

Laboratory of Microbiology and Infection Control
UZ Brussel

National Reference Centre for Shiga toxin/verotoxin- producing *Escherichia coli* (STEC/VTEC)

Annual report 2015

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1. NUMBER OF CONFIRMED STEC STRAINS

Figures 1 and 2 give an overview of the number of STEC strains annually identified at the NRC STEC/VTEC. From 1994 until 2007 an average of 45 (min. 27 (1994) – max. 53 (1999)) strains were identified each year. Since 2008 the yearly number of isolates increased more than twice, to a mean of 99 (min. 82 (2010) – max. 112 (2009)). We believe this increase in STEC isolates since 2008 can be partly explained by external factors. From 2008 until 2010 a research project was performed to study the incidence of STEC infections in the Brussels-Capital Region (Buvens *et al.* 2012). Furthermore in 2008 additional primers were added to our VTEC screening PCR assay to target the Shiga toxin (Stx) subtype 2f, which could not be detected before. In 2011 the Belgian National Reference Centers for human microbiology were officially established, leading to more samples for diagnosis being send to us by external laboratories. From May to July 2011, a large outbreak of STEC infections with a serotype that was reported only very rarely before, O104:H4, occurred in Germany. This exceptionally virulent strain combined virulence factors of enteroaggregative *E. coli* (EAggEC) and STEC making it a mixed enteroaggregative and Shiga toxin (Stx)- producing *E. coli* (“Agg-STECC”). (Frank *et al.* 2011) In order to intercept these highly virulent hybrid strains, all STEC isolated at the NRC are tested for the presence of the *aaiC* and *aggR* genes typical for EAggEC. In the previous years 2 Agg-STECC O104:H4 were detected, unrelated to the 2011 outbreak, 1 in 2012 and 1 in 2013. (De Rauw *et al.* 2014)

In 2015, 95 STEC strains were identified. 82 of them were ‘typical’ enterohemorrhagic *E. coli* (EHEC) isolates; *eg.* STEC positive for the additional virulence genes *eaeA* en *hlyA*. The *eaeA* gene codes for the virulence factor intimin which is correlated to the production of attaching and effacing (A/E) lesions. The *hlyA* gene codes for enterohaemolysin and is part of the EHEC virulence plasmid. 13 were atypical STEC; lacking one or both of these virulence determinants. In 2015, no STEC strain was found positive for *aaiC* and *aggR*.

Figure 1: Number of STEC strains annually identified at the NRC STEC/VTEC

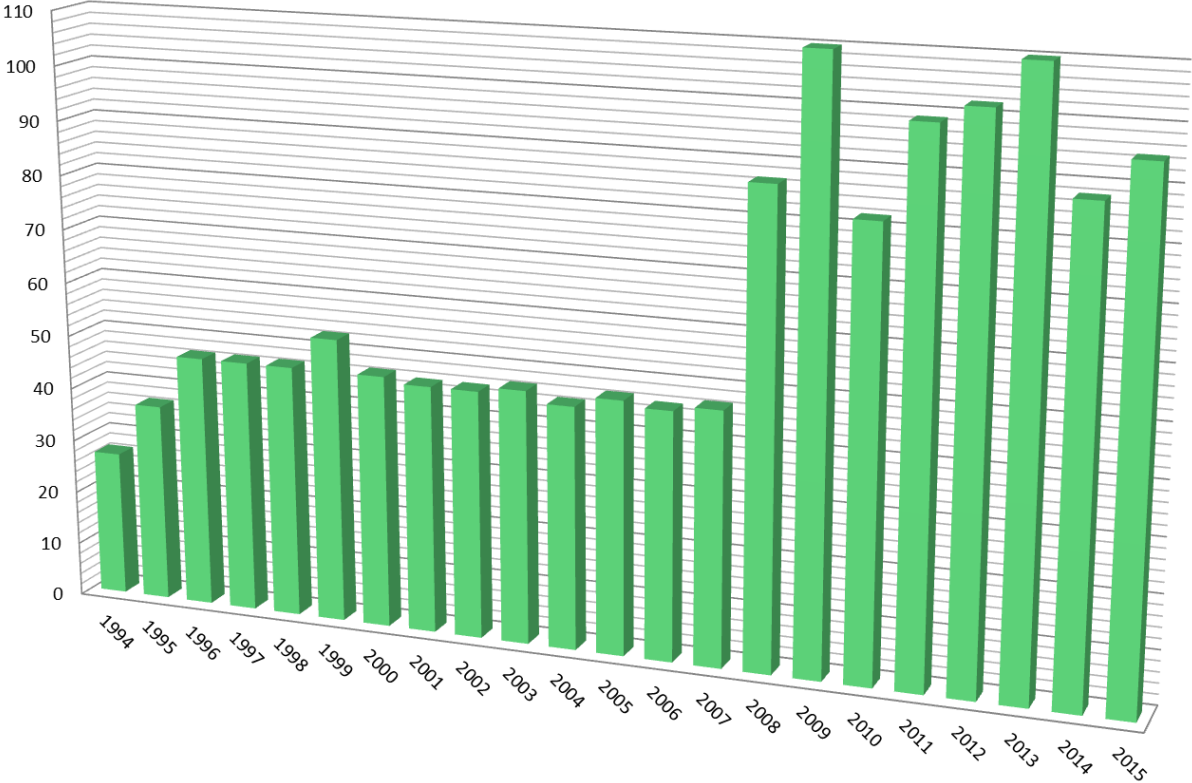
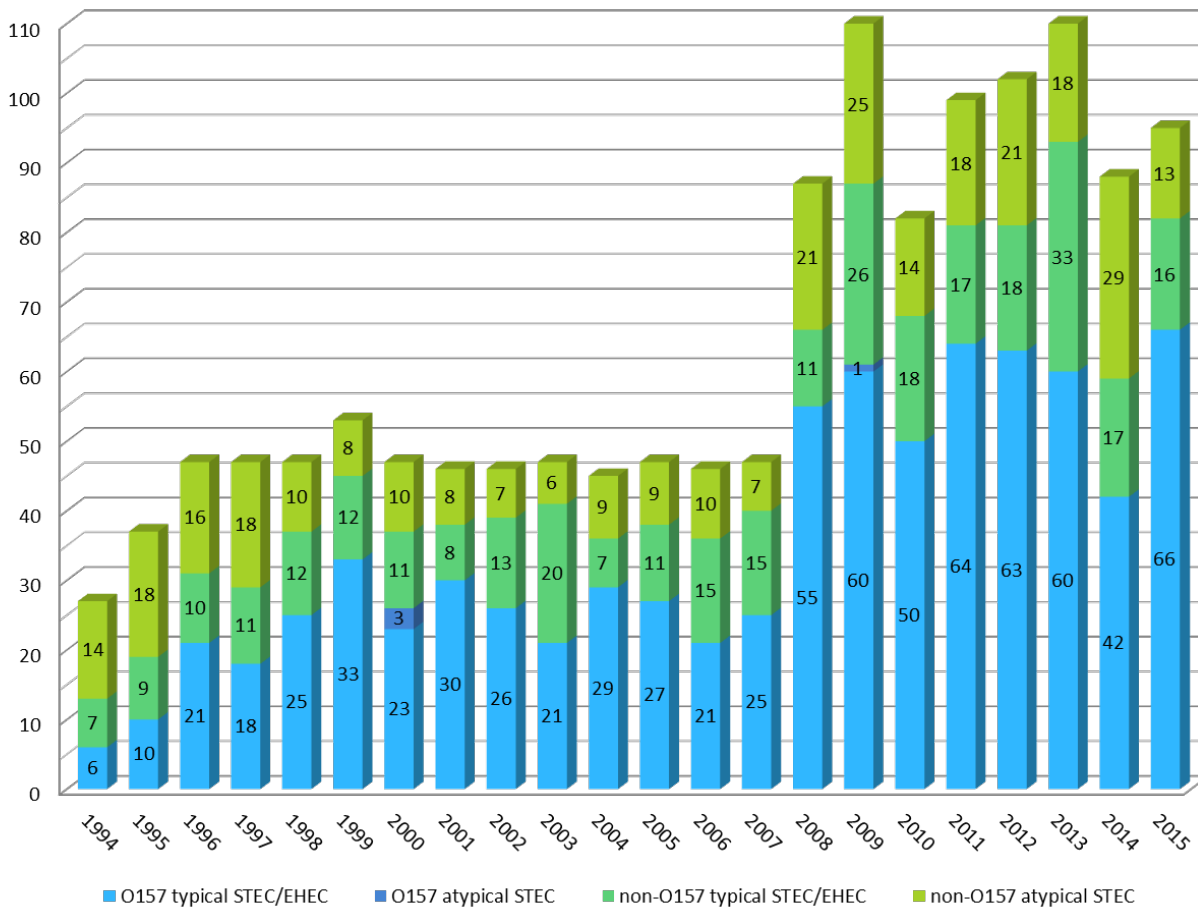


Figure 2: Typical and atypical STEC isolates



2. STEC SEROTYPES

STEC are classified based on their serotype (“O”-antigen:”H”-antigen). Most STEC infections are caused by STEC O157:H7, but non-O157 have also been associated with diarrheal disease worldwide. Figure 2 shows the annual distribution of O157 and non-O157 strains. 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 and only one isolate with this serotype was isolated, in 2008 (Table 1). Figure 3 gives an overview of the evolution of the 6 most important serogroups in Belgium, also called the “pathogenic gang of 6”: O26, O103, O111, O121, O145 and O157. Additionally 6 other non-O157 serogroups have been isolated frequently over the years: O146, O91, O128, O118, O63 and O182. Interestingly, O118 was detected regularly in the early years of screening but hasn’t been found since 2009; while O182 and O63 have only been isolated since 2003 and 2008, respectively

(Table 1). In 2008 primers for the detection of *stx2f* were added to the STEC PCR screening assay and all O63:H6 isolated at our NRC carry the *stx2f* gene, explaining why this serotype wasn't detected the years before.

In 2015 the majority (66/95; 69,5%) of the STEC strains belonged to serogroup O157 (serotype O157:H7 or O157:H-), all of them were typical EHEC (*eaeA+*, *hlyA+*). Additionally, one *E. coli* strain of serotype O157:H7 that was positive for *eaeA* and *hlyA* but did not possess the *stx* genes was isolated. Four of the 'top 5' non-O157 serogroups were represented: 4 O26 strains, 2 O103 strains, 2 O145 isolates and 1 STEC O111 (Table 1).

Table 1: Annual distribution of STEC O-serogroups. Total number of STEC (number of 'typical' STEC).

O-group ^a	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	TOTAL	
O1	1																						1 (0)	
O2		1													1	1								3 (0)
O3										1 (1)	1													2 (1)
O4		1 (1)														2 (1)								3 (2)
O5											1 (1)				1	2 (2)								4 (3)
O6		1									1													2 (0)
O7	1																							1 (0)
O8	1		1					1	1	1			1			2	1	1	1 (1)					11 (1)
O9						1																		1 (0)
O15											1					1	1							3 (0)
O20	1														1	1								3 (0)
O22	1		1																					2 (0)
O24																1 (1)								1 (1)
O26	4 (1)	1	3 (3)	2 (2)	5 (5)	7 (6)	3 (2)	2 (2)	4 (3)	9 (7)	4 (2)	2 (2)	7 (6)	5 (5)	7 (6)	12 (10)	6 (6)	7 (7)	7 (7)	10 (10)	3 (3)	6 (6)		116 (101)
O36															1									1 (0)
O38												1	1						1	1				4 (0)
O43										1														1 (0)
O45															1									1 (0)
O55		1				1								1			1	1	2		1	1		9 (0)
O63															1	3 (1)	4	1	1	3	2			15 (1)
O76							1								1					1	1			4 (0)
O78															1									1 (0)

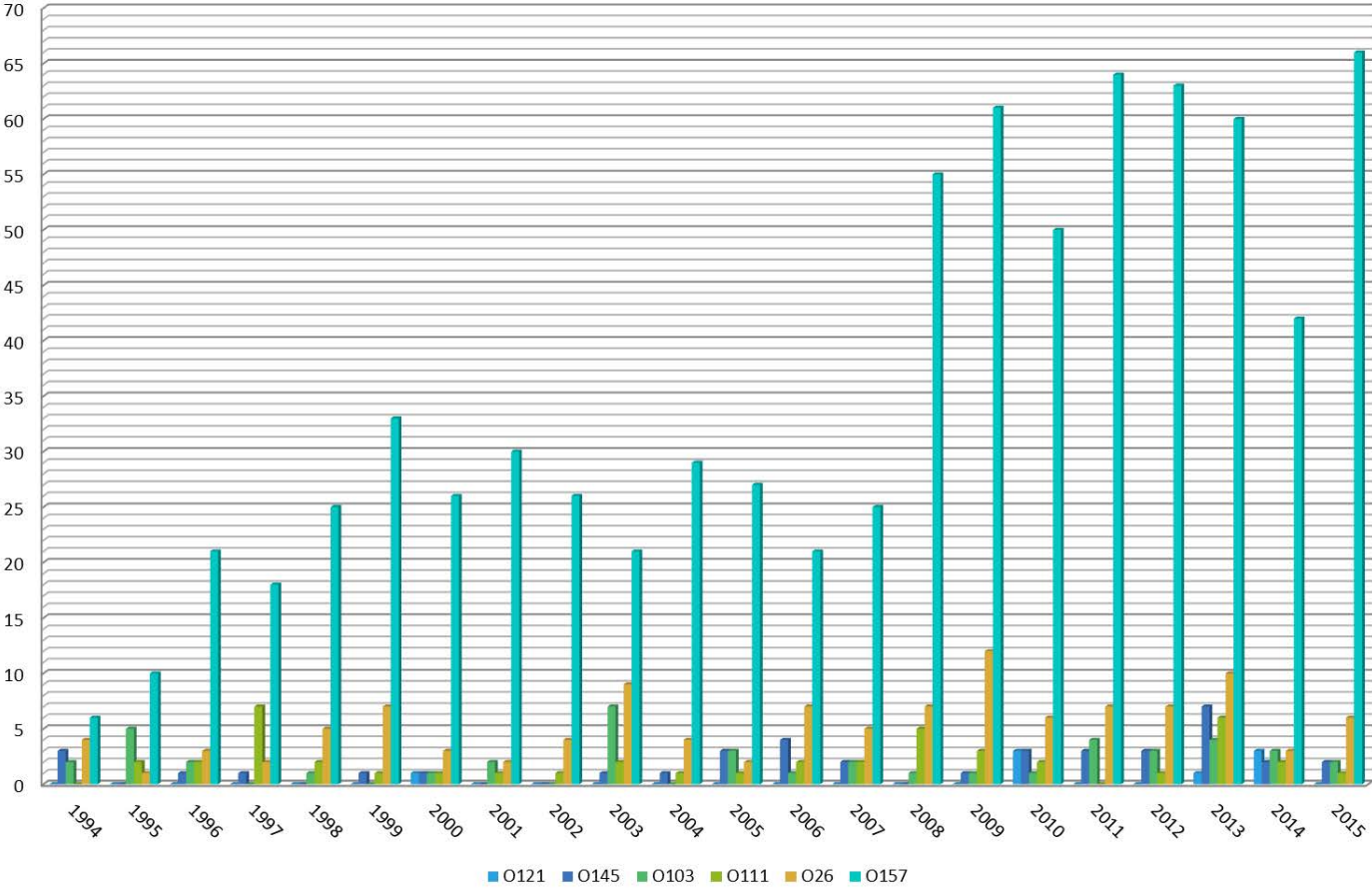
O128	1	1	2						1			1	1	1	2		1	1	1	4 (1)	1	18 (1)	
O132																				1		1 (0)	
O136															1							1 (0)	
O140			2																			2 (0)	
O145	3 (3)		1	1 (1)		1 (1)	1			1 (1)	1 (1)	3 (3)	4 (4)	2 (2)		1 (1)	3 (3)	3 (3)	3 (2)	7 (7)	2 (2)	2 (2)	39 (36)
O146	2			1	1	2 (1)		3		1	2	1	2	1	4		1	5	3	4	2	35 (1)	
O147								1 (1)														1 (1)	
O148																				1 (1)		1 (1)	
O150	1									1 (1)												2 (1)	
O152			1					1 (1)														2 (1)	
O153												1 (1)			1							2 (1)	
O156										1			1 (1)							1 (1)	1 (1)	1 (1)	5 (4)
O157	6 (6)	10 (10)	21 (21)	18 (18)	25 (25)	33 (33)	26 (23)	29 (29)	26 (26)	21 (21)	29 (29)	27 (27)	21 (21)	25 (25)	55 (55)	61 (60)	50 (50)	64 (64)	63 (63)	60 (60)	42 (42)	66 (66)	778 (774)
O162				2				1	1													4 (0)	
O163																					1	1 (0)	
O165			1		1 (1)									1 (1)								3 (2)	
O166												1			1						2	4 (0)	
O168								1 (1)						1								2 (1)	
O169	1 (1)																					1 (1)	
O172			1					1 (1)												1 (1)		3 (2)	
O174	2													1							1	4 (0)	
O175													1 (1)									1 (1)	
O176														1	1							2 (0)	
O177														1								1 (0)	
O178								1												1	1	3 (0)	

O179						1																1 (0)	
O181											1							1				2 (0)	
OX182										1	2 (2)	1 (1)		3 (3)		1 (1)		1 (1)	1 (1)	3 (3)	1 (1)	14 (13)	
OX183															1	1						2 (0)	
O186																					1	1 (0)	
E8686-77							1	1														2 (0)	
E7477/77										1 (1)	1											2 (1)	
Ount	1	6	8 (3)	4	4	2 (2)	2 (1)	4 (1)	4 (4)	1					1						1	38 (11)	
Orough	1	3 (1)	2					2	1	1				1	1	3	1	3 (1)			1	20 (2)	
NON-O157	1	1	2				1			1				1 (0)		3 (1)	1	14 (1)		2	12 (2)	12 (4)	51 (8)
TOTAL	27 (13)	37 (19)	47 (31)	47 (29)	48 (38)	53 (46)	47 (33)	46 (37)	46 (36)	47 (40)	45 (36)	47 (38)	46 (37)	47 (40)	103 (73)	112 (86)	82 (68)	100 (81)	102 (81)	111 (93)	88 (59)	95 (82)	1423 (1096)

^aOunt: untypable; Orough: auto-agglutinating; NON-O157: did not agglutinate with O157, O26, O103, O111, O121, O145 and was not typable with gnd-typing.

^bAgg-STEC O104:H4

Figure 3: Annual distribution of the ‘pathogenic gang of six’



3. HUS CASES

Table 2 gives an overview of the STEC serotypes detected in patients suffering from the haemolytic uremic syndrome (HUS) from 2011 until 2015. Every year the majority of HUS-cases were associated with STEC O157.

In 2015, STEC strains were isolated in 18 HUS cases: 15 STEC O157, 1 O26 and 2 NON-O157. Additionally, STEC infection could be confirmed in 4 HUS cases by detection of STEC O lipopolysaccharide in their serum samples (1xO26, 1xO103, 1xO111 and 1xO157).

Table 2: Number of HUS-associated STEC serotypes

Serotype	2011	2012	2013	2014	2015
O157:H-/H7	10	9	6	4	15
O157 serology^a	1	1	3	0	1

O26:Hunk	0	1	0	0	1
O26 serology^a	0	1	2	0	1
O55:H7	0	1	0	0	0
O61:H-	0	0	1	0	0
O63:H6	0	1	0	0	0
O103:Hunk	0	0	1	0	0
O103 serology^a	0	0	0	0	1
O104:H4	0	0	1	0	0
O111:H-	0	0	1	0	0
O111 serology^a	0	0	0	0	1 ^c
O145:Hunk/H-	1	1	4	0	0
O148:H8	0	1	0	0	0
O182:H25	0	0	1	0	0
Orough:H-^b	1	0	0	0	0
NON-O157^b	0	0	0	2	2
vtx PCR	0	1	0	0	0
TOTAL	13	17	20	6	22

^a In these HUS patients STEC O LPS was detected with antibodies in the serum sample, but no STEC could be isolated from the stool

^b Orough: auto-agglutinating; NON-O157: did not agglutinate with O157, O26, O103, O111, O121, O145 and was not typable with gnd-typing

^c A STEC O157 was isolated from the stool from the same patient

4. OUTBREAKS

STEC infections in Belgium are mainly sporadic, but over the years a few outbreaks have occurred. The outbreaks since 2007 are shortly presented.

4.1 STEC O145/STEC O26 – 2007, ANTWERP

In 2007, an outbreak with both STEC O145 and STEC O26 occurred among consumers of ice cream produced at a farm in the province of Antwerp. Five children developed HUS and 7 persons contracted severe diarrhoea. In 3 HUS cases STEC O145 infections were laboratory confirmed, one in association with STEC O26. (De schrijver *et al.* 2008)

4.2 Agg-STEC O104:H4 – 2011, GERMANY

No Belgian patients were involved in the outbreak itself, but two sporadic cases of *stx2a* positive Agg-STEC O104:H4 infection occurred in Belgium in 2012 and 2013 respectively. In both cases travel in a Mediterranean country preceded the infection. (De Rauw *et al.* 2014).

Germany: from May to July 2011, a large outbreak of STEC infections with a serotype that was reported only very rarely before, O104:H4, was observed. This outbreak differed from previous STEC outbreaks by its sheer size, the large percentage of patients with HUS and the high proportion of adults, mainly women. This exceptionally virulent strain combined virulence factors of EAggEC and STEC. (Frank *et al.* 2011) At the same time, a smaller outbreak of Agg-STE C O104:H4 took place in France. Comparison of the back tracing information from the French and German outbreaks led to the conclusion that fenugreek seeds imported from Egypt were the most likely source of infection although the outbreak strain could not be isolated from the seeds. (EFSA report 2011) Even though several nationalities were hit by this outbreak,

4.3 STEC O157 – 2012, LIMBURG

In 2012 an outbreak of STEC O157:H7 in Limburg was successfully traced back to the consumption of raw meat ('filet américain'). The outbreak involved 24 cases, of which 17 were laboratory-confirmed. Five cases developed HUS and fifteen were hospitalized. (Braeye *et al.* 2014)

4.4 STEC O157 – 2013, BELGIUM

From May until July 2013 14 stx2a positive typical EHEC O157:H7 strains with undistinguishable or closely related molecular typing profiles (PFGE and IS629-typing) were isolated at NRC STEC. The molecular fingerprints of the strains were identical to those of 5 STEC O157 isolated from meat at the National Reference Laboratory for Foodborne Outbreaks (NRL-FBO) in the same period. Two of the infected patients developed HUS, the others were affected with (bloody) diarrhoea. Eight patients originated from the province East-Flanders, the remaining six patients were from different parts of the country. Despite the molecular relatedness between the STEC isolates, no clear epidemiological link was found between the patients or the consumption of the meat.

4.5 STEC O157 – 2015, PROVINCE OF LUXEMBOURG

Between the end of August and October 2015 a small outbreak of stx2a + stx2c positive typical EHEC O157 related to children's day-care took place in Arlon in the province of Luxembourg between August and October. Seven children were involved all between 4 years and 8 months of age, 2 of them suffered from HUS. Some of the patients kept shedding STEC in their feces for several weeks; and for one patient it even took more than 7 weeks to clear the isolate from the stool. Stools of the

co-workers of the day-care were also screened for the presence of STEC, but none of them was infected with the outbreak strain. The source of the outbreak was not established.

On 18/9/2015 an urgent inquiry about the outbreak was made on the Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD), a web-based restricted-access communication platform released by the ECDC. But none of the neighbouring countries reported to have detected the outbreak strain.

5. 2015 CONCLUSIONS

The number of STEC infections identified at the NRC in 2015 stayed stable in comparison to the previous years. The majority of the strains belonged to the O157:H7/H- serotype. Most of the infections were sporadic, but from the end of August until October an outbreak of EHEC O157 occurred in a children's day-care centre in Arlon (Luxembourg) involving 7 infants.

6. REFERENCES

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