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# National VTEC Reference Laboratory

# Five-year review of VTEC laboratory characterisation in Ireland 2017-2021

Image Infection Control Today

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**Introduction**

The Public Health Laboratory, Dublin (incorporating the NRL-VTEC) has provided reference services for VTEC since 1998 and has received 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 standards by INAB. The routine methods of culture and PCR (for *vtx1 & vtx2* genes) was augmented with the introduction of WGS (whole genomic sequencing) in 2018. One isolate per VTEC case is now characterised by WGS & the data is shared nationally with HPSC & with EU-ECDC for investigation of multi-member state VTEC outbreaks. 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

https://www.inab.ie/fileupload/medical-testing/public-health-laboratory-dublin-334mt.pdf and

https://www.inab.ie/fileupload/testing/public-health-laboratory-dublin-101t.pdf

**VTEC NRL Requests:**

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 all samples are accompanied by a completed NRL-VTEC request form.

Each laboratory has been sent a customised NRL-VTEC 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 the PHL Dublin website.

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 you including an outbreak code (if relevant) and your *vtx* PCR result and CP value, this enables us to streamline our testing protocol and provide you with the fastest possible 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 the 5-year period from 2017 to 2021 4668 VTEC cases were detected and VTEC was isolated from 3808 isolates (81.5%). During this period VTEC O26 was the most common serogroup with VTEC O157 second. Non O157/O26 serogroups continued to increase in 2017 to 2019. The incidence dropped in 2020 (as did all VTEC) most likely due to COVID, however VTEC rates in Ireland remained the highest in Europe at 20/100000 in 2018. The EU average was 2.4/100000 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-verocytototoxin-escherichia-coli-annual-epidemiological-report-2018.pdf .

The proportion of cases in Ireland, where VTEC was detected by PCR but the organism could not be isolated remained stable over the 5 years at an average of 18.25%.

**Serogroups**

The serogroup of VTEC isolates is determined by a combination of PCR and serology for culture positive cases and subsequently confirmed by WGS from pure isolates. Serogroup is therefore not determined for culture negative cases.

Between the years 2017-2021, there were 3808 culture confirmed VTEC cases. 1342(35%) were VTEC O26, this percentage was relatively stable over the 5 years. 938 (25%) were VTEC O157, these ranged from range 18.5% (2021) to 30% (2018). This differs from the 2018 EU data where VTEC O157 accounted for the majority of VTEC cases, yet VTEC O26 accounted for the majority of HUS (haemolytic uremic syndrome) cases. The higher 2018 VTEC O157 percentage in Ireland was largely due to a single outbreak of >100 cases. 293 (8%) cases were VTEC O145 and 139 (3.6%) VTEC O103. The other 2 'big six' serogroups O111 & O104 had 50 (1.3%) & 1 isolates detected respectively. The remaining 1045 (27%) cases comprised 88 different serogroups (Table 2)

**Culture positivity**

The proportion of cases where VTEC is detected by PCR but the organism could not be isolated remains stable over the 5 years, at an average of 18.25% (16.9%-20.1%). This may reflect the culture independent methods now commonly utilised in primary diagnostic laboratories. It is more likely that samples positive for vtx*1+ vtx2* will be isolated p=*0.0004.* (graphpad prism, anova). With the exception of 4 isolates over the 5-year period all VTEC O26 and VTEC O157 were isolated.

**Table 1: Number of VTEC cases in Ireland 2017-2021**

| Year | serogroup | culture & PCR positive(%) | PCR positive & culture negative(%) | Total VTEC cases |
|---|---|---|---|---|
| **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** |
| | **Grand Total** | **3808 (81.6)** | **860 (18.4)** | **4668** |

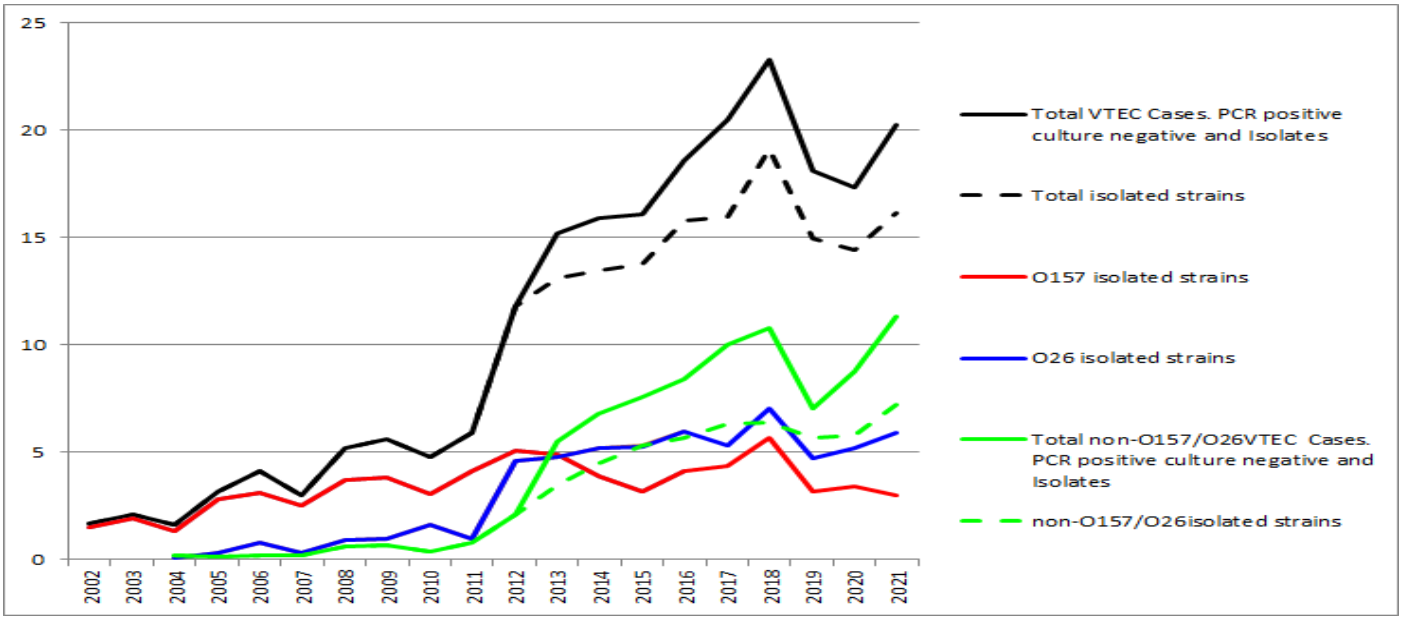



**Figure 1: Incidence/100000 of VTEC cases in Ireland 2002-2021**

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

| Serogroup | 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 | 20 | 26 | 27 | 27 | 38 |
| O103:H8 | | | 1 | | |
| O104:H7 | 1 | 0 | | | 1 |
| O107:H7 | 0 | 1 | | | |
| O108:H2 | 1 | 2 | | | 1 |
| O109:H16 | 0 | 1 | | | 1 |
| O11:H5 | 1 | 0 | | | |
| O110:H31 | 1 | 0 | | | |
| O111:H2 | | | 1 | | |
| O111:H8 | 9 | 11 | 5 | 9 | 15 |
| O112:H12 | 0 | 1 | | | |
| O112:H21 | | | | 1 | |
| O112AB:H2 | | | 2 | 1 | |
| O112AB:H21 | | | 1 | | |
| O113:H21 | 1 | 0 | 2 | 3 | |
| O113:H4 | 8 | 9 | 5 | 5 | 2 |
| O113:H7 | | | | 1 | |
| O113:H17 | | | | | 1 |
| O115:H2 | | | | 1 | |
| O115:H25 | 0 | 1 | | | |
| O117:H4 | 1 | 0 | | | |
| O117:H7 | 1 | 4 | 5 | 3 | |
| O117:H14 | | | | 2 | |
| O118/O151:H2 | 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 | 2 | | | | 1 |
| O126:H20 | 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 | 1 | | 4 | 1 | 2 |
| O128AC:H4 | 1 | | | | |
| O128AC:H12 | | | 1 | | |
| O130:H11 | 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:H48 | 1 | | 1 | | |
| O145:H25 | | | 1 | | |
| O145:H28 | 62 | 47 | 66 | 53 | 63 |
| O145:H34 | 1 | | | | |
| O146:H21 | 19 | 18 | 16 | 29 | |
| O146:H28 | 1 | | 1 | 1 | |
| O149:H1 | 1 | 2 | 1 | | |
| O15:H27 | | | 1 | | |
| O150:H2 | 5 | 5 | 1 | 2 | 4 |
| O153/O178:H19 | 1 | | | | 1 |
| O153/O178:H7 | 2 | 2 | 1 | 1 | |
| O154:H31 | | 1 | | | |
| O155:H21 | | | 2 | 1 | |
| O156:H25 | 4 | 1 | 2 | | |
| O157:H7 | 143 | 163 | 152 | 273 | 207 |
| O159:H42 | | | 2 | | |
| O162:H33 | 1 | | | | 1 |
| O165:H25 | 2 | | 2 | 1 | |
| O166:H28 | 6 | 4 | 4 | 7 | 3 |
| O167:H26 | 1 | 1 | 2 | | 1 |
| O168:H8 | 3 | 1 | 2 | 1 | 1 |
| O17/O44:H18 | 1 | 1 | | | |
| O171:H2 | | 2 | | | |
| O171:H8 | | | 1 | | |
| O171:H25 | 1 | | 1 | | |
| O174:H2 | | 1 | | | |
| O174:H8 | | | 2 | | 1 |
| O174:H21 | 5 | 1 | 4 | 6 | 6 |
| O174:H8 | 3 | | | | |
| O176:H4 | 2 | 1 | | 2 | 2 |
| O176:H17 | | | | | 1 |

| | | | | | |
|---|---|---|---|---|---|
| O177:H7 | | | 1 | | |
| O177:H11 | 4 | 3 | 1 | 2 | 1 |
| O177:H25 | 11 | 2 | 3 | 1 | 1 |
| O177:H45 | | 2 | | | |
| O179:H8 | | | 1 | 1 | |
| O181:H16 | 2 | | 2 | 1 | 1 |
| O182:H25 | 15 | 14 | 11 | 5 | 12 |
| O183:H18 | 9 | 4 | 6 | 6 | 4 |
| O183:H28 | | | | 1 | |
| O183:H2 | | | 1 | | |
| O184:H2 | | | | | 1 |
| O185:H2 | | | 1 | | 1 |
| O187:H28 | 2 | | | | |
| O2:H6 | | | | | 1 |
| O2:H25 | | 1 | | | |
| O21:H6 | | | | 1 | |
| O22:H14 | 2 | | | 1 | |
| O22:H16 | | | | | 1 |
| O24:H4 | | | | | 1 |
| O26:H11 | 283 | 248 | 225 | 335 | 251 |
| O3:H21 | | | 1 | | |
| O30:H25 | 1 | | | | |
| O38:H26 | 2 | | 1 | | 3 |
| O4:H2 | | | | 1 | |
| O43:H2 | | | | | 2 |
| O45:H2 | | | | 3 | |
| O5:H9 | 11 | 5 | 16 | 13 | |
| O5:H19 | | 1 | 1 | | |
| O5:H- | 1 | | 1 | | 18 |
| O50/O2:H27 | 1 | 1 | | | |
| O50/O2:H6 | 1 | 1 | 2 | 5 | 1 |
| O55:H12 | 10 | 3 | 5 | 5 | 4 |
| O55:H7 | 2 | 5 | 4 | 4 | 1 |
| O55:H9 | | | | 1 | |
| O6:H10 | | | 1 | 1 | 1 |
| O6:H31 | | | | 1 | |
| O6:H39 | 1 | | | | |
| O63:H6 | 1 | | | | |
| O65:H2 | | | | | 1 |
| O71:H19 | | | | 1 | |
| O75:H5 | | | | 1 | |
| O75:H8 | | 1 | | 1 | |
| O76:H7 | | | 1 | | |
| O76:H19 | | 9 | 5 | 8 | 6 |
| O78:H4 | 18 | 7 | 4 | | 4 |

| | | | | | |
|---|---|---|---|---|---|
| O78:H17 | | | | | 2 |
| O79:H14 | | | 2 | 5 | |
| O8:H8 | | | 1 | | |
| O8:H9 | 1 | 2 | 5 | 2 | 1 |
| O8:H28 | 2 | 1 | | | |
| O8:H21 | | | 2 | | |
| O8:H14 | | 1 | | | |
| O8:H19 | | 2 | 1 | 5 | 3 |
| O8:H20 | | | 1 | | |
| O8:H30 | 1 | | | | |
| O80:H2 | | 2 | | | |
| O81:H21 | | 1 | | | |
| O84:H2 | 12 | 8 | 11 | 6 | 10 |
| O86:H2 | | 1 | | | |
| O86:H21 | 1 | | 1 | | 1 |
| O87:H16 | | | | 2 | 2 |
| O88:H25 | | 1 | | | |
| O9:H9 | | | 1 | | |
| O9:H30 | | | 1 | | |
| O90:H40 | 7 | 3 | 5 | 6 | 5 |
| O91:H10 | | | | 1 | |
| O91:H14 | 20 | 15 | 23 | 14 | 144 |
| O91:H21 | 1 | | | | |
| O96:H19 | | | | 1 | |
| O98:H21 | 2 | 1 | | 2 | 3 |
| O-Untypeable:H11 | | | 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 | 2 | | 2 | 1 | |
| O-Untypeable:H14 | | | 1 | | 1 |
| O-Untypeable:H35 | | | | | 1 |
| O-Untypeable:H40 | 2 | | | 1 | |
| O-Untypeable:H7 | 2 | | 1 | | 1 |
| O-Untypeable:H16 | 0 | 1 | | | |
| O-Untypeable:H45 | 0 | 2 | | | |
| O-Untypeable:H8 | 0 | 1 | 1 | 1 | |
| Other | 2 | | | | |
| **Grand Total** | **770** | **687** | **712** | **911** | **758** |

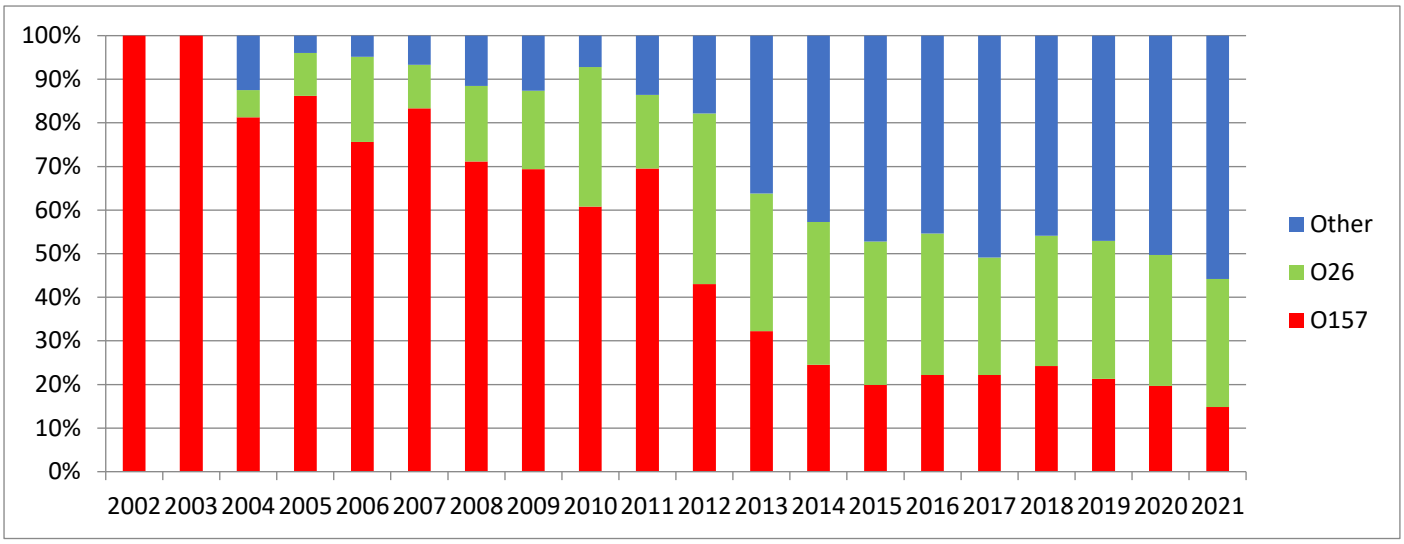



**Figure 2: VTEC serogroup distribution Human VTEC isolates 2002-2021**

**Verotoxins**

VTEC pathogenicity is expedited by verotoxins (*vtx*). There are two forms 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 of verotoxin is determined by PCR but the subtypes are determined by WGS. Therefore, the presence of *vtx1* and *vtx2* is determined for both culture positive and culture negative cases (4668), but verotoxin subtype is known only for the culture positive cases (3808).

The proportion of verotoxin subgroups remained relatively stable over the 5 years, with 27% *vtx1*, 34% *vtx2* and 39% *vtx1+ vtx2* (table 3, fig 3). Therefore 73% of VTEC cases in Ireland carry *vtx2* which is traditionally associated with more severe clinical illness.

**Verotoxin Subtypes**

Verotoxin subtypes was determined by WGS on the culture positive cases (n=3808). 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 <5years. From this Irish data we see that only a very small proportion (4.1% in 2017 & 6.7% in 2019, averaging 5.54%) of VTEC cases do not carry the potential virulent subtypes; vtx*1a, vtx2a, vtx2c,* or *vtx2d* (Table 4).

**Table 3: Verotoxin genotypes of all Human VTEC isolates (culture positive or negative) 2017-2021**

| Toxin genotype | 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 |
| *vtx1* | 202 | 65 | 193 | 33 | 219 | 53 | 189 | 64 | 208 | 51 |
| *vtx2* | 244 | 83 | 218 | 64 | 246 | 61 | 275 | 97 | 254 | 73 |
| Vtx1+2 | 324 | 46 | 276 | 43 | 247 | 37 | 447 | 42 | 296 | 52 |
| **Total** | **770 (80%)** | **194 (20%)** | **687 (83%)** | **140 (17%)** | **712(82.5%)** | **151(17.5%)** | **911 (82%)** | **200 (18%)** | **758 (81%)** | **175 (19%)** |
| **Total** | **964** | | **827** | | **863** | | **1111** | | **933** | |

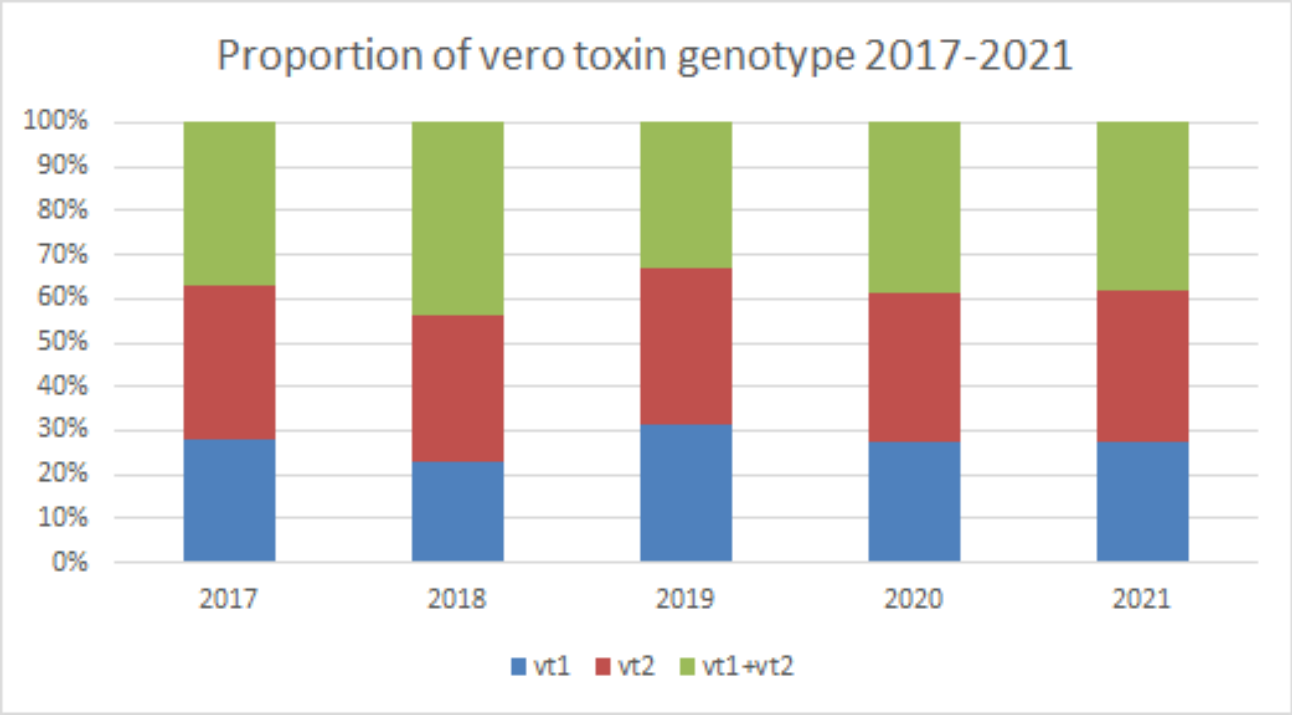



**Fig 3**: Verot**oxin genotypes Human VTEC isolates 2017-2021**

**Table 4: Toxin subtypes Human VTEC isolates 2017-2021**

| Year | Toxin genotype | *vtx1a* | vtx1c | vtx1d | *Vtx1a+c* | vtx2a | vtx2b | vtx2c | vtx2d | vtx2e | vtx2f | vtx2g | *Vtx2a+2c* | *Vtx2a+2d* | *Vtx2b+2c* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2017 | ***vtx1*** | 172 | 23 | 1 | 1 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | ***vtx1+2*** | 263 | 21 | 0 | 0 | 204 | 29 | 48 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| | ***vtx2*** | -- | -- | -- | -- | 201 | 12 | 16 | 0 | 4 | 1 | 0 | 2 | 0 | 0 |
| 2018 | ***vtx1*** | 147 | 31 | 1 | 0 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | ***vtx1+2*** | 406 | 31 | 0 | 0 | 268 | 45 | 123 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | ***vtx2*** | -- | -- | -- | -- | 189 | 17 | 28 | 10 | 2 | 1 | 1 | 0 | 0 | 1 |
| 2019 | ***vtx1*** | 176 | 33 | 3 | 0 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | ***vtx1+2*** | 217 | 23 | 0 | 0 | 160 | 40 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ***vtx2*** | -- | -- | -- | -- | 170 | 12 | 24 | 8 | 8 | 0 | 2 | 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 |
| | ***vtx2*** | -- | -- | -- | -- | 171 | 12 | 8 | 3 | 2 | 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 |
| | ***vtx2*** | -- | -- | -- | -- | 169 | 14 | 23 | 8 | 1 | 4 | 4 | 1 | 0 | 0 |

**Note:** Data for culture positive cases only

**VTEC isolates from Water**

In the period from 2017 to 2021 VTEC was isolated from 51 water samples (table 5). 39/51 (76%) were from private wells, 2/51 were labelled as potable water with source not defined, 1/51 was rain water, 1/51 was a bathing water, 1 river water and 7/51 water type was unspecified. Between 7-15 water samples were positive for VTEC each year, there were a variety of serogroups detected, VTEC O157 (27%) and VTEC 0136 (27%) accounting for 14 samples each, VTEC O26 was isolated from just 3 samples. However VTEC O136 accounted for only 8 (0.2%) of clinical cases. But as it was the joint most prevalent serogroup in water, we compared the VTEC O136 sequences from water and clinical isolates along with VTEC O136 from meat, milk and horticulture samples (n=11) held in the Dept. of Agriculture Food & Marine VTEC Database at Backweston laboratories. However there was no cluster linkage between the water, clinical, milk or meat isolates. (Fig 4 below; provided by Dr Brian Byrne, Dept. of agriculture Backweston). Thus the small number of clinical VTEC O136 cases do not appear related to contaminated water exposure. This correlates with the clinical cases originating from an urban setting and the water samples submitted from a rural setting. Most contamination of private wells is caused by run off from agricultural land, and O136 is a common VTEC serogroup in cattle. However, there was no evidence here to support contamination of water supplies by cattle. These trends in VTEC O136 need to be monitored closely going forward.

47/51 of the water samples submitted were linked to clinical VTEC samples submitted contemporaneously and WGS comparison performed. In approximately half of the instances where VTEC was isolated from water, the serogroup was different from that of the clinical case. This may indicate intermittent dynamic VTEC contamination of the water. Where the VTEC serogroup isolated from water matched that of clinical cases, in general they were genetically related (Fig 5).

**Table 5: VTEC Serogroup of water isolates 2017-2021**

| Serogroup | 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 |
| O146:H21 | 1 | | | | 1 |
| O15:H16 | | 1 | | | |
| O157:H7 | 1 | 4 | 3 | 4 | 2 |
| O165:H25 | | 1 | | | |
| O168:H8 | 2 | | 1 | | |
| O26:H11 | 1 | 1 | | | 1 |
| O8:H21 | | | | 1 | |
| O8:H28 | 1 | | | | |
| O84:H2 | 1 | | | | |
| Grand Total | 8* | 15 | 9 | 10 | 10 |

*8 VTEC organisms isolated from 7 water samples

**Table 6: Verotoxin genotypes and subtypes of water isolates 2017-2021**

| Toxin genotype | Number | ***vtx1a*** | **vtx1c** | **vtx2a** | **vtx2c** | **vtx2d** | **vtx2g** |
|---|---|---|---|---|---|---|---|
| *vtx1* | 7 | 5 | 2 | | | | |
| *vtx2* | 13 | 13 | | 8 | 4 | | |
| Vtx1+2 | 32 | | | 23 | 1 | 2 | 5 |
| Total | 52* | 18 | 2 | 31 | 5 | 2 | 5 |

*52 isolates from 51 water samples

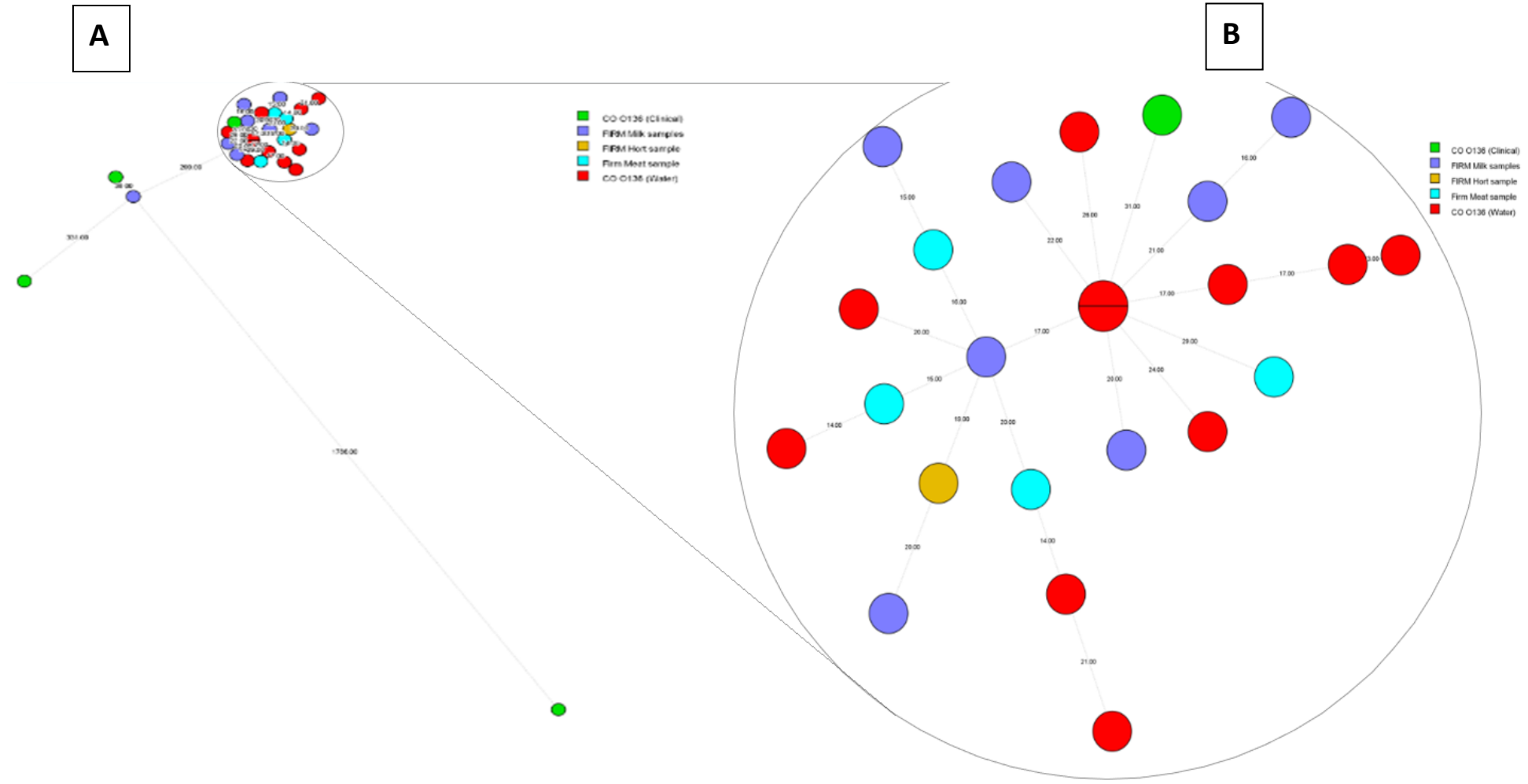
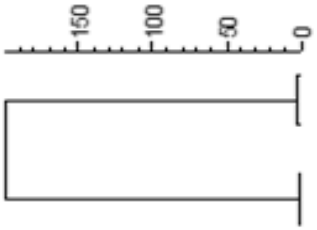



**Fig 4: (A) Minimum spanning Tree of all VTEC O136 isolates. (B) Expanded branch excluding the 3 clinical genetically diverse isolates**




| Sample Type | Serogroup | Genotype | Sequence type |
|---|---|---|---|
| Stool | O157:H7 | *vtx2* | 11 |
| Water | O157:H7 | *vtx2* | 11 |
| Stool | O103:H2 | *Vtx1+2* | 17 |
| Water | O103:H2 | *Vtx1+2* | 17 |

**Fig 5: Dendrogram of WGS from clinical and water samples.**

2 O157 VTEC isolates from both clinical and water samples have just one allele difference. Similarly 2 O103 VTEC isolates had 0 allele differences. Isolates with ≤6 allele differences are likely to have been exposed to the same source or linked by person-to-person transmission.

**Whole Genome Sequencing**

Between 2017 and 2021 >3000 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 sequence generated must pass multiple QC parameters including coverage (50X), core percent and N50 among others. Once a sample has passed QC cgMLST is performed and the new sample compared to the existing database. Where clusters are observed a cluster report is distributed immediately. Between 2017 and 2021 57 VTEC WGS reports were issued, there 2 types of reports; 1: An outbreak report, where a cluster is noted among isolates that are clustered in time and/or location and are therefore likely to be an outbreak, and a 2: a surveillance note where an emerging cluster is observed over time, these could reflect the emergence of a clonal group or could indicate a persistent reservoir in the environment.

Figs 5, 6, 7, and 8 show dendrograms of VTEC isolates generated from cgMLST data, Fig 5 shows all VTEC isolates from 2017-2021, isolates are coloured by serogroup, and it is evident that isolates cluster by serogroup. Figures 6, 7, and 8 show VTEC O157, O26 and O145 respectively, coloured by region of origin.

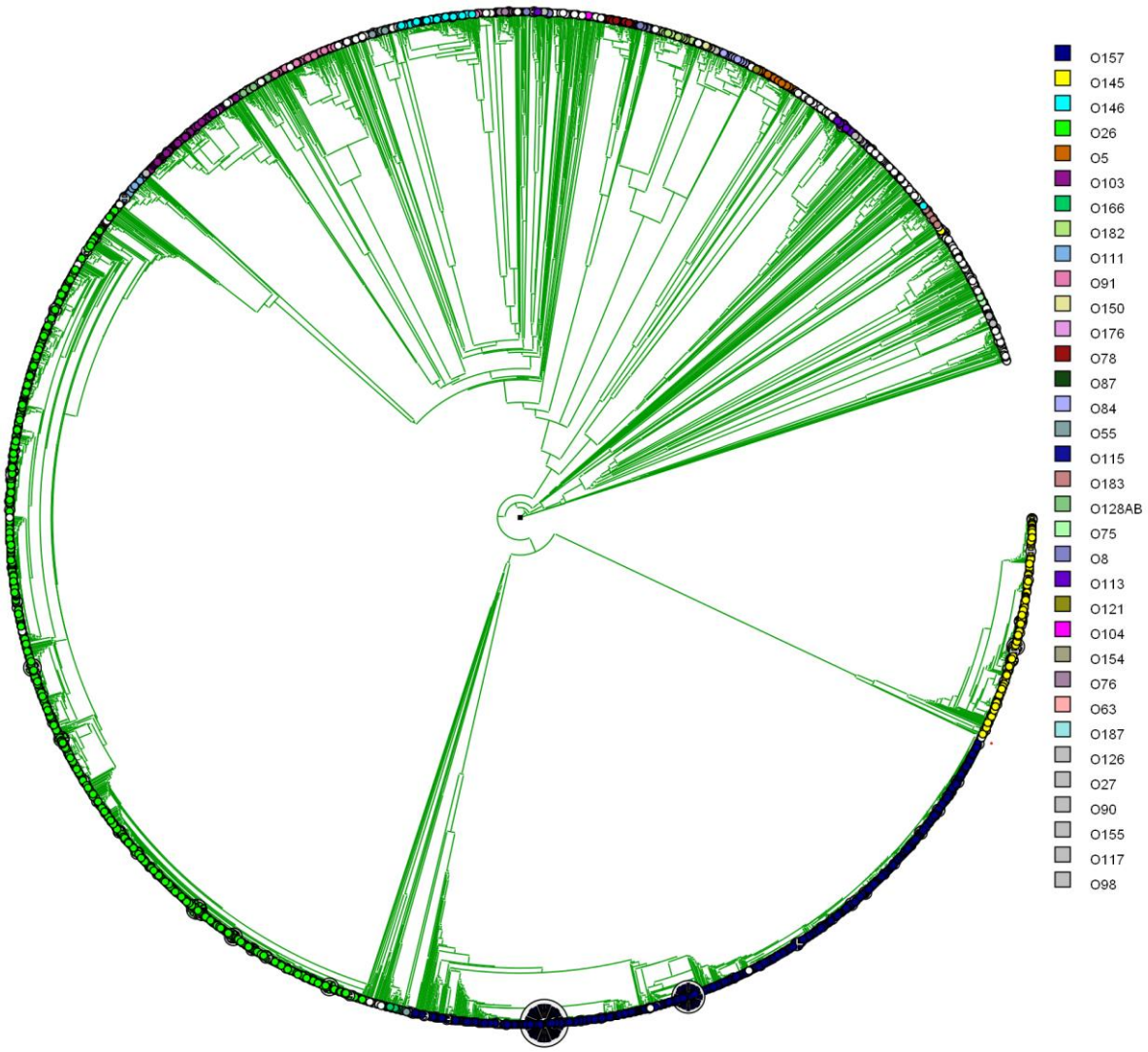



**Fig 5: cgMLST Dendrogram of all VTEC isolates 2017-2021 coloured by serogroup**

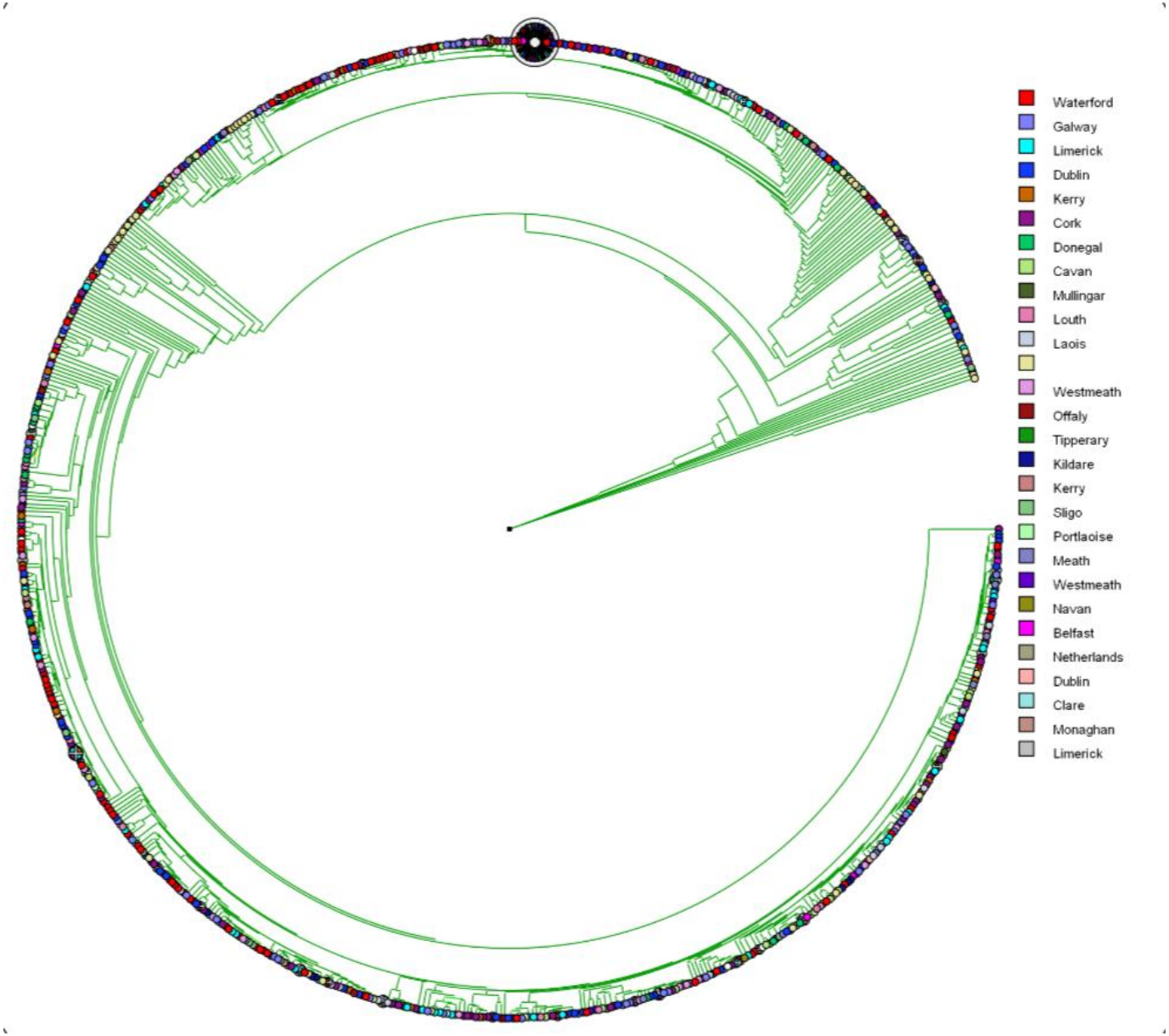



**Fig 6: cgMLST Dendrogram of VTEC O157 isolates 2017-2021, colour by area of residence**

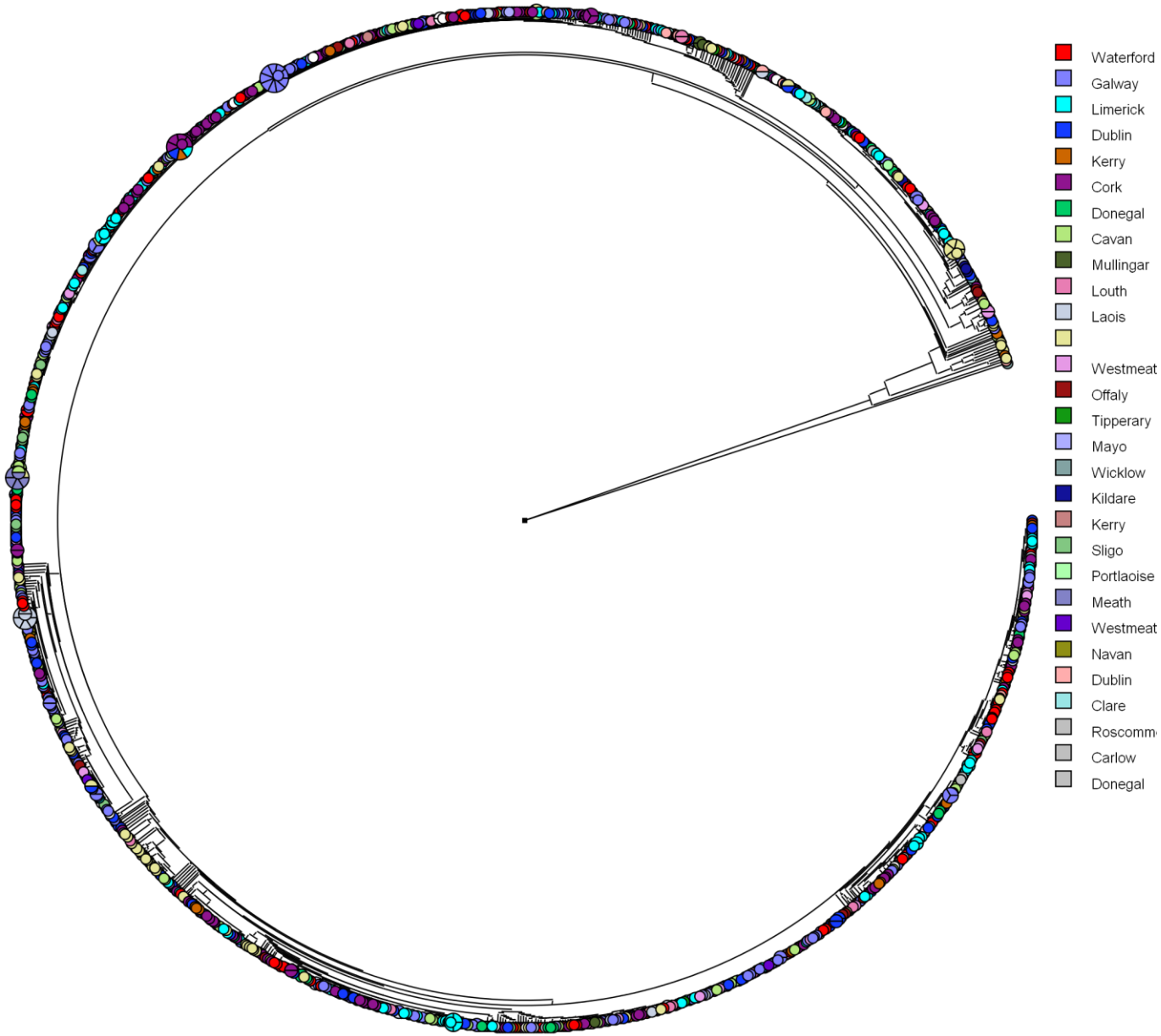



**Fig 7: cgMLST Dendrogram of VTEC O26 isolates 2017-2021, colour by area of residence**

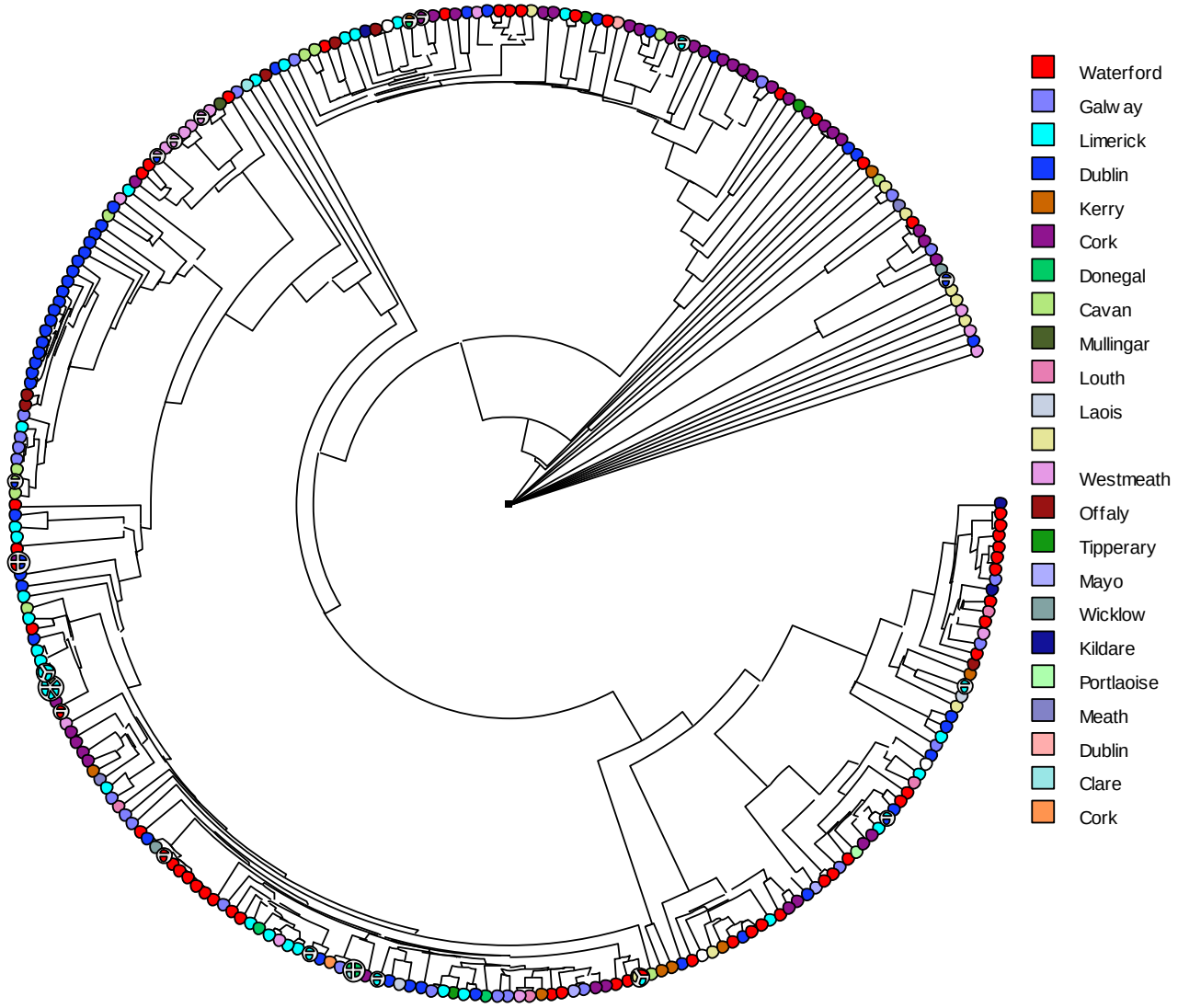



**Fig 8: cgMLST Dendrogram of VTEC O145isolates 2017-2021, colour by area of residence**