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NATIONAL REFERENCE CENTRE FOR SHIGA TOXIN/VEROTOXIN- PRODUCING *ESCHERICHIA COLI* (NRC STEC/VTEC)

ANNUAL REPORT 2022

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BACKGROUND

Shiga toxin-producing *Escherichia coli* (STEC) infections, also known as vero(cyto)toxin-producing *Escherichia coli* (VTEC) infections, are the cause of sporadic and epidemic watery or bloody diarrhoea worldwide. A small portion of patients, mainly children, develop hemolytic uremic syndrome (HUS) (Launders et al. 2016).

The main reservoir for STEC is cattle but other ruminants such as sheep, goats and deer can also be carriers of STEC. Transmission can occur through direct or indirect contact with animals or their environments, consumption of contaminated food or water, and person-to-person contact (Kintz et al. 2017).

STEC strains are classified based on their *E. coli* O:H-serotype (Orskov and Orskov 1984). STEC O157:H7/H- causes most of the STEC infections but other serotypes (termed non-O157) have also been associated with diarrheal disease worldwide. In the United States the 6 most common non-O157 groups are O26, O45, O103, O111, O121 and O145 (Gould et al. 2009). However, in Belgium O45 is very rare.

STEC's main pathogenic mechanism is the production of Shiga toxins (Stx) also called verocytotoxins (Vtx), which cause cell death by blocking the protein synthesis. The Stx family can be divided in two major antigenically distinct types, Stx1 and Stx2, which show distinct immunogenic and genetic properties. Stx variants are presently organized into a taxonomic system of three Stx1 (a, c, d and e) and seven Stx2 (a, b, c, d, e, f, and g) subtypes. Yet, novel Stx1e and Stx2 (h, i, j, k, l, m, n and o) subtypes have recently been described (Bai et al. 2021; Yang et al. 2020; Hughes et al. 2019). STEC strains can produce either only one Stx or a combination of different Stx subtypes. The Stx-coding genes (*stx*) are carried by bacteriophages that can easily be lost or acquired by horizontal transfer (Scheutz 2014; Scheutz et al. 2012). Determination of the Stx variants is clinically relevant as some Stx2 subtypes – *stx2a* and *stx2d* – seem to be more often associated with severe human illness, especially when they are present in combination with the *eae* virulence gene coding for intimin (Brandal et al. 2015; Werber and Scheutz 2019).

Since the outbreak of STEC/enteroaggregative *E. coli* (EAEC) O104:H4 in Germany in 2011 (Buchholz et al. 2011), the so-called cross-pathotype or hybrid strains are emerging. Besides STEC/EAEC, other hybrid STEC isolates have been reported, such as STEC/extraintestinal pathogenic *E. coli* (ExPEC) (Cointe et al. 2021), STEC/enterotoxinogenic *E. coli* (ETEC) (Bai et al. 2019) and STEC/uropathogenic *E. coli* (UPEC) (Gati et al. 2019).

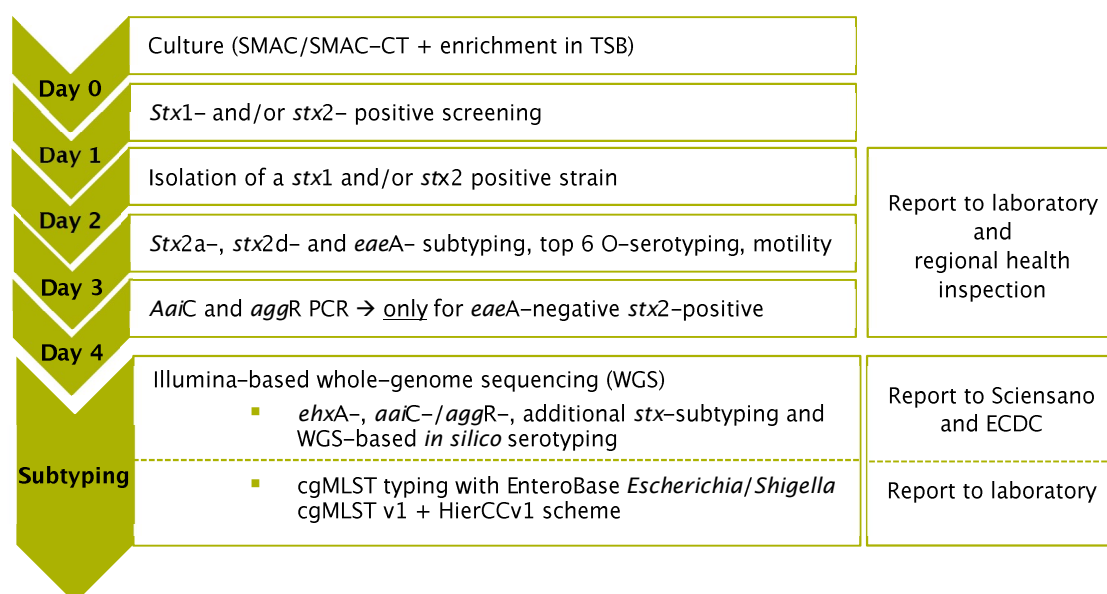
This report summarises the typing data of human STEC strains reported to the Belgian NRC in 2022 and compares it to previous years¹. It is to be noted that the NRC STEC only reports cases of STEC infection from whom STEC was isolated (see page 3), toxin production genes were directly detected in faeces (HUS cases only) or *E. coli* serogroup-specific (lipopolysaccharide (LPS)) antibody response was observed (HUS cases only) (see page 10). The NRC STEC does not report non-HUS cases from whom toxin production genes were directly detected in faeces without STEC isolation as per EU decision 2018/945.

¹ For more information, please contact us on lmiknrc@uzbrussel.be.

WHOLE-GENOME SEQUENCING FOR CHARACTERIZATION OF STEC AT THE NRC STEC

WGS has been implemented at the NRC in 2019. The method is performed on all STEC strains provided to the NRC, replacing the traditional quarterly subtyping methods (Figure 1). In addition, the sequencing data is analysed using the *Escherichia/Shigella* cgMLST v1 + HierCCv1 typing scheme in EnteroBase (Zhou et al. 2020). This analysis enables confirmation of epidemiologically linked cases on the one hand and clustering of cases that were not identified based on traditional typing data complemented with epidemiological data on the other hand. The cluster threshold is set at hierarchical clustering level HC5, i.e. all strains in this cluster have links no more than 5 alleles apart. The sequences are available in EnteroBase². To that end, a search can be performed by clicking the **Field** dropdown and selecting *Lab Contact*; and typing in *Laboratory of Microbiology, UZ Brussel* in the **Value** field.

Figure 1: Screening and virulence typing algorithm used at the Belgian NRC STEC from 2019 onwards



² <https://enterobase.warwick.ac.uk/>

NUMBER OF STEC STRAINS ANNUALLY ISOLATED

Figure 2 gives an overview of the number of STEC strains annually identified at the NRC STEC from 2008 to 2022. On average 108 strains were identified each year (min. 81 (2020) – max. 179 (2022)). In 2020, as for other gastrointestinal pathogens, a decrease in number of STEC strains was observed probably due to the COVID-19 pandemic.

In 2022, 179 different STEC strains were isolated from 177 Belgian patients (Table 1). The implementation of gastrointestinal molecular panels by a number of clinical laboratories has probably led to the increase in number of STEC observed.

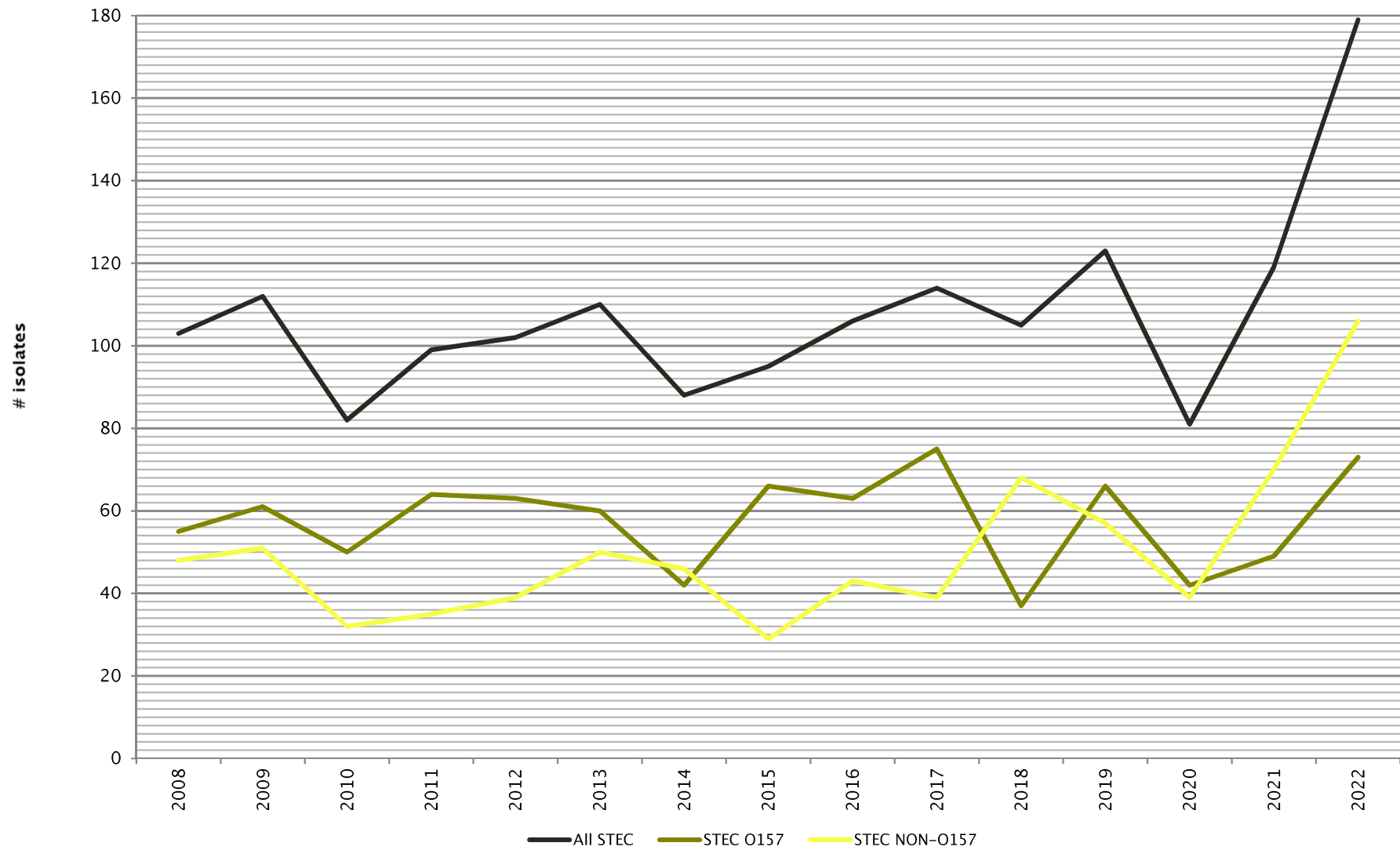
Four cases, including two cases of HUS, died in 2022. The serotypes of the isolated STEC strains were O26:H11 (2-year-old HUS case), O80:H2 (59-year-old HUS case), O157:H- (85-year-old case of bloody diarrhoea) and O177:H25 (90-year-old case of diarrhoea).

One hundred thirty-three of them were typical STEC (*eaeA*+, *hlyA*+) . Forty-six isolates were atypical STEC; lacking one or both of these virulence determinants. One strain with serotype O136:H20 (*stx1c*, *eaeA*-negative) was found positive for EAEC virulence gene *aaiC* and negative for *aggR*.

Table1: Number of STEC strains annually identified at the NRC STEC in relation to the number of cases with STEC infection (2017–2022)

Total numbers	2017	2018	2019	2020	2021	2022
STEC strains	114	105	123	81	119	179
Culture-positive cases of STEC infection	112	104	122	78	119	177

Figure 2: Number of STEC strains annually isolated at the NRC STEC (2008–2022)



STEC SEROTYPES

Figure 2 shows the annual distribution of O157 and non-O157 strains. Fifty-three percent of the STEC strains typed at the Belgian NRC STEC between 1994 and 2022 were of the O157:H7/H- serotype (n=1183).

O-serogroups of 994 out of the 1065 STEC non-O157 could be determined, classifying them in 101 different O-serogroups and 4 additional O-genotypes (OgN9, OgN10, OgN15 and OgN-RK14)³ (Figure 3). The remaining 71 non-O157 strains could not be serotyped with the methods used at the moment of analysis⁴ (NT non-O157). It is to be noted that in 2021 one strain has been counted in as NT non-O157 as *stx2f* was not detected anymore after sub-cultivation for WGS.

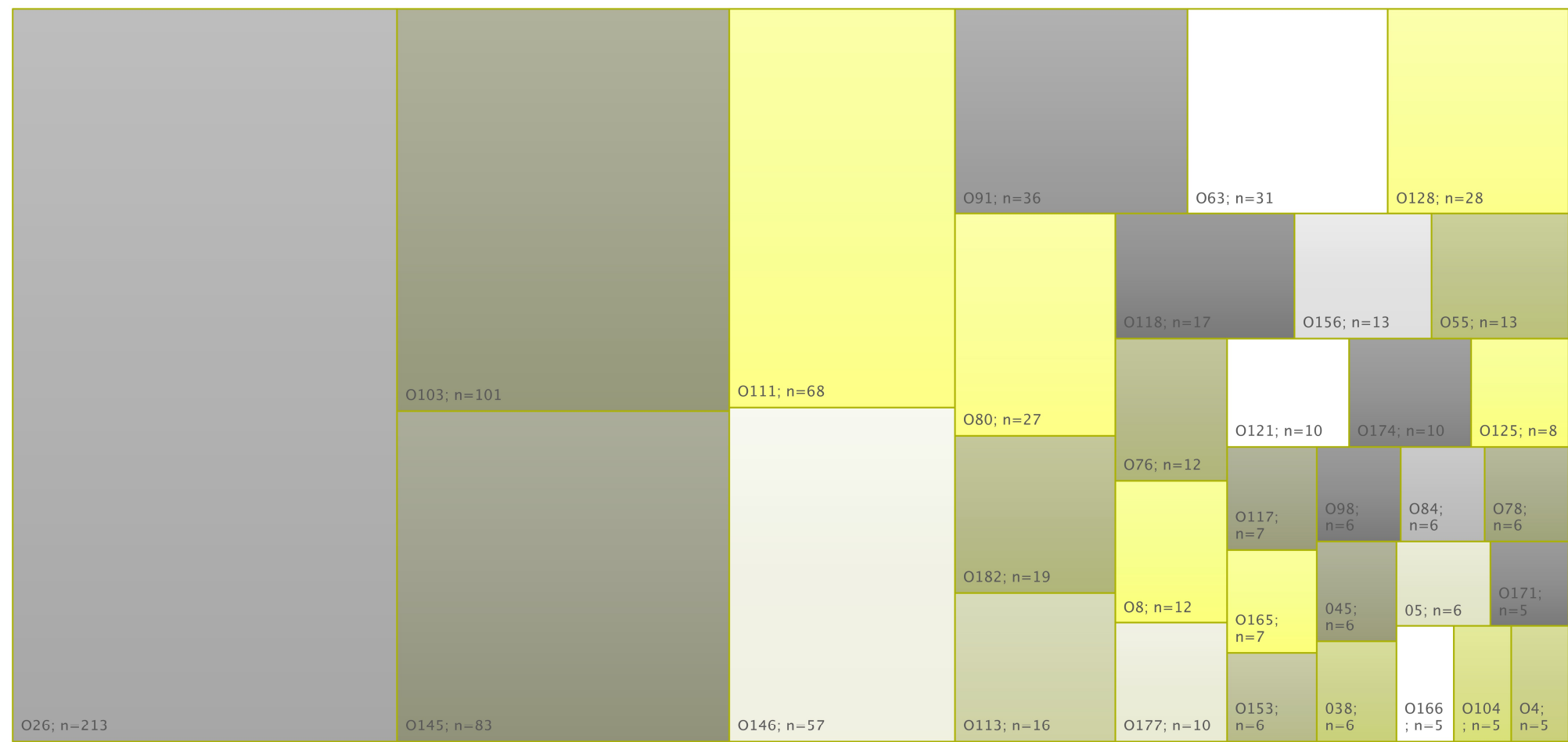
Figure 4 gives an overview of the evolution of the 6 most important serogroups worldwide, also called the “pathogenic gang of 6”: O26 (n_{total}=213), O103 (n_{total}=101), O111 (n_{total}=68), O121 (n_{total}=10), O145 (n_{total}=83) and O157 (n_{total}=1183). Additionally eight other non-O157 serogroups have been isolated frequently over the years: O63 (n_{total}=31), O80 (n_{total}=27), O91 (n_{total}=36), O113 (n_{total}=18), O118 (n_{total}=17), O128 (n_{total}=28), O146 (n_{total}=57) and O182 (n_{total}=19). Interestingly, O118 was detected regularly in the early years of screening but has not been found from 2010 to 2016 and only once in 2017. Serogroups O63, O80 and O182 have only been isolated since 2008 and 2003, respectively. In 2008, primers for the detection of *stx2f* were added to the STEC PCR screening assay and all STEC O63 isolated at our NRC carry the *stx2f* gene, explaining why this serotype was not detected the years before. Seventeen STEC O80 strains were isolated from 2018 to 2022 while only nine were detected at the NRC between 2008 and 2016 (De Rauw et al. 2019). The identification of STEC O80:H2 is of importance as this hybrid pathotype has recently emerged in France and in other European countries and has been associated with HUS as well as HUS associated with bacteraemia (Ingelbeen et al. 2018; Nuesch-Inderbinen et al. 2018; Rodwell et al. 2021; Cointe et al. 2021).

In 2022, the majority (73/179; 40.8 %) of the STEC strains belonged to serogroup O157 (serotype O157:H7/H-). Four of the ‘top 5’ non-O157 serogroups were represented: 24 O26 serogroup, 7 O103 serogroup, 9 O111 serogroup and 11 O145 serogroup (Figure 4).

³O-genotyping was performed by Dr. Lang and Dr. Fruth at the Robert Koch Institute as described in Lang et al. (2019).

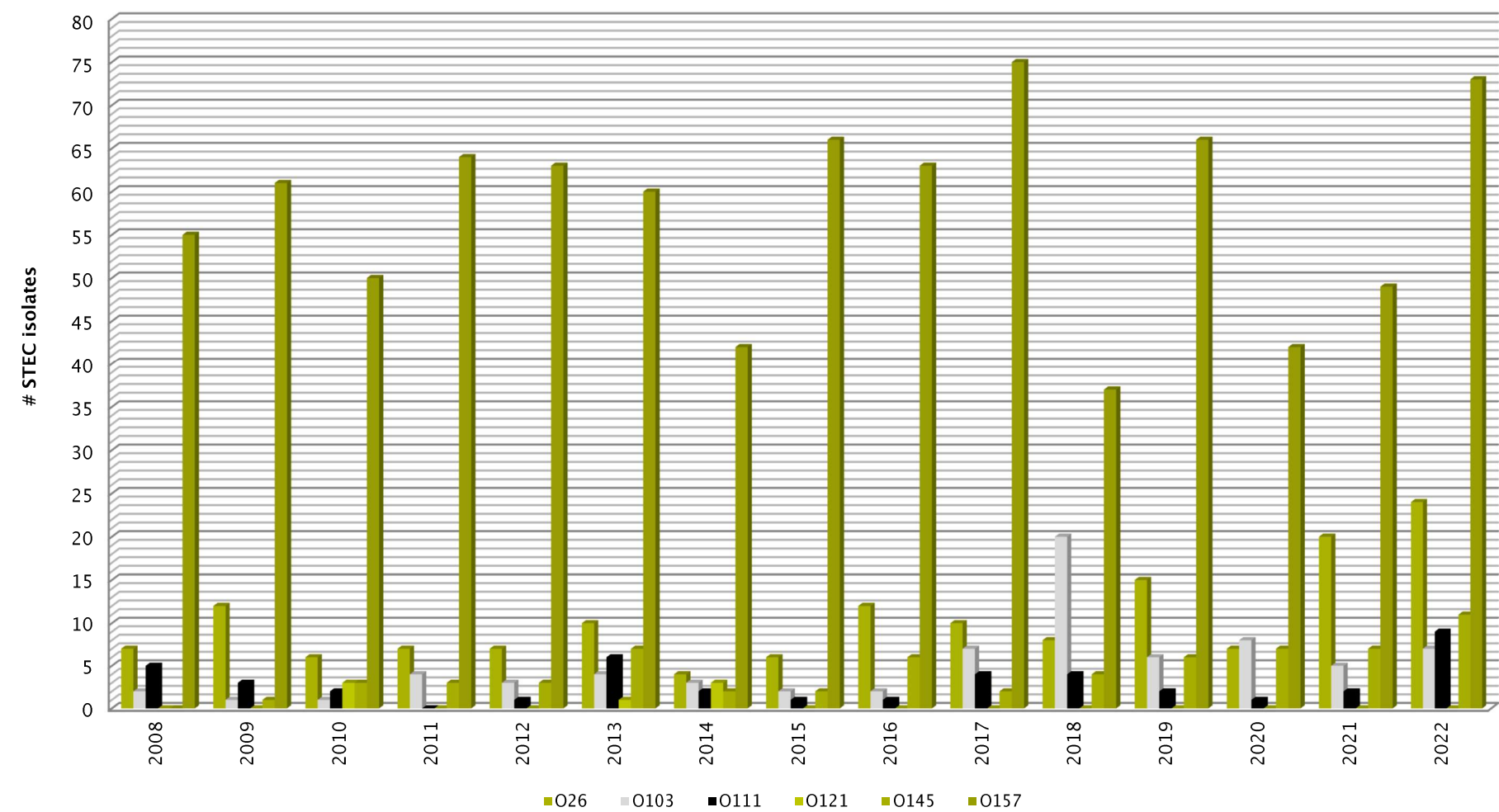
⁴*Gnd*-sequencing for O-typing from 2015 to 2018 (Gilmour et al. 2007); WGS-based *in silico* serotyping for O:H-typing from 2019 to present (*E.coli* functional genotyping plugin in BioNumerics v.8.1. (Applied Maths, BioMérieux, France)). In 2022, WGS has been performed retrospectively on all NT non-O157 strains isolated between 2011 and 2018 (n=48). Therefore, the numbers of NT non-O157 differ from previous annual reports.

Figure 3: Occurrence of the STEC non-O157-serogroups (1994–2022)⁵



⁵ Serogroups detected ≤ 4 times, including NT non-O157, are not included in Figure 3.

Figure 4: Annual distribution of the ‘pathogenic gang of six’ (2008–2022)



SHIGA TOXIN SUBTYPES

In 2022, 48.0 % of the isolates were *stx2* positive (86/179), 19.6 % (35/179) were *stx1* positive and 32.4 % were *stx1* and *stx2* positive (58/179). The majority of the STEC strains were at medium (41,3 %) or high (39,1 %) risk for developing HUS (Table 2) (De Rauw et al. 2018). The predominating *stx* subtype profiles were: *stx1a* (30), *stx1a+stx2a* (19), *stx1a+stx2c* (27), *stx1c+stx2d* (11), *stx2a* (38), *stx2c* (19) and *stx2f* (10) (Table 3). The most common *stx* subtype profile, *stx2a* alone, was harboured by four different serotypes, with O157:H7/H–predominating (n=15/38; 39.5 %) (Table 4).

Table 2: Risk classification for the development of HUS among STEC isolates (2017–2022)

Risk for HUS development	2017	2018	2019	2020	2021	2022
High	52	59	62	30	49	70
Medium	44	23	42	37	51	74
Low	18	23	19	14	19	35
TOTAL	114	105	123	81	119	179

Table 3: Shiga toxin subtype profiles detected among STEC isolates (2017–2022)

<i>Stx</i> subtype	2017	2018	2019	2020	2021	2022
<i>stx1a</i>	16	21	17	13	16	30
<i>stx1c</i>	1	2	2	1	3	5
<i>stx1d</i>	1	0	0	0	0	0
<i>stx1a+stx2a</i>	25	22	23	7	12	19
<i>stx1a+stx2a+stx2c</i>	0	4	0	0	0	0
<i>stx1a+stx2b</i>	4	2	1	2	2	1
<i>stx1a+stx2c</i>	19	12	26	24	31	27
<i>stx1a+stx2d</i>	0	1	1	0	0	0
<i>stx1c+stx2b</i>	3	2	2	2	1	11
<i>stx2a</i>	21	19	24	16	33	38
<i>stx2a+stx2c</i>	6	6	10	4	2	7
<i>stx2a+stx2d</i>	0	0	0	0	0	2
<i>stx2b</i>	0	1	3	2	2	3
<i>stx2b+stx2c</i>	0	0	0	0	0	1
<i>stx2b+stx2d</i>	0	0	0	1	0	1
<i>stx2c</i>	16	2	7	6	6	19
<i>stx2c+stx2d</i>	0	0	0	0	1	0
<i>stx2d</i>	0	7	4	2	1	3
<i>stx2e</i>	0	0	0	0	0	2
<i>stx2f</i>	2	4	3	1	9	10
TOTAL	114	105	123	81	119	179

Table 4: Number of *stx* subtype profiles reported amongst the top 10 STEC serogroups in Belgium, 2021–2022

<i>Serogroup</i>	<i>stx</i> subtype	2021	2022
O26	<i>stx1a</i>	4	10 ⁶
	<i>stx2a</i>	16	10 ⁷
	<i>stx1a+stx2a</i>	0	2
O63	<i>stx2f</i>	3	5
O80	<i>stx1a</i>	0	1
	<i>stx2a</i>	1	0
	<i>stx2d</i>	1	2
O91	<i>stx1a</i>	1	0
	<i>stx1a+stx2b</i>	2	1
O103	<i>stx1a</i>	4	6 ⁸
	<i>stx1a+stx2a</i>	1	1
O111	<i>stx1a</i>	0	5 ⁹
	<i>stx1c</i>	1	0
	<i>stx1a+stx2a</i>	1	4 ¹⁰
O128	<i>stx2b</i>	1	2
	<i>stx1c+stx2b</i>	0	1
O145	<i>stx2a</i>	7	8 ¹¹
	<i>stx2a+stx2c</i>	0	1
	<i>stx2a+stx2d</i>	0	1
	<i>stx2f</i>	0	1
O146	<i>stx1c+stx2b</i>	0	7 ¹²
O157	<i>stx1a+stx2a</i>	8	11 ¹³
	<i>stx1a+stx2c</i>	31	26 ¹⁴
	<i>stx2a</i>	4	15 ¹⁵
	<i>stx2a+stx2c</i>	2	4 ¹⁶
	<i>stx2c</i>	4	17 ¹⁷

⁶ Two isolate genomes within HC5_212630; two isolate genomes within HC5_212689.

⁷ Two isolate genomes within HC5_205177; two isolate genomes within HC5_220334.

⁸ Two isolate genomes within HC5_197304.

⁹ Three isolate genomes within HC5_212686.

¹⁰ Two isolate genomes within HC5_208219.

¹¹ Three isolate genomes within HC5_197308.

¹² Two isolate genomes within HC5_231652.

¹³ Six isolate genomes within HC5_111396; three isolate genomes within HC5_177415.

¹⁴ Four isolate genomes within HC5_220336; five isolate genomes within HC5_205173; two isolate genomes within HC5_205435; two isolate genomes within HC5_212684.

¹⁵ Three isolate genomes within HC5_116498 (2022–FWD–00032); two isolate genomes within HC5_117163; three isolate genomes within HC5_197305; two isolate genomes within HC5_205175.

¹⁶ Two isolate genomes within HC5_197303.

¹⁷ Two isolate genomes within HC5_212677.

HEMOLYTIC UREMIC SYNDROME

The annual total numbers of HUS cases reported to the NRC STEC from 2017 to 2022 are presented in Table 6. It is to be noted that this number is higher in 2022 compared to previous years (41 cases vs. an average of 23.6 cases over the years 2017–2021). This is an unexpected observation, as clinical laboratories are systematically sending samples from all HUS cases to the NRC over the years in order to exclude STEC associated HUS. This is of importance for the reimbursement of eculizumab for patients with atypical HUS.

Table 6: Number of cases in relation to HUS (2017–2022)

Total numbers	2017	2018	2019	2020	2021	2022
Cases of STEC infection	119	112	127	81	127	187
HUS cases	27	19	26	19	27	41
Culture-positive HUS cases	20	11	21	16	19	31
Percentage of HUS cases	22,7	17,0	20,5	23,5	21,3	21,9
UNK clinical status	36	14	18	17	18	9

As expected, the majority (84,3 %) of the STEC strains isolated from HUS cases were at high risk for developing HUS (Table 7).

Table 7: Risk classification for the development of HUS among STEC isolates of HUS cases (2017–2022)

Risk for HUS development	2017	2018	2019	2020	2021	2022
High	16 ^a	9 ^a	20	13 ^{c,d}	17	27 ^e
Medium	2	0	1	3	2	3
Low	3 ^a	3 ^b	0	2 ^{c,d}	1	2 ^e
TOTAL	21	12	21	18	19	32

^aCo-infection with two STEC strains (low risk O145 *stx1a* and high risk O157 *stx2a*); ^bCo-infection with two STEC strains (low risk O103 *stx1a* and high risk O111 *stx1a+stx2a*); ^cCo-infection with two STEC strains (low risk O103 *stx1a* and high risk O103 *stx1a+stx2a*); ^dCo-infection with two STEC strains (low risk O98 *stx1a* and high risk O157 *stx2a*); ^eCo-infection with two STEC strains (low risk O26 *stx1a* and high risk O145 *stx2a*).

Table 8 gives an overview of the STEC serotypes detected in Belgian patients suffering from HUS from 2017 until 2022. Every year the majority of HUS cases was associated with STEC O157, except in 2021. That year, the majority of the HUS cases was associated with O26:H11. Out of the 12 O26:H11 HUS cases, three cases belonged to cluster HC5 168530 and seven cases to cluster HC5 177396 (2021-FWD-00080).

In 2022, 31 STEC strains were isolated from the faecal samples of 30 HUS cases: 11 STEC O157:H7/H–, 10 STEC O26:H11, 4 O145:Hunk and 6 different non-O157. One HUS patient was contaminated with two STEC strains (O26:H11 *stx1a* and O145:Hunk *stx2a*). In addition, a STEC O80:H2 strain was only isolated from blood of an HUS case. The *stx* subtype profiles found in these 32 strains were *stx1a* (2), *stx1a+stx2c* (7), *stx1c+stx2b* (1), *stx2a* (15), *stx2c* (2), *stx2d* (2) and *stx2a+stx2c* (3). The subtype profile *stx2a* alone was detected in four serotypes including O26:H11 (n=7), O45:H2 (n=1), O145:Hunk (n=3) and O157:H7 (n=4).

Additionally, STEC infection could be confirmed in one HUS patient by detection of antibodies against STEC O LPS (O145) in the serum sample only and in nine HUS patients by detection of *stx1* (n=1), *stx2* (n=6), *stx2f* (n=1) or *stx1+stx2* (n=1) in stool samples without isolation of the STEC strain.

Table 8: Number of HUS-associated STEC serotypes (2017–2022)

Characteristic	2017	2018	2019	2020	2021	2022
O2/O50:H6^a						1
O26:Hunk	3	0	0	0	0	0
O26:H11		2	4	4	12	8
O26:H11 + O145:Hunk^b						1
O26:H11 + O111 serology^c						1
O26 serology^d	1	0	0	0	0	0
O45:H2			1	0	0	1
O55:H7	0	0	0	0	0	0
O55:H12		1	0	0	0	0
O61:H-	0	0	0	0	0	0
O63:H6	0	0	0	0	0	0
O74:H42			1	0	0	0
O80:H2	0	0	2	0	0	1
O80:H2 + O26 serology^c			1	0	0	0
O103:Hunk	1	3	0	0	0	0
O103:H2				1 ^g	0	1
O103:Hunk + O111:H-^b		1	0	0	0	0
O103 serology^d					1	0
O104:H4^e	0	1	0	0	1	0
O111:H8						1
O111 serology^d	0	0	1	0	0	0
O113:H4				1	0	1 ^h
O145:Hunk/H-	1	0	1	5	1	3
O145 serology^d			1	1	0	1
O150:H2			1	0	0	0
O156:H25	1	0	0	0	0	0
O157:H7/H-	12	3	9	5	3	11
O157:H- + O98:H21^a				1	0	0
O157:H7 + O145:H-^a	1	0	0	0	0	0
O157 serology^c	2	3	1	0	1	0
O177:H25			1	0	1	1
OgN9:H2					1	0
Stx positive^f	4	5	2	2	6	9
TOTAL	27	19	26	19	27	41

^aO2/O50 are genetically nearly identical *wzx*/*wzy*-genes; *wzm*/*wzt*-genes; ^bCo-infection with two STEC strains; ^cIn these HUS patients STEC could be isolated from stool but the serogroup does not correspond to the STEC O LPS detected in the serum sample; ^dIn these HUS patients STEC O LPS was detected with antibodies in the serum sample, but no STEC could be isolated from stool; ^eEAEC–STEC; ^fThe sample was found positive for *stx* genes, but it was not possible to isolate a STEC strain. *Stx*PCR on non-cultivable faecal samples from HUS patients was routinely introduced in 2017; ^gTwo STEC O103 (*stx1a* and *stx1a stx2a*; cgMLST 150264) were isolated from the stool of a same patient; ^hVanesse et al. (2023) reported a severe clinical case of HUS with neurological involvement associated with STEC O113:H4 (Vanesse et al. 2023).

OUTBREAKS

STEC infections in Belgium are mainly sporadic, but over the years a few outbreaks have occurred. An overview of the number of STEC clusters based on molecular relatedness is presented in Table 9.

Table 9: Number of molecular clusters of STEC (2019–2022)

Serogroup	2019			2020			2021			2022		
	Total #	Isolate #	Range	Total #	Isolate #	Range	Total #	Isolate #	Range	Total #	Isolate #	Range
O26	1	4	4	1	2	2	2	12	5–7	4	8	2
O157	8	26	2–10	4	13	2–4	4	25	2–17	12	36	2–6
Other	2	6	3	2	9	4–5	1	2	2	5	12	2–3

The outbreaks that occurred in 2022 are briefly presented below (Figure 5 and Table 10).

STEC O26 – 2022, BELGIUM, FOUR SMALL CLUSTERS

May 2022, Namur

Two sibling cases of STEC **O26:H11 *stx2a eae*-positive** were reported to the NRC in May 2021. Both cases, aged 4 and 6 years old, developed HUS. The strains belonged to cgMLST **HC5|205177**. No source of contamination was found.

August 2022, West Flanders

Two sibling cases of STEC **O26:H11 *stx1a eae*-positive** were reported to the NRC in August 2022. Both paediatric patients suffered from diarrhoea. The strains belonged to cgMLST **HC5|212630**. The source of contamination could not be identified.

August –September 2022, East Flanders

Two child-care related cases of STEC **O26:H11 *stx1a eae*-positive** were reported to the NRC between August and September 2022. The case with known clinical manifestations developed diarrhoea. The strains belonged to cgMLST **HC5|212689**. No source of contamination was found.

October – November 2022, Limburg

Two sibling cases of STEC **O26:H11 *stx2a eae*-positive** were reported to the NRC between October and November 2022. Both paediatric patients developed HUS. The strains belonged to cgMLST **HC5|220334**. The source of contamination could not be identified.

STEC O103 – 2022, EAST FLANDERS AND FLEMISH FLANDERS

O103:H2 *stx1a* has been isolated from two cases with diarrhoea between April and May 2022 (cgMLST **HC5|197304**). The isolate genomes had 4 allelic differences (AD). The *eaeA* gene was not detected in one of the two isolates. No epidemiological link could be found between the cases.

STEC O111 – 2022, BELGIUM, TWO CLUSTERS

July 2022, Antwerp and Flemish Brabant

STEC O111:H8 *stx1a stx2a eae*-positive has been isolated from two cases in July 2022 (cgMLST HC5|208219). The paediatric case (<1 y) had bloody diarrhoea while the adult case (>65 y) was asymptomatic. No epidemiological link was found between the patients.

August –September 2022, Walloon Brabant

Three child-care related cases of STEC O111:H8 *stx1a eae*-positive were reported to the NRC between August and September 2022. Two out of the three paediatric cases had diarrhoea. The third case was asymptomatic. The strains belonged to cgMLST HC5|212686. The source of contamination could not be identified was found.

STEC O145 – 2022, HAINAUT AND WALLOON BRABANT

In March 2022, a STEC O145:H- *stx2a eae*-positive strain was isolated from stool of a 5-year-old case of HUS. One month later, based on cgMLST analysis, two additional isolates from two sibling cases were identified as closely related and belonged to cgMLST HC5|197308. The strains were isolated from a 2-year-old boy that developed HUS while his 6-year-old brother was asymptomatic. No epidemiological link could be found between the cases except for two sibling cases.

STEC O146 – 2022, WEST FLANDERS

Two cases of O146:H21 *stx1c stx2b eae*-negative were reported to the NRC in December 2022 (HC5|231652). Both cases had diarrhoea. Consumption of meat was identified as the most probable source of contamination.

STEC O157 – 2022, BELGIUM, SEVERAL CLUSTERS

In 2022, multiple clusters of STEC infection were detected in Belgium:

July – August 2022, Flemish Brabant and West Flanders

Three child-care related cases of STEC O157:H7 *stx1a stx2a eae*-positive were reported to the NRC in July 2022. Two cases developed HUS while the third case was asymptomatic. The cases were originating from West Flanders. Three additional isolates, with the same IS629-profile as the child-care-related cases, were identified at the end of July and the beginning of August. The three cases were from a same local authority in Flemish Brabant. Two cases, including a case of HUS, attended a girls camp while the other case was a cooking staff at the boys camp. There was no contact between the cases at both camps. An additional case of HUS attended the girls camp but no strain could be isolated. Based on cgMLST analysis, all six isolate genomes could be identified as being closely related and belonged to cgMLST HC5|111396. Interestingly, in September 2021, an outbreak of STEC O157:H7 (HC5|111396) linked to the consumption of cucumber salad was reported in France (2021-FWD-00077). The cucumbers were imported from Belgium. Based on this information, the cases were questioned again by the health inspectors. Cucumber could be identified as probable source of contamination as it was consumed by at least one child at the day-care centre and regularly at both the girls and the boys camp.

April – December 2022, Flemish Brabant, East Flanders and France

One case of STEC **O157:H7 *stx2a eae*-positive** could be related to a multi-country outbreak based on cgMLST analysis (cgMLST **HC5|116498**) (2022-FWD-00032). The Belgian case developed bloody diarrhoea mid-May. The patient did not travel before the start of the symptoms. France reported sixteen confirmed cases between April and May 2022. Halal food was suggested as possible vehicle of infection by the French health inspectors.

In December 2022, two additional HUS cases were identified based on cgMLST analysis. The isolate genomes are closely related to the French cluster representative with 4 AD. No epidemiological link could be found between the Belgian cases.

April 2022, East Flanders

Two cases of STEC **O157:H- *stx2a eae*-positive** were reported to the NRC In April 2022 (cgMLST **HC5|117163**). Both cases had diarrhoea. For the 2 years old case, consumption of turkey meat was identified as the most probable source of contamination. No epidemiological link could be found between the cases.

April – July 2022, Brussels–Capital Region and Flemish Brabant, Liège

O157:H7 *stx1a stx2a eae*-positive has been isolated from a 4-year-old case of HUS in April 2022 (cgMLST **HC5|177415**). Two additional strains, isolated from cases with bloody diarrhoea in July 2022, were identified with 4 AD compared to the first isolate. No epidemiological link was found between the patients.

April 2022, Flemish Brabant and Hainaut

Two cases of STEC **O157:H7 *stx2a stx2c eae*-positive** were reported to the NRC in April 2022 (cgMLST **HC5|197303**). One case 5-year-old case developed HUS whereas the other 2-year-old case had diarrhoea. No epidemiological link could be found between the cases.

March – August 2022, Limburg and Luxembourg

In March 2022, a 6-year-old case of HUS infected with STEC **O157:H7 *stx2a eae*-positive** was reported to the NRC. Two additional strains, isolated from adult cases with bloody diarrhoea in August 2022, were identified with 4 AD compared to the first isolate (cgMLST **HC5|197305**). No epidemiological link was reported between the patients.

May – July 2022, Flemish Brabant and West Flanders

Four cases of STEC **O157:H- *stx1a stx2c eae*-positive** were reported to the NRC between May and June 2022 (age range: 6 to 93 years old). The three cases with known clinical manifestations developed bloody diarrhoea. One additional strain, isolated from a 12-year-old case with bloody diarrhoea in July 2022, was identified with 5 AD compared to the cluster representative (cgMLST **HC5|205173**). No epidemiological link was reported between the patients.

May 2022, Liège

Two cases of STEC **O157:H- *stx2a eae*-positive** were reported to the NRC in May 2022 (cgMLST **HC5|205175**). One case 4-year-old case developed HUS. No epidemiological link could be found between the cases.

June 2022, Antwerp and East Flanders

In June 2022, two cases of bloody diarrhoea infected with STEC **O157:H- stx1a stx2c eae-positive** were reported to the NRC (cgMLST HC5|205435). No epidemiological link was reported between the cases.

August 2022, Antwerp

STEC **O157:H- stx2c eae-positive** has been isolated from two sibling cases in August 2022 (cgMLST HC5|212677). Both patients, 5 and 7 years of age, suffered from bloody diarrhoea. The source of contamination could not be identified.

August 2022, Antwerp and East Flanders

Two cases of STEC **O157:H- stx1a stx2c eae-positive** were reported to the NRC in August 2022 (cgMLST HC5|212684). None of the cases was hospitalized. No epidemiological link was reported between the cases.

September – November 2022, Antwerp, Flemish Brabant and West Flanders

Four cases of STEC **O157:H- stx1a stx2c eae-positive** were reported to the NRC between September and November 2022 (cgMLST HC5|220336). Two hospitalized cases, aged 55 and 69 years, had bloody diarrhoea. The clinical manifestations were not known for the other two cases, aged 2 (no HUS, hospitalization status unknown) and 8 years (HUS status unknown, hospitalized). No epidemiological link was reported between the cases.

Figure 5: Minimum spanning tree of cgMLST data from 179 Belgian STEC genomes analysed in 2022. *Clusters of at least two STEC cases are highlighted based on Hierarchical Clustering of cgMLST data. The analysis was carried out in EnteroBase, using the 'Create MLST GrapeTree' tool and selecting the Escherichia/Shigella cgMLST v1 + HierCC v1 scheme and the MSTree V2 algorithm. Nodes are colour-coded by HC5 cluster as labelled.*

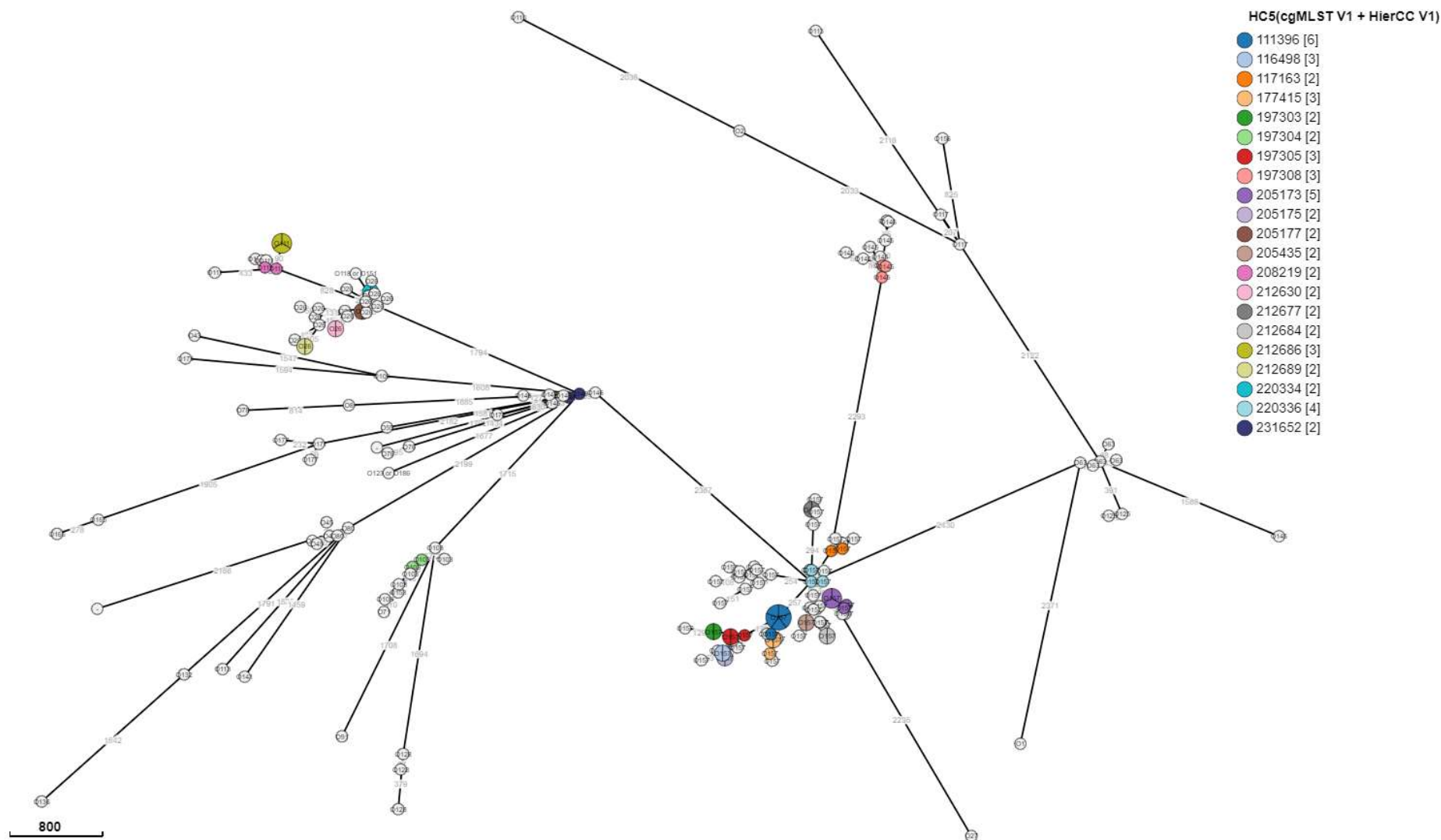


Table 10: Results obtained after traditional typing and Hierarchical Clustering of cgMLST (HierCC) of 179 STEC genomes analysed in 2022

Serotype	#HUS cases/ # cases	HierCC HC5	IS629 -type	Stx-subtype combination	Epidemiological link	Suspected source	Region
O26:H11	2/2	205177	/	<i>stx2a</i>	Familial	Not identified	Namur
O26:H11	0/2	212630	/	<i>stx1a</i>	Familial	Not identified	West Flanders
O26:H11	0/2	212689	/	<i>stx1a</i>	Child-care centre	Not identified	East Flanders
O26:H11	2/2	220334	/	<i>stx2a</i>	Familial	Not identified	Limburg
O103:H2	0/2	197304	/	<i>stx1a</i>	No link	Not identified	East Flanders and Flemish Flanders
O111:H8	0/2	208219	/	<i>stx1a stx2a</i>	No link	Not identified	Antwerp, Flemish Brabant
O111:H8	0/3	212686	/	<i>stx1a</i>	Child-care centre	Not identified	Walloon Brabant
O145:Hunk	2/3	197308	/	<i>stx2a</i>	No link/ familial link	Not identified	Hainaut, Walloon Brabant
O146:H21	0/2	231652	/	<i>stx1c stx2b</i>	No link	Meat	West Flanders
O157:H7	3 ^a /6	111396	AH	<i>stx1a stx2a</i>	Child-care centre /camp	Vegetable	Flemish Brabant, West Flanders
O157:H7	2/3	116498	B2	<i>stx2a</i>	No link	Not identified	Flemish Brabant, East Flanders
O157:H-	0/2	117163	BV	<i>stx2a</i>	No link	Turkey meat	East Flanders
O157:H7	1/3	117415	BU	<i>stx1a stx2a</i>	No link	Not identified	Brussels-Capital, Flemish Brabant, Liège
O157:H7	1/2	197303	R	<i>stx2a stx2c</i>	No link	Not identified	Flemish Brabant, Hainaut
O157:H7	1/3	197305	B2	<i>stx2a</i>	No link	Not identified	Limburg, Luxembourg
O157:H-	0/5 ^b	205173	O	<i>stx1a stx2c</i>	No link	Not identified	Flemish Brabant, West Flanders
O157:H-	1/2	205175	BX	<i>stx2a</i>	No link	Not identified	Liège
O157:H-	0/2	205435	AW	<i>stx1a stx2c</i>	No link	Not identified	Antwerp, East Flanders
O157:H-	0/2	212677	BY	<i>stx2c</i>	Familial	Not identified	Antwerp
O157:H-	0/2	212684	AW	<i>stx1a stx2c</i>	No link	Not identified	Antwerp, East Flanders
O157:H-	0/4 ^c	220336	AW	<i>stx1a stx2c</i>	No link	Not identified	Antwerp, Flemish Brabant, West Flanders

HierCC HC5: hierarchical cluster level HC5, which includes all strains with links no more than 5 alleles apart – other than missing data; HUS: hemolytic-uremic syndrome; ^a1 additional case of HUS attending a camp but no strain isolated; ^bone 85-year-old case of diarrhoea died; ^cHUS status of one hospitalized case is unknown.

An overview of the international clusters of STEC based on molecular relatedness is presented in Table 11.

Table 11: International clusters of STEC in 2022

Serotype	Stx-subtype combination	HierCC HC5	Country	# of confirmed cases	Suspected source	EpiPulse ID
O26:H11	<i>stx2a</i>	204886	France	1 BE + 1 FR	Not identified	/
O26:H11	<i>stx2a</i>	211372	United Kingdom	1 BE + 1 UK	Turkey	/
O156:H7	<i>stx1a</i>	215555	United Kingdom	1 BE + 3 UK	Not identified	/
O157:H7	<i>stx2a</i>	116498	France (16)	3 BE + 16 FR	Halal food	2022-FWD-00032
O157:H-	<i>stx1a stx2c</i>	211462	United Kingdom	1 BE + 2 UK	Turkey	/

HierCC HC5: hierarchical cluster level HC5, which includes all strains with links no more than 5 alleles apart – other than missing data; HUS: hemolytic-uremic syndrome.

CONCLUSION

The number of STEC infections identified at the NRC STEC in 2022 increased greatly compared to 2021. This increase might be due to the implementation of gastrointestinal molecular panels, enhanced by the COVID-19 pandemic, by clinical laboratories that did not routinely test for STEC before.

Out of the 179 isolates, 40.8 % belonged to the O157:H7/H- serotype with O26 as second most detected (13.4 %). While O157 remains stable compared to other years, an increase in non-O157 has been observed in 2022. Though most of the infections were sporadic, multiple small clusters of STEC, mainly O157, have been observed.

Finally, an increased total number of HUS cases reported to the NRC STEC was observed in 2022. This unexpected observation cannot be related to the implementation of gastrointestinal molecular panels as clinical laboratories are systematically sending samples from all HUS cases to the NRC over the years.

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