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National VTEC Reference Laboratory

Annual Report of VTEC in Ireland 2022

Image Infection Control Today

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Introduction

The Public Health Lab-Dublin (incorporating the NRL-VTEC) has been providing reference services for Verotoxigenic E. coli (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 HSE Community Healthcare East (CHO 6).

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. The VTEC WGS service was the recipient of the HSE Excellence awards for innovation in service in 2018 https://healthmanager.ie/2019/03/hse-award-for-new-laboratory-service/ For Full scope of accreditation see http://www.inab.ie/FileUpload/Testing/Public-Health-Laboratory-Dublin-101T.pdf

To facilitate work flow 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. Each laboratory has been sent a customised request form, if you have not received this please e mail phl.dublin@hse.ie and we will send it to you, alternatively current request forms can be downloaded from http://www.hse.ie/eng/services/list/5/publichealth/publichealthlabs/Public_Health_Laboratory_Dublin/Request_Forms.html 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 *vtx* PCR result and CP value, this enables us to streamline our testing protocol and provide you with the fastest turnaround time.

If you have any queries about our services or the content of this report please do not hesitate to contact us.

Summary

In 2022, 992 VTEC cases were detected and VTEC was isolated from 760 (77%), this is a slight increase on 2021, in actual numbers but with increasing population the incidence/100000 is slightly decreased from 20.2/100000 in 2021 to 19.3 in2022. VTEC O26 remained the most common serogroup with VTEC O157 second. Non O157/O26 serogroups continued to increase in 2022, although the increase was small. VTEC rates in Ireland remain the highest in Europe at 19.3/100000 in 2022. The EU average was 2.4/100000 in 2018 with Norway, the country with the second highest rates less than half that of Ireland at 9.3/100000. https://www.ecdc.europa.eu/sites/default/files/documents/shiga-toxin-verocytotoxin-escherichia-coli-annual-epidemiological-report-2018.pdf The proportion of cases where VTEC is detected by PCR but the organism could not be isolated remains stable over the 6 years at an average of 20% (23.5% in 2022).

Serogroups

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 PCR positive but culture negative cases.

In 2022 there were 760 culture confirmed VTEC cases. 231(30%) were VTEC O26, this is a decrease from 36.5% in 2021. 168 (22%) were VTEC O157 this is an increase from 18% in 2021. 43 cases were VTEC O145 and 25 VTEC O103. The remaining 282 cases comprised numerous different serogroups (Tables 1, 2 & Figures 1 & 2).

Table 1: Number of VTEC cases in Ireland 2017-2022

Year	serogroup	cult pos and PCR pos(%)	PCR pos cult neg(%)	Total pos
2017	O157	207(100)	0(0)	207
	O26	251(99.6)	1(0.4)	252
	Other	300(63.6)	174 (36.7)	474
	Total	**758(81.2)**	**175(18.8)**	**933**
2018	O157	273(99.6)	1(0.4)	274
	O26	335(99.4)	2(0.6)	337
	Other	303(60.6)	197(39.4)	500
	Total	**911(82)**	**200(18)**	**1111**
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**
	Grand Total	**4568 (80.8)**	**1092 (19.2)**	**5660**

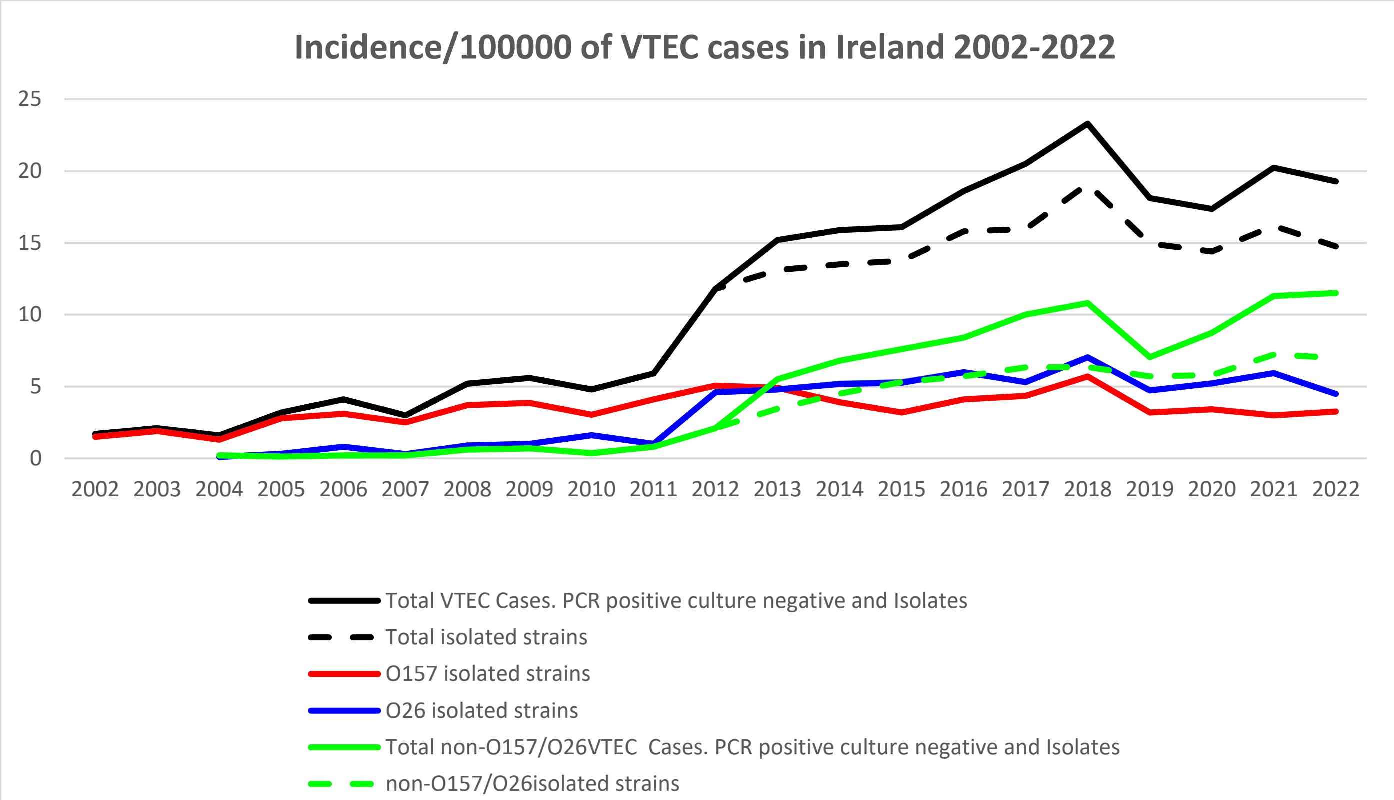

Figure 1: Incidence/100000 of VTEC cases in Ireland 2002-2022

Table 2: Serogroup of VTEC cases in Ireland 2017-2021

Serogroup	2022	2021	2020	2019	2018	2017
Unknown		6	8	11	10	26
O10:H25		0	1			
O100:H20						1
O100:H30		1			1	
O101:H33		1			1	
O103:H2	24	20	26	27	27	38
O103:H11	1					
O103:H8				1		
O106:H45	2					
O104:H7		1	0			1
O107:H7		0	1			
O108:H2	2	1	2			1
O109:H16		0	1			1
O11:H5		1	0			
O110:H31		1	0			
O111:H2				1		
O111:H8	11	9	11	5	9	15
O112:H12		0	1			
O112:H21					1	
O112AB:H2				2	1	
O112AB:H21				1		

O113:H21	2	1	0	2	3	
O113:H4	11	8	9	5	5	2
O113:H7					1	
O113:H17						1
O115:H2					1	
O115:H25		0	1			
O117:H4		1	0			
O117:H7	4	1	4	5	3	
O117:H14	1				2	
O118/O151:H2	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:H2			1			
O125AC:H6	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	10	1		4	1	2
O128AC:H4		1				
O128AC:H12				1		
O130:H11	2	1	1	2	3	4
O130:H26				1		
O133/O186:H2				1		
O136:H12			1	2		2
O136:H16					1	
O136:H20			1	1		
O138:H46	1					
O138:H48		1		1		
O145:H25				1		
O145:H28	43	62	47	66	53	63
O145:H34		1				
O146:H10						
O146:H21	31	19	18	16	29	
O146:H28	3	1		1	1	
O148:H8	1					
O148:H10	1					
O149:H1	2	1	2	1		

O15:H27				1		
O150:H2	3	5	5	1	2	4
O153:H15	1					
O153:H21	2					
O153:H40	2					
O153/O178:H19		1				1
O153/O178:H7		2	2	1	1	
O154:H31			1			
O155:H21	2			2	1	
O156:H25		4	1	2		
O157:H7	168	143	163	152	273	207
O157:H16	1					
O159:H42				2		
O162:H33	1	1				1
O165:H7	1					
O165:H25	3	2		2	1	
O166:H28	7	6	4	4	7	3
O167:H26		1	1	2		1
O168:H8	2	3	1	2	1	1
O17/O44:H18		1	1			
O171:H2			2			
O171:H8				1		
O171:H25		1		1		
O172:H25	3					
O174:H2			1			
O174:H8				2		1
O174:H21	3	5	1	4	6	6
O174:H8	3	3				
O176:H4	5	2	1		2	2
O176:H17						1
O177:H7				1		
O177:H11	1	4	3	1	2	1
O177:H25	4	11	2	3	1	1
O177:H45			2			
O178:H7	1					
O179:H8	2			1	1	
O181:H16	2	2		2	1	1
O182:H25	18	15	14	11	5	12
O183:H18	6	9	4	6	6	4
O183:H28					1	
O183:H2				1		
O184:H2						1
O185:H2	1			1		1
O187:H28		2				
O2:H6						1
O2:H25			1			
O21:H2	1					

O21:H6					1	
O21:H21	1					
O22:H14		2			1	
O22:H16						1
O23:H16	1					
O24:H4						1
O26:H11	231	283	248	225	335	251
O27:H30	2					
O3:H12	1					
O3:H21				1		
O30:H25		1				
O38:H26	1	2		1		3
O4:H2					1	
O43:H2						2
O45:H2					3	
O49:H10	1					
O5:H9	11	11	5	16	13	
O5:H19	1		1	1		
O5:H-	1	1		1		18
O50/O2:H27	1	1	1			
O50/O2:H6	2	1	1	2	5	1
O55:H12	4	10	3	5	5	4
O55:H7	1	2	5	4	4	1
O55:H9					1	
O6:H10	1			1	1	1
O6:H31					1	
O6:H39		1				
O63:H6		1				
O65:H2						1
O69:H32	1					
O7:H14	1					
O70:H11	3					
O71:H2	1					
O71:H8	2					
O71:H11	3					
O71:H19					1	
O75:H5					1	
O75:H8			1		1	
O76:H7	1			1		
O76:H19	3		9	5	8	6
O78:H4	23	18	7	4		4
O78:H17						2
O79:H14	1			2	5	
O8:H8				1		
O8:H9	1	1	2	5	2	1
O8:H28	3	2	1			
O8:H21	1			2		

O8:H14			1			
O8:H19	2		2	1	5	3
O8:H20				1		
O8:H30		1				
O80:H2			2			
O81:H21			1			
O84:H2	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	2					
O9:H30				1		
O90:H40	10	7	3	5	6	5
O91:H10					1	
O91:H14	26	20	15	23	14	144
O91:H21		1				
O96:H19					1	
O98:H21	1	2	1		2	3
O-Untypeable:H11	3			3	3	
O-Untypeable:H15		4		1	3	
O-Untypeable:H18				1		
O-Untypeable:H2		1		1	1	1
O-Untypeable:H20		1		1		1
O-Untypeable:H21		1			1	
O-Untypeable:H25		1				1
O-Untypeable:H28		3				
O-Untypeable:H4	1	2		2	1	
O-Untypeable:H14				1		1
O-Untypeable:H35						1
O-Untypeable:H40		2			1	
O-Untypeable:H7	1	2		1		1
O-Untypeable:H16			1			
O-Untypeable:H45	1		2			
O-Untypeable:H8			1	1	1	
O-Untypeable:H56	1					
Other		2				
Grand Total	**760**	**770**	**687**	**712**	**911**	**758**

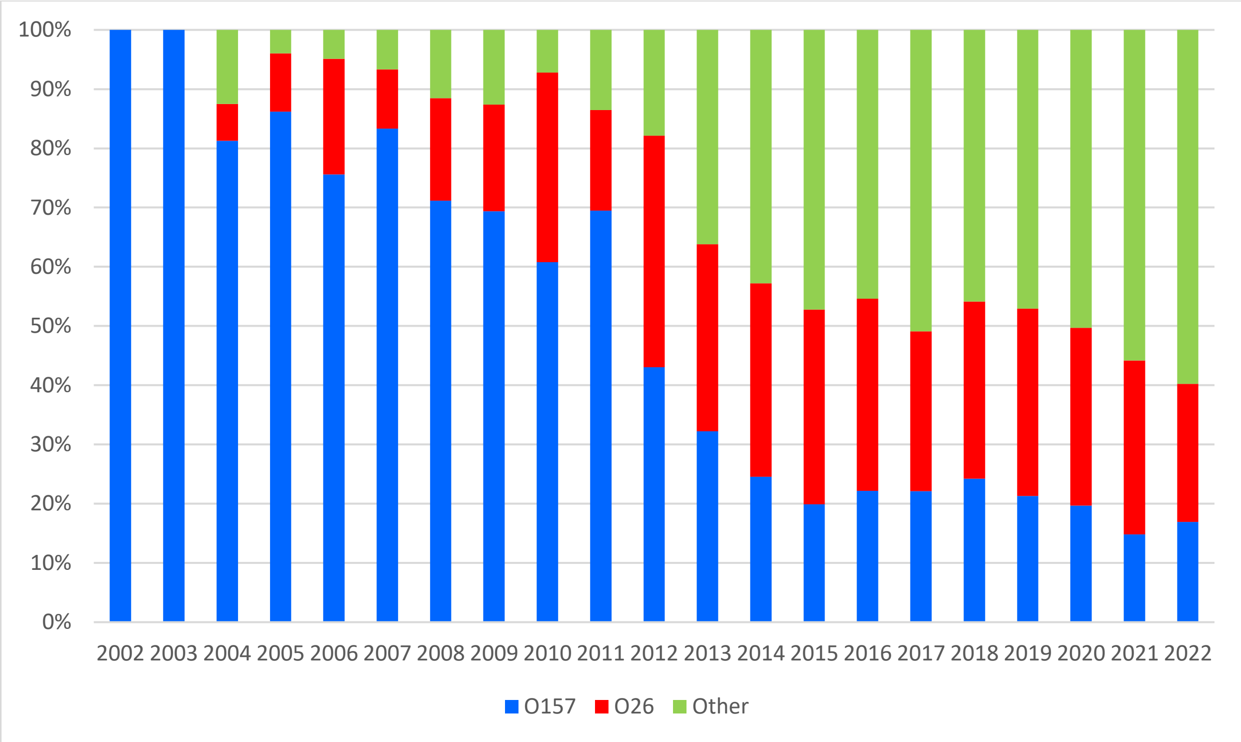

Figure 2: VTEC serogroup distribution Human VTEC isolates 2002-2021 (as a % of total culture pos & neg)

Toxins

VTEC pathogenicity is caused by verotoxins (*vtx*). There are two types of verotoxins, *vtx1* and *vtx2*. Both are encoded on a lamboid lysogenic bacteriophage. Either *vtx1* or *vtx2* or both together can be present. There are 3 subtypes of *vtx1; vtx1a, vtx1c, vtx1d* and 7 subtypes *of vtx2; vtx2a, vtx2b, vtx2c, vtx2d, vtx2e, vtx2f,* and *vtx2g.* Multiple subtypes can be present. The presence any type of toxin is determined by PCR and the subtypes are determined by WGS. Therefore, the presence of *vtx1* and *vtx2* is determined for culture positive and culture negative cases (992), but detection of toxin subtype is possible only for the culture positive cases (760). The proportion of toxin serogroups remained relatively stable over the past number of years. 2022 saw 27.3% *vtx1*, 32% *vtx2* and 40.7% *vtx1+ vtx2* (table 3, fig 4).

Toxin Subtypes

Toxin subtypes are determined by WGS therefore there is only data available for culture positive cases. The presence of *vtx2* subtypes *vtx2a, vtx2c,* and *vtx2d* have been associated with increased risk of HUS development, however *vtx1a,* has also been associated with more severe illness, particularly in those aged <5 years. *Vtx1a* and *vtx2a* remain the most common toxin subtypes (Table 4).

Culture positivity

The proportion of cases where VTEC is detected by PCR but the organism could not be recovered on culture (ie PCR positive but culture negative) remains stable over the 6 years at an average of 20% (17%-23.5%), 23.5% in 2022. It is more likely that samples positive for vtx*1+ vtx2* will be isolated p<0.001***.*** (graphpad prism, anova, Fig 3).

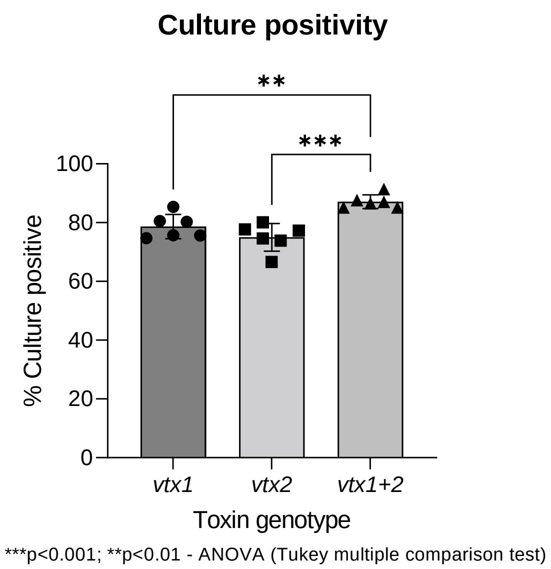

Figure 3: Analysis of culture positivity

Table 3: Toxin genotypes Human VTEC isolates 2017-2021

Toxin genotype	**2022**		**2021**		**2020**		**2019**		**2018**		**2017**	
	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	205	66	202	65	193	33	219	53	189	64	208	51
vtx2	211	106	244	83	218	64	246	61	275	97	254	73
Vtx1+2	344	60	324	46	276	43	247	37	447	42	296	52
Total (%)	**760(76.5)**	**232 (23.5)**	**770(79.8)**	**194 (20.2)**	**687 (83)**	**140 (17)**	**712 (82.5)**	**151 (17.5)**	**911 (82)**	**200 (18)**	**758 (76.5)**	**175 (23.5)**
Total	**992**		**964**		**827**		**863**		**1111**		**933**	

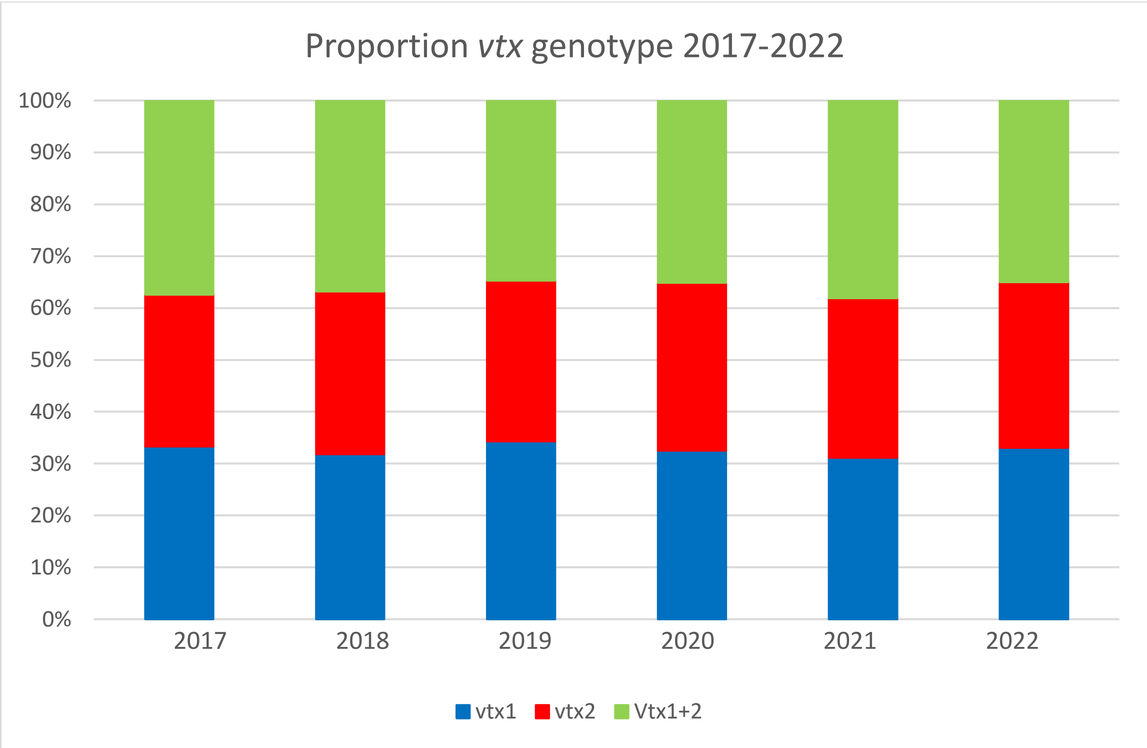

Fig 4: **Toxin genotypes Human VTEC isolates 2017-2022**

Table 4: Toxin subtypes Human VTEC isolates 2017-2021 detected by WGS

Year	**Toxin genotype**	***vtx1a***	**vtx1c**	**vtx1d**	***Vtx1a+c***	**vtx2a**	**vtx2b**	**vtx2c**	**vtx2d**	**vtx2e**	**vtx2f**	**vtx2g**	***Vtx2a+2c***	***Vtx2a+2d***	***Vtx2b+2c***	***Vtx2a+2b***
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

Note: Data for culture positive cases only

Food and Water Isolates

In 2022 140 water samples and 14 food samples were tested for VTEC. VTEC was not detected in any of the food samples. VTEC was detected in 11 water samples, 10 were culture positive and one PCR only positive and culture negative). 10/11 VTEC positive waters were tested in response to a VTEC positive clinical case. However, only 2/10 had a genetic relatedness to corresponding clinical cases. This is not an unusual occurrence and usually indicates an ongoing contamination of the water source. In the period from 2017 to 2022, VTEC was isolated from 61 water samples (table 5). Between 7-15 isolates were isolated each year, 10 in 2022. There were a variety of serogroups detected, VTEC O157 and VTEC 0136 accounting for 16 and 15 samples respectively, VTEC O26 was isolated from just 5 samples.

Table 5: Serogroup of water isolates 2017-2022

Serogroup	**2022**	**2021**	**2020**	**2019**	**2018**	**2017**
O-unidentifiable:H11						1
O103:H2					3	
O109:H16			1			
O113:H4			2			
O116:H28				2		
O116:H8						1
O136:H12		1	4	3	2	4
O136:H16	1					
O146:H21	1	1				1
O15:H16			1			
O157:H7	2	1	4	3	4	2
O165:H25			1			
O168:H8	1	2		1		
O177:H25	1					
O26:H11	2	1	1			1
O27:H30	1					
O5:Unknown	1					
O8:H21					1	
O8:H28		1				
O84:H2		1				
Grand Total	10#	8*	15	9	10	10

*8 VTEC organisms isolated from 7 water samples

In addition to the 10 isolated strains in 2022, 1 water sample was positive for *vtx2* by PCR but culture negative

Table 6: Toxin genotypes and subtypes of water isolates 2022

Toxin genotype	Number	***vtx1a***	**vtx1c**	**vtx2a**	***Vtx2b***	**vtx2g**
vtx1	1	1				
vtx2	6			6	1	1
Vtx1+2	3	2	1	2	1	
Total	10	3	1	8	2	1

Whole Genome Sequencing

In 2022 all clinical and water VTEC isolates were 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 (≈4000). Isolates with ≤6 allele differences are likely to have been exposed to the same source or linked by person-to-person transmission, based on this cut-off, 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. In 2022 75 cluster reports were issued, ranging from 2 related isolates to a general outbreak with 27 cases. 7 surveillance notes were also issued in 2022.

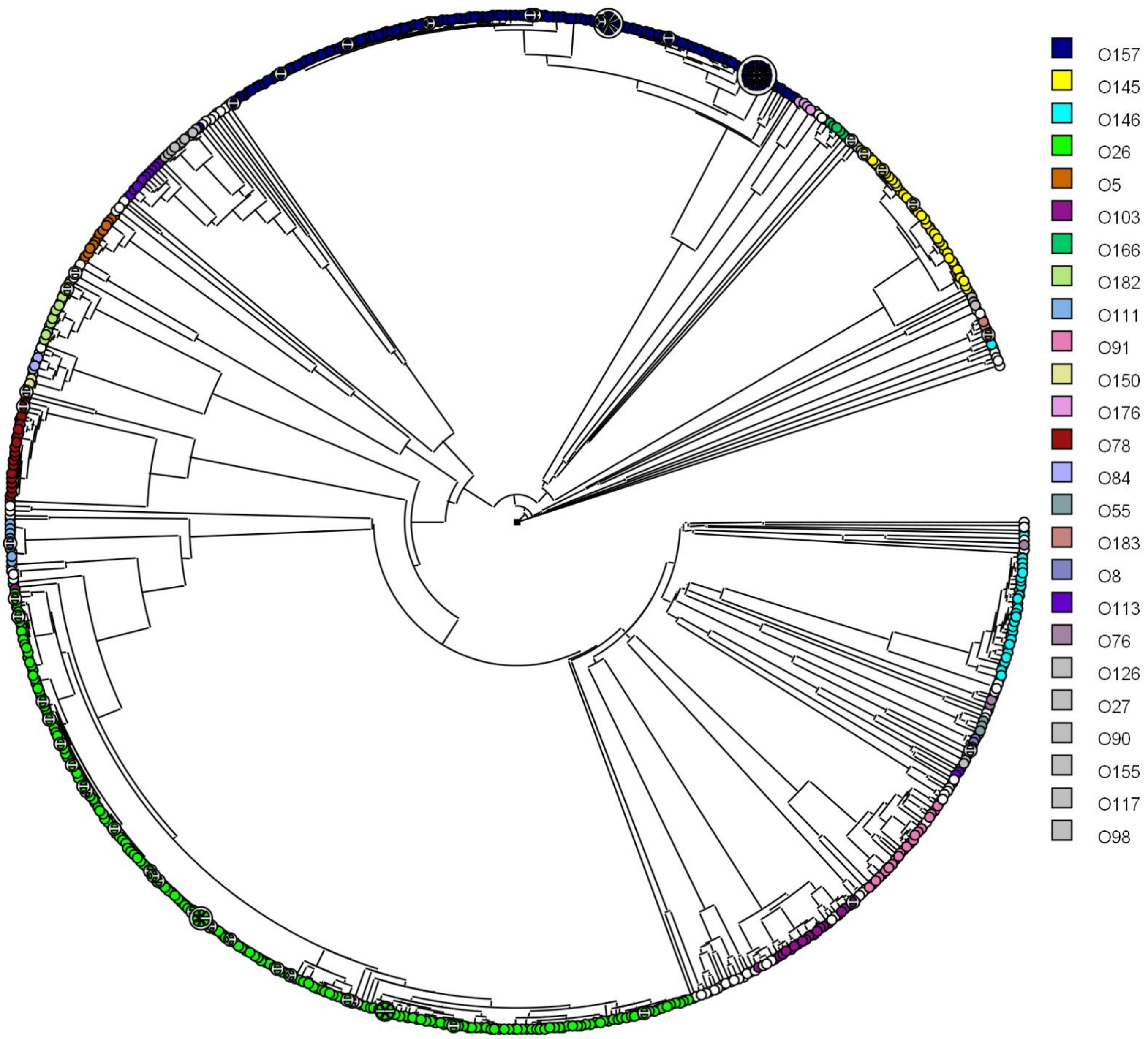

Fig 5: cgMLST Dendrogram of all clinical 760 VTEC isolates 2022

Isolates are coloured by serogroup.

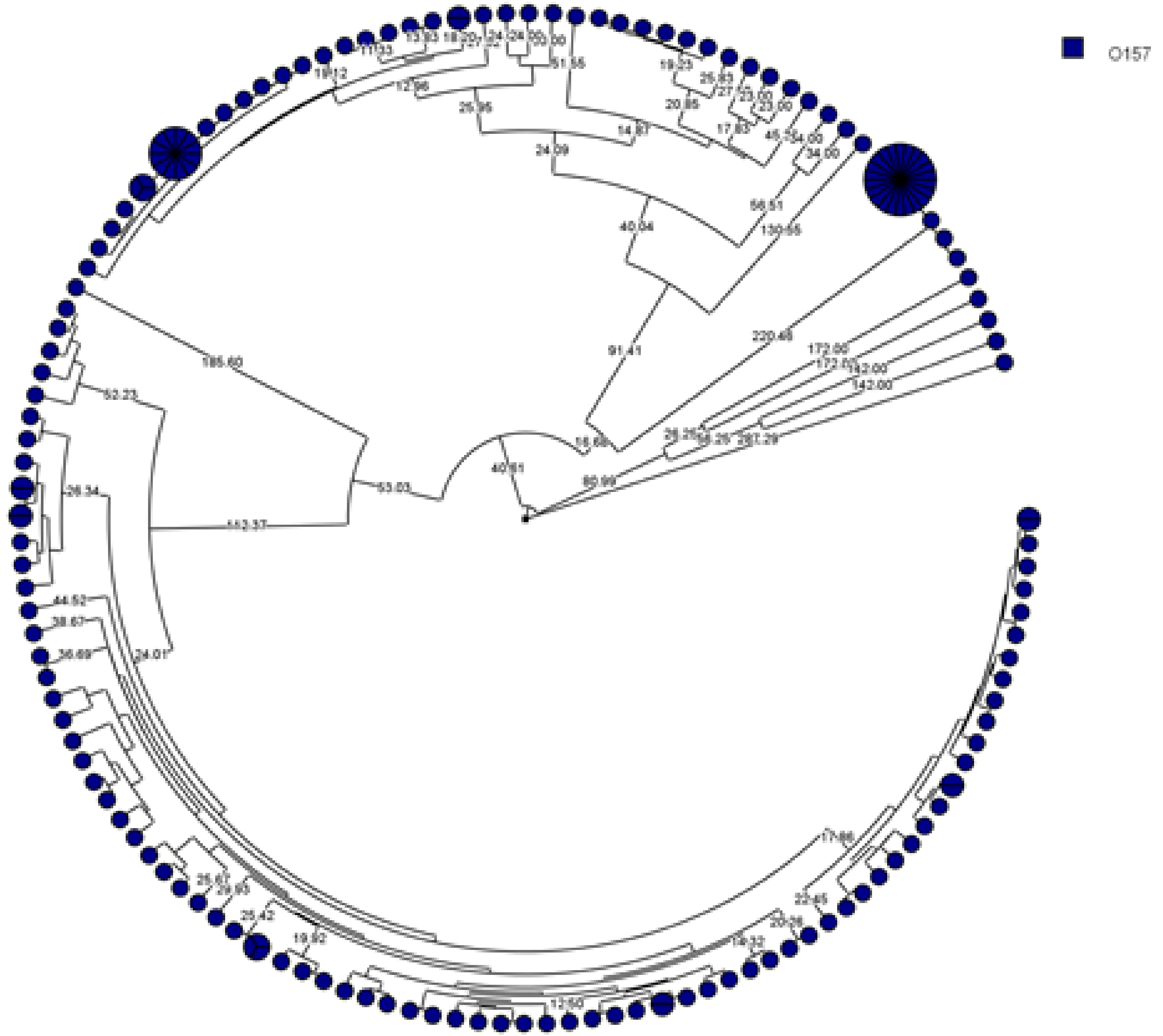

Fig 6: cgMLST Dendrogram of 168 VTEC O157 isolates from 2022

The number of allele difference by cgMLST can be seen as branch labels.

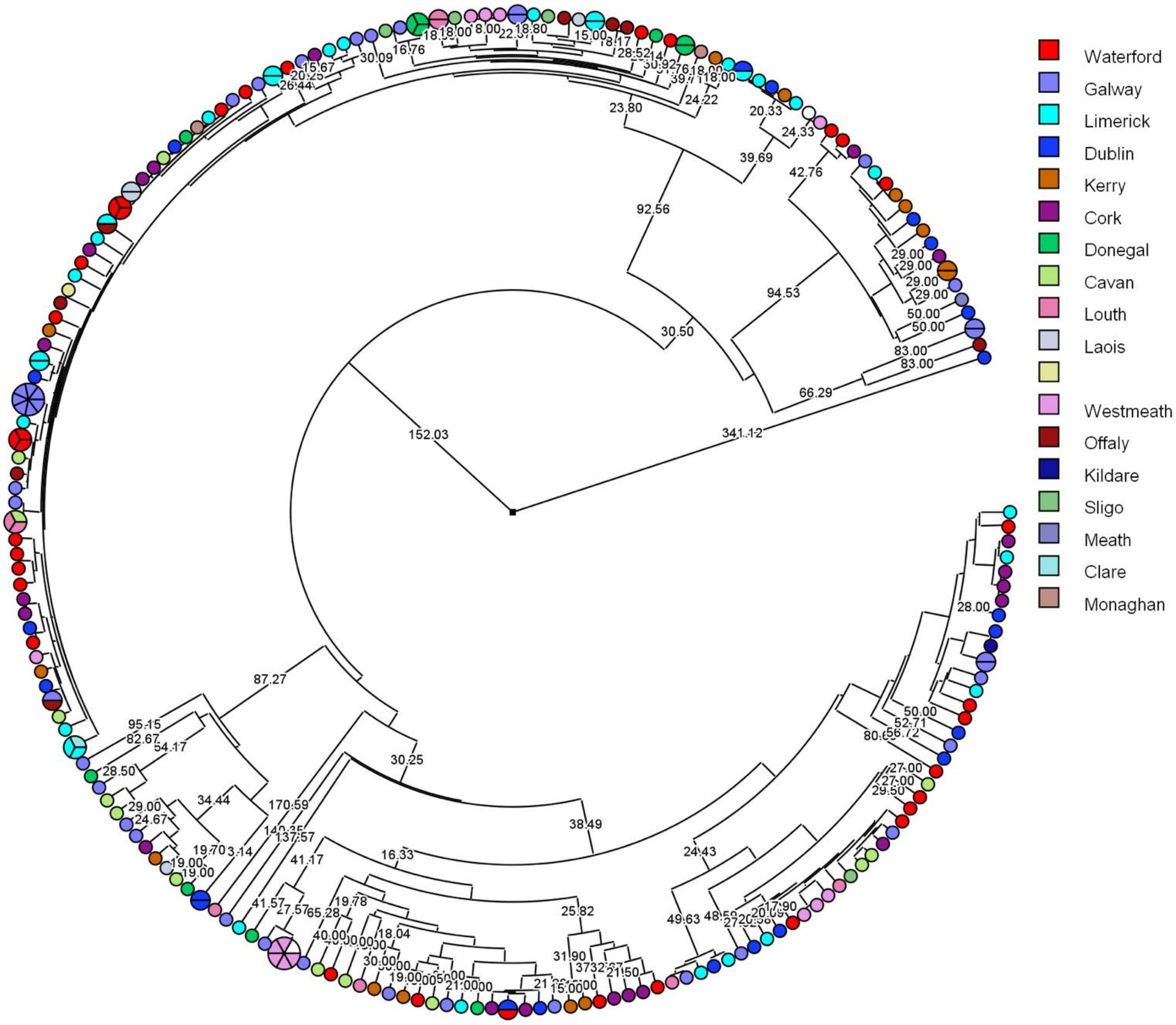

Fig 7: cgMLST Dendrogram of 231 VTEC O26 isolates from 2022

The number of allele difference by cgMLST can be seen as branch labels. Nodes are coloured by county of origin.