ml of MTSB (novobiocin 16mg/l) and incubated at 37oC overnight. 10ul of the enriched culture is plated onto MacConkey agar, and STEC CHROMagar™. Resulting colonies are tested for stx1 and stx2 by Real-time PCR. In addition, automated DNA extraction was carried out from the overnight of MTSB (novobiocin 16mg/l) and PCR for stx1 and stx2 is carried out on the resulting elutate.

The sensitivity of culture methods are lower than that of RT PCR, however, information on whether an organism is viable, is of crucial importance to the clinical interpretation and the public health implications. Thus, a combination of 2 different PCRs and culture, provides the highest sensitivity, specificity, PPV and NPV (98.21%, 100% 100% and 99.43% respectively). This testing algorithm this fast (24-hour turnaround time) and reliable and provides the required viability information. A fast and reliable reference laboratory method is essential to inform public health interventions and should complement the guidance on the Public Health management of VTEC. Please see this link for the Public Health Managment of VTEC.

Following a positive PCR for stx1 and/or stx2, PCR and/or serology for O26 and O157 is carried out on a single isolated colony. Further serotyping and virulence determinants such as stx1 and stx2 subtyping are determined by whole genome sequencing (WGS). WGS is performed on Illumina MiSeq or NextSeq and analysis including cgMLST done through Ridom SeqSphere+ software.

To facilitate workflow efficiency, we request that urgent samples or large numbers of samples for referral are preceded by a phone call to NRL-VTEC and that all samples are accompanied by a completed NRL-VTEC request form. Current request forms can be downloaded from here. We also ask that as many of the fields as possible are completed. Mandatory is ‘External lab ID’, ‘Name’, ‘DOB’. Preferably include clinical details (especially if HUS). In addition, we appreciate your including stx PCR result and CP value, this enables us to streamline our testing protocol and provide you with the fastest turnaround time.

Outbreak codes will only be created on PHL-LIMS following receipt of the required information (Appendix 1) this should be e mailed to PHL (phl.dublin@hse.ie) before sending samples.

Results

The incidence of VTEC in Ireland peaked in 2018 at 23.3/100000. Incidence fell during 2019 and 2020 mainly due to COVID. In 2021 the incidence rose again to 20.24/100000, but there has been a year-on-year decline since then to 15.45/100000 in 2024 (Fig 1).

The serogroup of VTEC isolates is determined by a combination of PCR and serology for culture positive cases and confirmed by WGS. Serogroup is therefore not determined for culture negative cases. In 2023 and 2024 there were 674 and 629 culture confirmed VTEC cases respectively. 207(31%) and 219 (35%) respectively were VTEC O26, this is in line with that has been seen in previous years. 129 (19%) and 112 (18%) were VTEC O157 this is a slight decrease from 22% in 2022. 41 and 33 cases respectively were VTEC O145 and 12 and 18 cases respectively were VTEC O103, this is a decrease from 2022. The remaining 283 and 247 cases comprised numerous different serogroups (Table 2).

Table 1: Number of VTEC cases in Ireland 2019-2024

Year	Serogroup	cult pos and PCR pos (%)	PCR pos cult neg (%)	Total pos
2019	O157	152(100)	0(0)	152
	O26	225(1000	0(0)	225
	Other	335(68.9)	151(31.1)	486
	Total	712(82.5)	151(17.5)	863
2020	O157	163(100)	0(0)	163
	O26	248(100)	0(0)	248
	Other	276(66.3)	140(33.7)	416
	Total	687(83)	140(17)	827
2021	O157	143(100)	0(0)	143
	O26	283(100)	0(0)	283
	Other	344(63.9)	194(36.1)	538
	Total	770(80)	194(20)	964
2022	O157	168(100)	0(0)	168
	O26	231(100)	0(0)	231
	Other	361(61)	232(39)	593
	Total	760(77)	232(23)	992
2023	O157	129(100)	0(0)	129
	O26	207(100)	0(0)	207
	Other	338 (57)	257(43)	595
	Total	674 (72)	257 (28)	931
2024	O157	112(100)	0(0)	112
	O26	219(100)	0(0)	219
	Other	298	183	481
	Total	629 (77)	183 (23)	812

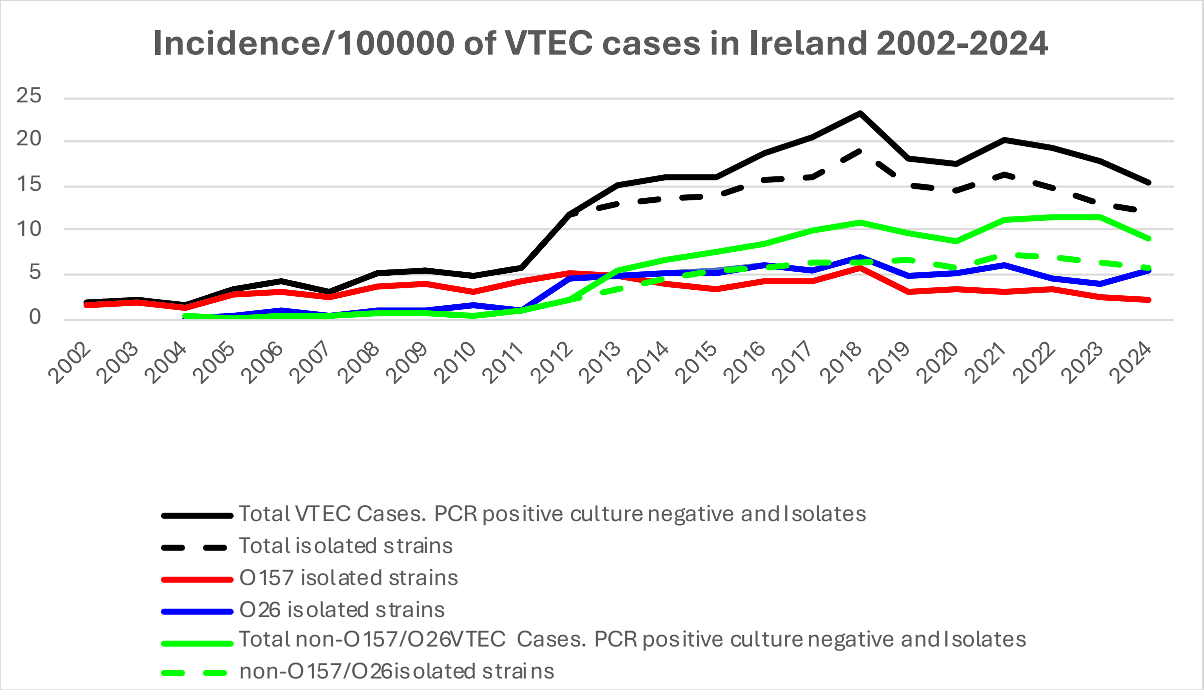

Figure 1: Incidence/100000 of VTEC cases in Ireland 2002-2024

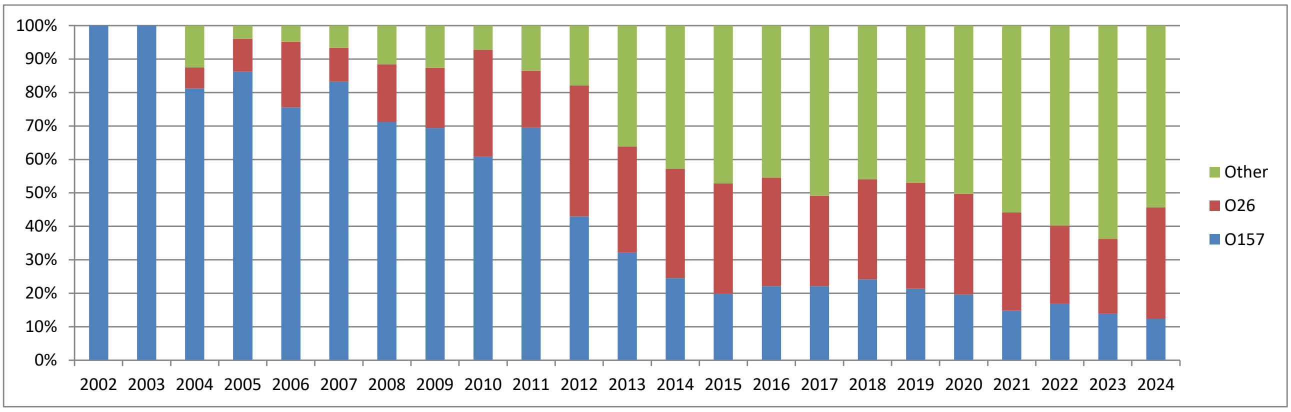

Figure 2: VTEC serogroup distribution Human VTEC isolates 2002-2024 (as a % of total culture pos & neg)

Table 2: VTEC serogroups 2017-2024

Serogroup	2024	2023	2022	2021	2020	2019	2018	2017
Unknown				6	8	11	10	26
O1:H20		3						
O1:H7		1						
O1:H45	1							
O10:H25				0	1			
O100:H20								1
O100:H30		1		1			1	
O101:H33				1			1	
O103:H2	17	12	24	20	26	27	27	38
O103:H11			1					
O103:H8	1					1		
O106:H45			2					
O104:H7				1	0			1
O107:H7				0	1			
O108:H2	2	4	2	1	2			1
O108:H25	1							
O109:H16		1		0	1			1
O11:H5				1	0			
O110:H31				1	0			

O111:H2						1		
O111:H8	8	13	11	9	11	5	9	15
O111:H21		1						
O112:H12				0	1			
O112:H21							1	
O112AB:H2	1					2	1	
O112AB:H21						1		
O113:H21	1	1	2	1	0	2	3	
O113:H4	10	11	11	8	9	5	5	2
O113:H7							1	
O113:H17								1
O115:H2							1	
O115:H25				0	1			
O116:H28		1						
O117:H4				1	0			
O117:H7	4	6	4	1	4	5	3	
O117:H8		1						
O117:H14			1				2	
O118/O151:H2	3	6	5	1			1	
O119:H4						1		
O121:H2								1

O121:H15						1		
O121:H19				1				
O122AB:H2						1		
O123:H10					1			
O123:H11						1		
O123:H16	2	1						
O123:H2					1			
O125AC:H6	1	2	1	2				1
O126:H20			1	1				
O126:H8						1		
O127:H4					2			
O127:H21				1				
O127:H40							1	
O128AB:H2				2	14	10	8	8
O128AB:H4				1				
O128AB:H34					1			
O128AC:H2	14	10	10	1		4	1	2
O128AC:H4				1				
O128AC:H12						1		
O130:H11	1	1	2	1	1	2	3	4
O130:H26						1		

O133/O186:H2						1		
O136:H12	1	1			1	2		2
O136:H16							1	
O136:H20		1			1	1		
O138:H46			1					
O138:H48	1	1		1		1		
O142:H38		1						
O145:H25	1					1		
O145:H28	30	41	43	62	47	66	53	63
O145:H34	2			1				
O146:H10								
O146:H21	16	18	31	19	18	16	29	
O146:H28	2	5	3	1		1	1	
O148:H8		1	1					
O148:H10			1					
O149:H1			2	1	2	1		
O15:H27						1		
O150:H18		1						
O150:H2	2	4	3	5	5	1	2	4
O150:H8	2							
O153:H2	1							

O153:H15	3	5	1					
O153:H21			2					
O153:H25	1							
O153:H40	1	1	2					
O153/O178:H19				1				1
O153/O178:H7	2			2	2	1	1	
O154:H31	1	1			1			
O155:H21			2			2	1	
O156:H25				4	1	2		
O157:H7	112	129	168	143	163	152	273	207
O157:H16			1					
O159:H42						2		
O160:H21	1							
O162:H33	1	3	1	1				1
O165:H7			1					
O165:H25	2	2	3	2		2	1	
O166:H28	7	9	7	6	4	4	7	3
O167:H26		4		1	1	2		1
O168:H8	1	2	2	3	1	2	1	1
O17/O44:H18	3			1	1			
O171:H2	1	1			2			

O171:H8						1		
O171:H25				1		1		
O172:H25			3					
O174:H2					1			
O174:H8						2		1
O174:H21	4	7	3	5	1	4	6	6
O174:H8	1	1	3	3				
O176:H4	8	4	5	2	1		2	2
O176:H17								1
O177:H7						1		
O177:H11	1	7	1	4	3	1	2	1
O177:H25	2	2	4	11	2	3	1	1
O177:H45					2			
O178:H7		1	1					
O178:H19		1						
O179:H8			2			1	1	
O181:H16	1		2	2		2	1	1
O182:H25	2	17	18	15	14	11	5	12
O183:H18	7	6	6	9	4	6	6	4
O183:H28	1						1	
O183:H2						1		

O184:H2								1
O185:H2			1			1		1
O187:H28				2				
O187:H52		1						
O2:H6								1
O2:H25					1			
O20:H7		3						
O21:H2	2		1					
O21:H6							1	
O21:H21			1					
O22:H14	1			2			1	
O22:H16								1
O23:H16			1					
O24:H4								1
O26:H11	219	207	231	283	248	225	335	251
O27:H30	2		2					
O3:H12		1	1					
O3:H21						1		
O30:H25				1				
O37:H2	1							
O38:H26	2	1	1	2		1		3

O4:H2							1	
O43:H2	1	3						2
O45:H2	1						3	
O49:H10			1					
O5:H9		13	11	11	5	16	13	
O5:H19			1		1	1		
O5:H-	12	5	1	1		1		18
O50/O2:H27	1	2	1	1	1			
O50/O2:H6	3		2	1	1	2	5	1
O54:H45	1							
O55:H12	5	5	4	10	3	5	5	4
O55:H7	3		1	2	5	4	4	1
O55:H9							1	
O6:H10		1	1			1	1	1
O6:H31							1	
O6:H39				1				
O63:H6		3		1				
O65:H2								1
O66:H25	1							
O68:H5	1							
O69:H32			1					

O7:H14			1					
O70:H11			3					
O71:H2		1	1					
O71:H8			2					
O71:H11			3					
O71:H19							1	
O75:H5							1	
O75:H7	1	1						
O75:H8		1			1		1	
O76:H7	1		1			1		
O76:H19	5	13	3		9	5	8	6
O78:H4	19	25	23	18	7	4		4
O78:H17								2
O79:H14	2	1	1			2	5	
O8:H4		1						
O8:H8						1		
O8:H9	1		1	1	2	5	2	1
O8:H28	2	2	3	2	1			
O8:H21			1			2		
O8:H14					1			
O8:H19	3	4	2		2	1	5	3

O8:H20						1		
O8:H30		1		1				
O80:H2	1	3			2			
O81:H21					1			
O84:H2	13	7	5	12	8	11	6	10
O86:H2					1			
O86:H21				1		1		1
O87:H16							2	2
O88:H25					1			
O9:H9						1		
O9:H19	1		2					
O9:H30						1		
O90:H40	1	3	10	7	3	5	6	5
O91:H10							1	
O91:H14	24	18	26	20	15	23	14	144
O91:H21	1			1				
O91:H28	1							
O92:H33		1						
O96:H7		1						
O96:H19	1						1	
O98:H21	4	2	1	2	1		2	3

O-Untypeable:H4	2	1	1	2		2	1	
O-Untypeable:H8	2	1			1	1	1	
O-Untypeable:H11	1	1	3			3	3	
O-Untypeable:H14		2				1		1
O-Untypeable:H15				4		1	3	
O-Untypeable:H18						1		
O-Untypeable:H19		1						
O-Untypeable:H2				1		1	1	1
O-Untypeable:H20				1		1		1
O-Untypeable:H21	1			1			1	
O-Untypeable:H25				1				1
O-Untypeable:H28	2			3				
O-Untypeable:H35								1
O-Untypeable:H40		1		2			1	
O-Untypeable:H7			1	2		1		1
O-Untypeable:H16					1			
O-Untypeable:H45	1		1		2			

O-Untypeable:H56			1					
Other				2				
Grand Total	629	674	760	770	687	712	911	758

Toxins

VTEC pathogenicity is expedited by shigatoxins (*stx*). There are two forms of shigatoxins, *stx1* and *stx2*. Both are encoded on a lamboid lysogenic bacteriophage. Either *stx1* or *stx2* or both together can be present. There are 3 subtypes of *stx1; stx1a, stx1c, stx1d* and 7 subtypes *of stx2; stx2a, stx2b, stx2c, stx2d, stx2e, stx2f,* and *stx2g*. Multiple subtypes can be present. The presence of any type of toxin is determined by PCR and the subtypes are determined by WGS. Therefore, the presence of *stx1* and *stx2* is known for culture positive and culture negative cases (931 and 812 cases in 2023 and 2024 respectively), but toxin subtype known only for the culture positive cases (674 and 629 cases respectively). The proportion of toxin genotypes remained relatively stable over the past number of years, with a dip in *stx1+ stx2* in 2023. 2023 saw 31.8% *stx1*, 33.7% *stx2* and 34.5% *stx1+ stx2*. 2024 had 29.9% *stx1*, 31.4% *stx2* and 38.7% *stx1+ stx2* (table 3, fig 3) [REF].

Toxin Subtypes

Toxin subtypes are determined by WGS therefore there is only data available for culture positive cases (674 and 629 cases respectively). The presence of *stx2* subtypes *stx2a, stx2c,* and *stx2d* have been associated with increased risk of HUS development, however *stx1a,* has also been associated with more severe illness, particularly in those aged <5 years. *stx1a* and *stx2a* remain the most common toxin subtypes (Table 4).

Table 3: Toxin genotypes Human VTEC isolates 2019-2024

Toxin genotype	**2024**		**2023**		**2022**		**2021**		**2020**		**2019**	
	Culture positive	Culture Negative	Culture positive	Culture Negative	Culture positive	Culture Negative	Culture positive	Culture Negative	Culture positive	Culture Negative	Culture positive	Culture Negative
vtx1	185	58	214	84	202	65	205	66	202	65	219	53
vtx2	195	60	209	104	244	83	211	106	244	83	246	61
Vtx1+2	249	65	251	69	324	46	344	60	324	46	247	37
Total (%)	629(77)	183(23)	674 (73)	257 (28)	770(80)	194 (20)	760(77)	232 (23)	770(79)	194 (21)	712 (82)	151 (18)
Total	812		931		992		964		827		863	

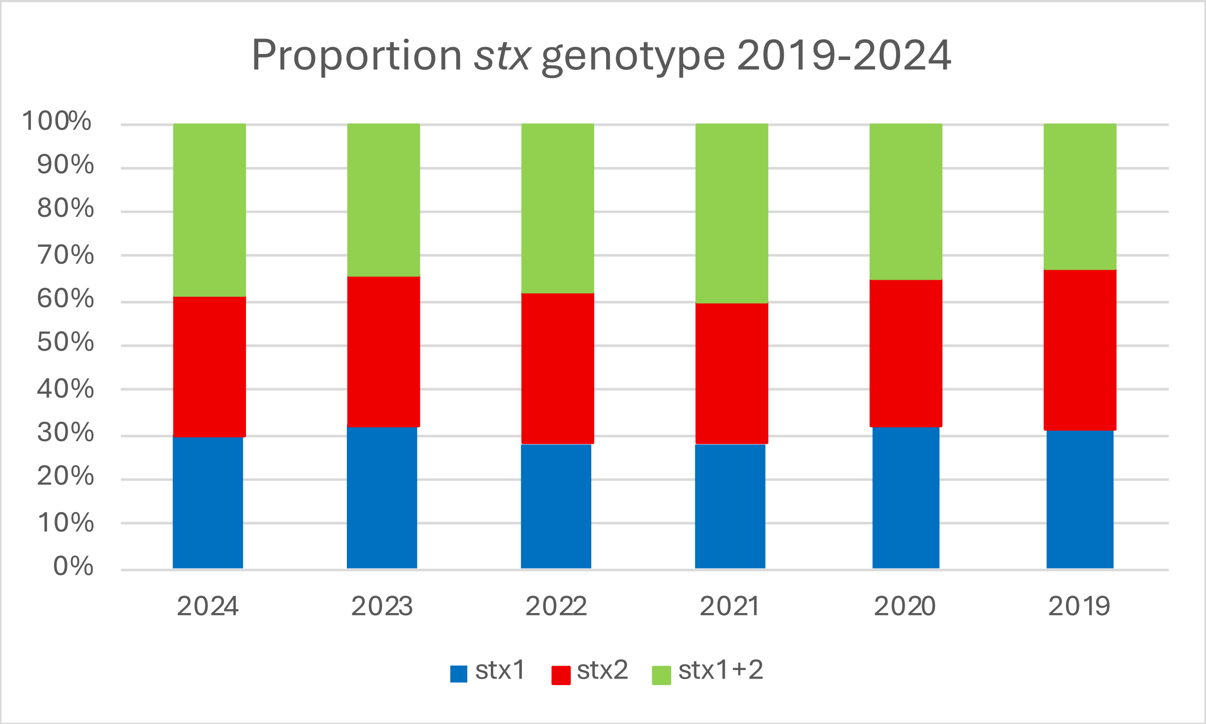

Figure 3: stx genotypes 2019-2024

Water Isolates

In the period from 2017 to 2024, VTEC was isolated from 78 water samples (table 5). Between 7-15 isolates were isolated each year, there were 10 in 2023 and 7 in 2024. There were a variety of serogroups detected, notable is the fact that no VTEC O157 or VTEC O26, the most common clinical serogroups, were isolated from water in 2023 or 2024. In addition to the strains isolated from water there were 8 samples in 2023 and 12 2024 where VTEC was detected by PCR but not isolated.

Table 4: Toxin subtypes Human VTEC isolates 2017-2024

Year	Toxin genotype	vtx1a	vtx1c	vtx1d	Vtx1a+c	vtx2a	vtx2b	vtx2c	vtx2d	vtx2e	vtx2f	vtx2g	Vtx2a+2c	Vtx2a+2d	Vtx2b+2c	Vtx2a+2b	Vtx2a+2g
2017	vtx1	172	23	1	1	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	263	21	0	0	204	29	48	1	0	0	0	1	0	0	0	--
	vtx2	--	--	--	--	201	12	16	0	4	1	0	2	0	0	0	--
2018	vtx1	147	31	1	0	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	406	31	0	0	268	45	123	0	0	0	0	0	1	0	0	--
	vtx2	--	--	--	--	189	17	28	10	2	1	1	0	0	1	0	--
2019	vtx1	176	33	3	0	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	217	23	0	0	160	40	35	0	0	0	0	0	0	0	0	--
	vtx2	--	--	--	--	170	12	24	8	8	0	2	0	0	0	0	--
2020	vtx1	138	30	4	0	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	221	24	0	0	165	36	38	1	0	0	0	0	0	0	0	--
	vtx2	--	--	--	--	171	12	8	3	2	0	0	0	0	0	0	--
2021	vtx1	158	38	1	0	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	291	27	1	0	227	42	31	0	0	0	0	1	0	0	0	--
	vtx2	--	--	--	--	169	14	23	8	1	4	4	1	0	0	0	--
2022	vtx1	145	58	2	0	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	308	35	0	0	208	54	76	1	0	0	0	3	0	0	0	--
	vtx2	--	--	--	--	155	24	13	10	2	1	4	1	0	0	1	--
2023	vtx1	157	55	3	--	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	216	36	--	--	176	49	25	1	--	--	--	--	--	--	--	--
	vtx2	--	--	--	--	152	14	18	15	5	5	3	2	1	1	--	1
2024	vtx1	134	47	3	--	--	--	--	--	--	--	--	--	--	--	--	--
	vtx1+2	208	40	--	--	158	60	22	--	--	--	--	--	--	--	--	--
	vtx2	--	--	--	--	136	17	14	15	1	5	1	1	--	--	--	--

Table 5: Serogroup of water isolates 2017-2024

Serogroup	2024	2023	2022	2021	2020	2019	2018	2017
O-untypable:H11								1
O1:H10	1							
O103:H2							3	
O109:H16					1			
O113:H4					2			
O116:H28	1					2		
O116:H8								1
O136:H12	1	1		1	4	3	2	4
O136:H16			1					
O146:H21	1	1	1	1				1
O149:H1	1							
O15:H16					1			
O157:H7		3	2	1	4	3	4	2
O165:H25					1			
O168:H8		1	1	2		1		
O171:H2	1							
O177:H25			1					
O187:H52		1						
O26:H11			2	1	1			1
O27:H30			1					
O38:H26	1							
O5:H9		1	1					
O6:H10		1						
O8:H21							1	
O8:H28		1		1				
O84:H2				1				
Grand Total	**7**	**10**	**10**	**8**	**15**	**9**	**10**	**10**

Food Isolates

In 2024 VTEC was isolated from a single food sample. This was from a raw milk sample and the isolate was O Ungroupable:H21 and was *stx1d* positive.

Whole Genome Sequencing

Since 2017 all clinical and water VTEC isolates are characterised by WGS. WGS gives information on serotype, toxin type, toxin subtype, virulence genes, sequence type(ST) and AMR. Core genome MLST (cgMLST) was used to determine genetic relatedness between isolates and thus identify outbreaks/clusters. Each time a sequencing run is carried out, the new sequences are compared to all of the isolate sequences in the database. If a cluster is identified, a cluster report is generated and sent to relevant stakeholders including referring hospital, Dept. of Public Health and HPSC. Where the presence of a clonal strain is seen, a surveillance note is issued

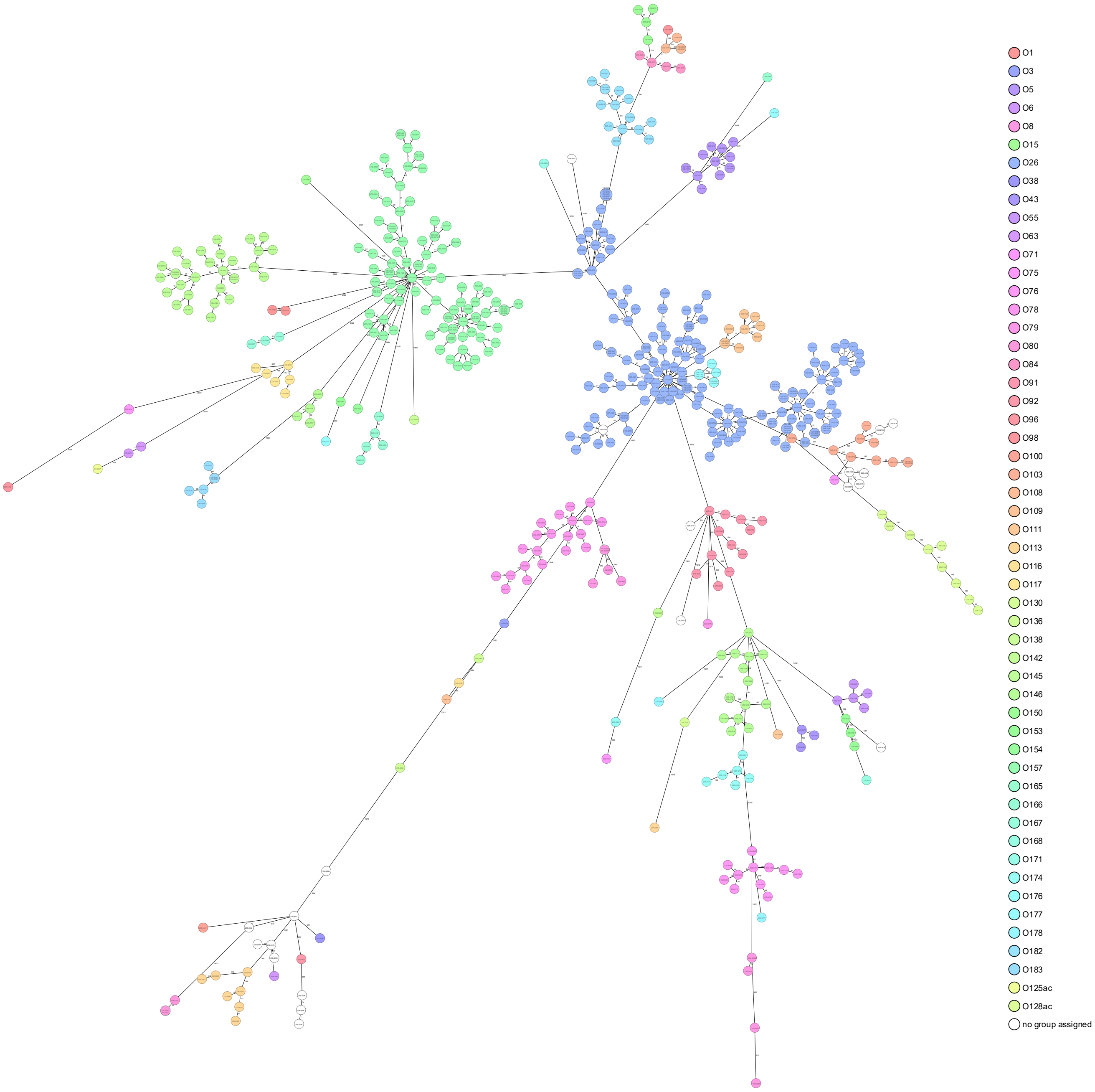

Figure 4: Minimum Spanning Tree of 2023 isolates coloured by serogroup

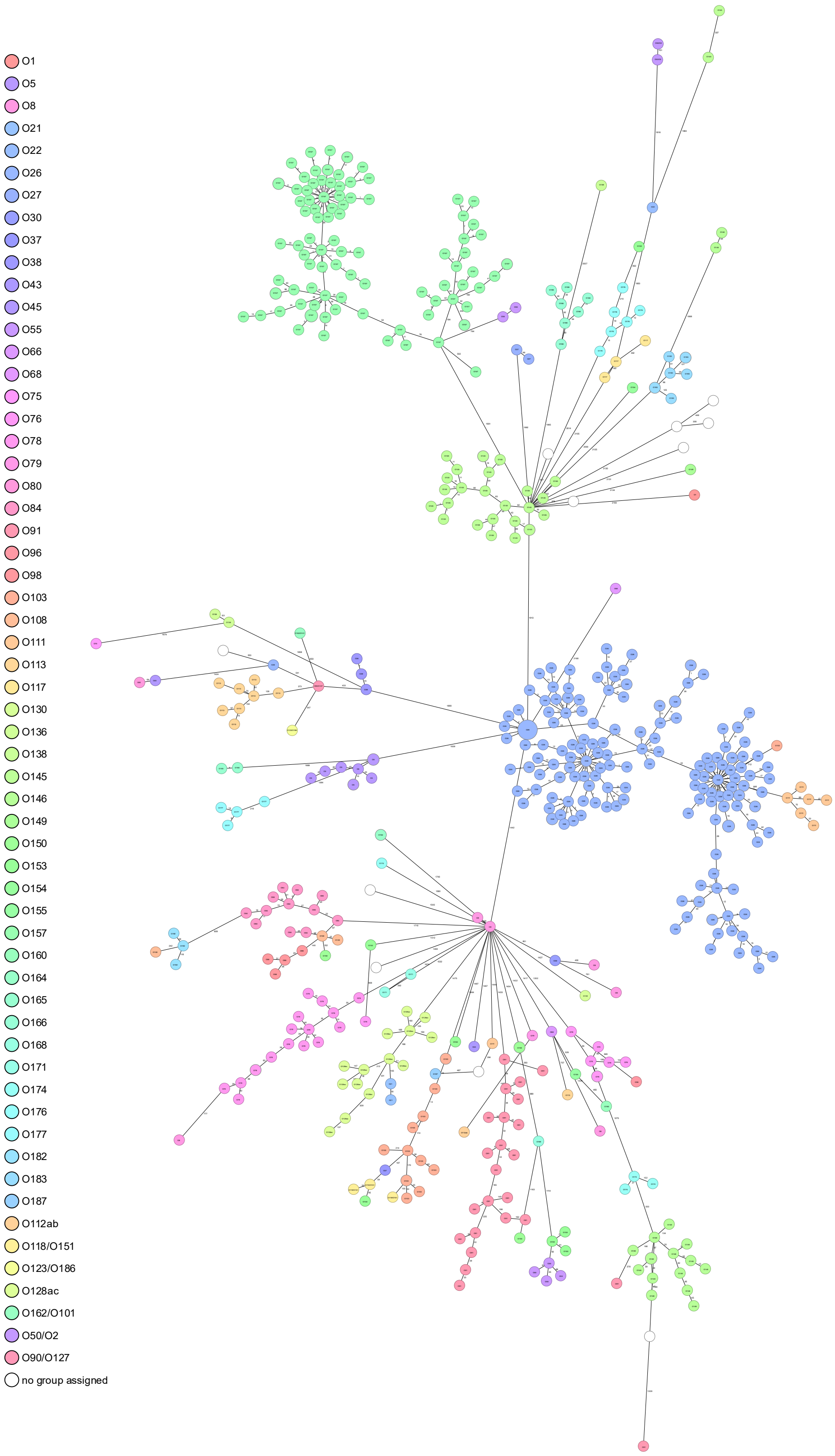

Figure 5: Minimum Spanning Tree of 2024 isolates coloured by serogroup

Apendix 1

PHL-LIMS Outbreak code information

Outbreak Code	
Index Case Name	
Index Case DOB	
Suspected organism	
Number of clinical samples expected	
Number of water samples expected	
Number of food samples expected	
Area medical officer/ PH consultant	
Other relevant information	