

Laboratory of Microbiology and Infection Control, UZ Brussel

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

**ANNUAL REPORT 2016**

---

K. De Rauw

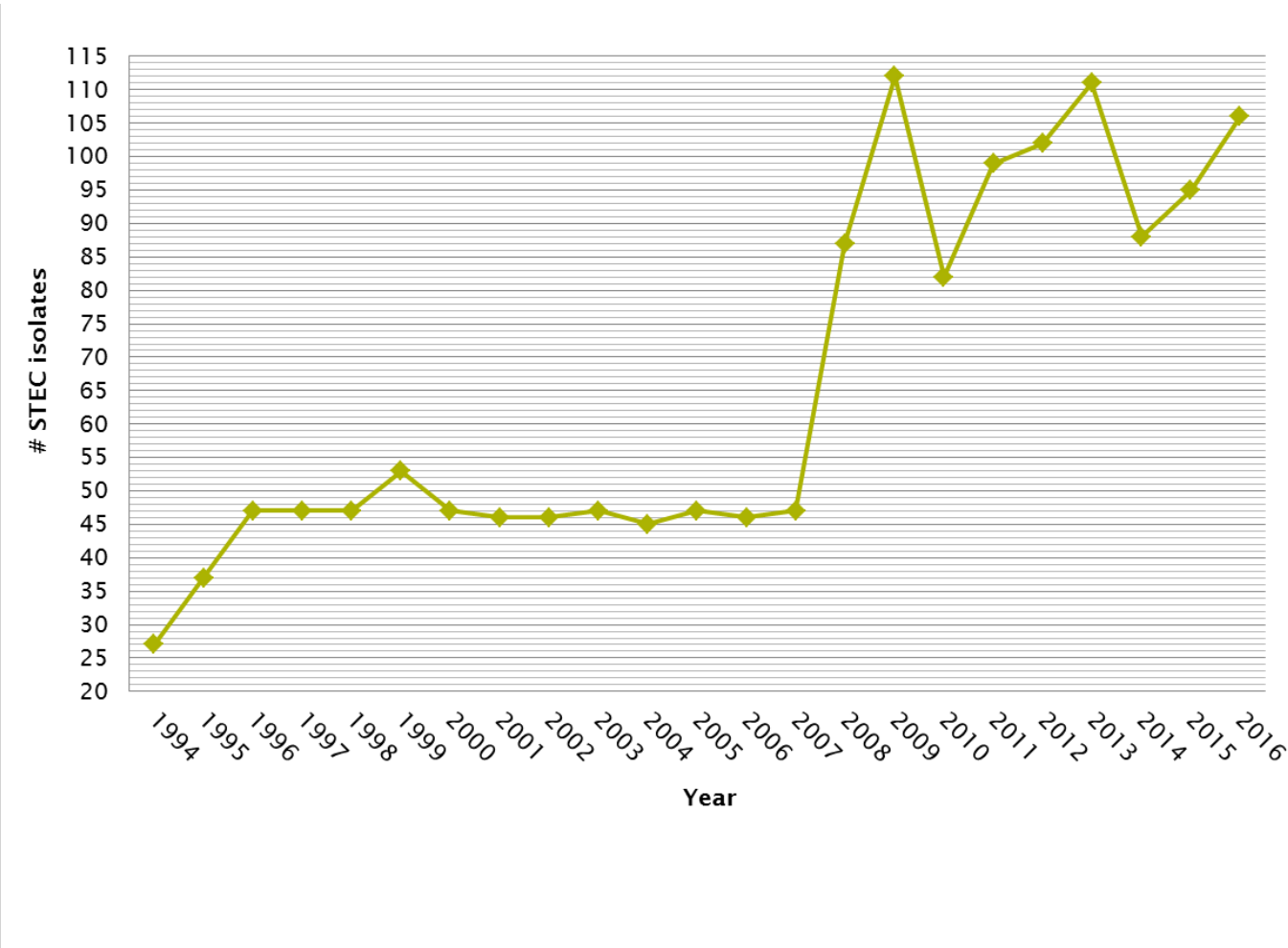
Prof. Dr. D. Piérard

## NUMBER OF STEC STRAINS ANNUALLY ISOLATED

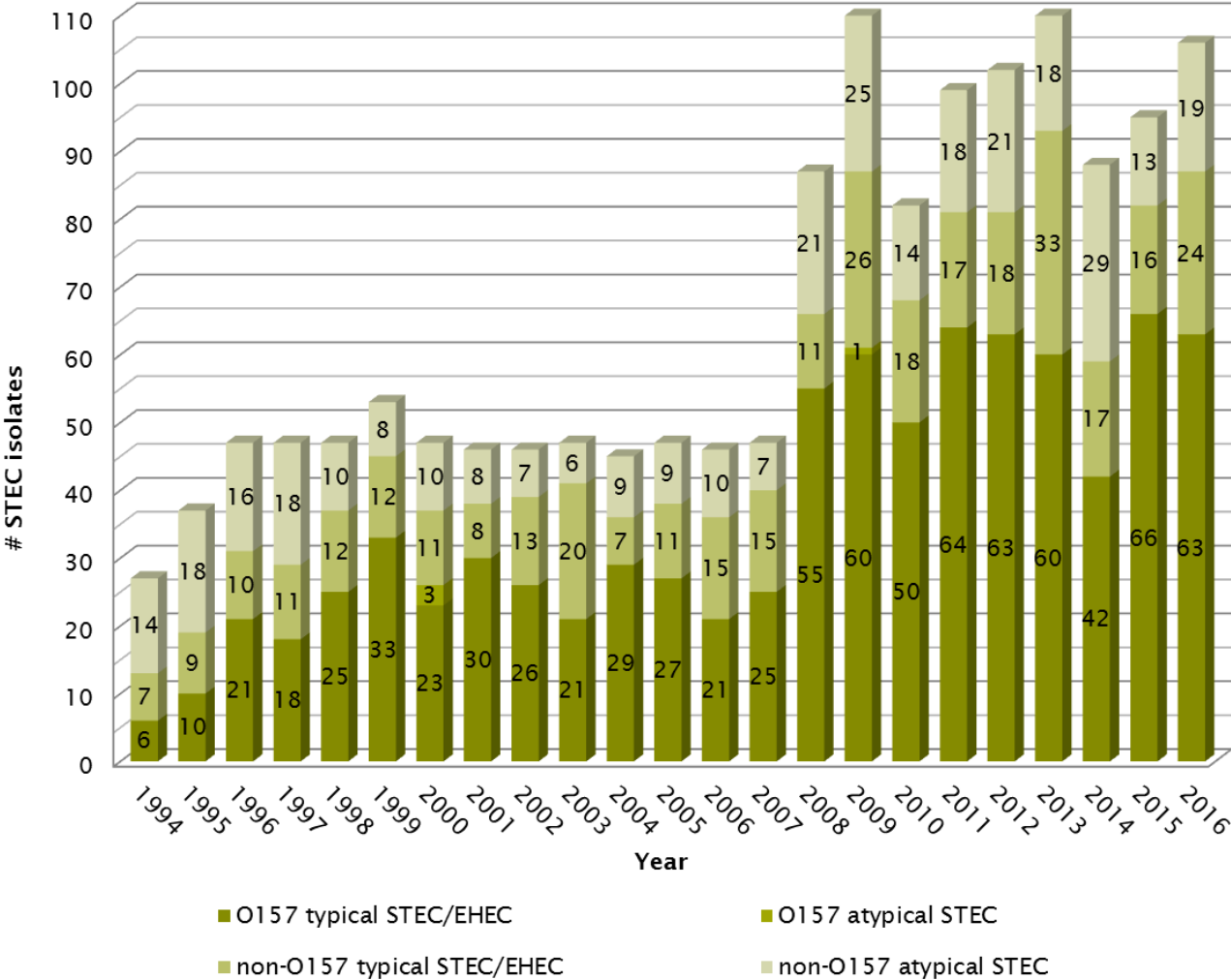
Figures 1 and 2 give an overview of the number of Shiga toxin-producing *Escherichia coli* (STEC) strains annually identified at the NRC STEC/VTEC. From 1994 until 2007 an average of 45 (min. 27 (1994) – max. 53 (1999)) strains were identified each year. Since 2008 the yearly number of isolates increased more than twice, to a mean of 100 (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 STEC 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.

In 2016, 106 different STEC strains were isolated from 105 Belgian patients. In the fecal sample of one patient two nearly identical STEC O157:H- were found; one strain carried the *stx* subtypes *stx1a* and *stx2c* while the second one only possessed the *stx1a* subtype. Eighty seven of them were 'typical' enterohemorrhagic *E. coli* (EHEC) isolates; *eg.* STEC positive for the additional virulence genes *eaeA* and *hlyA* (Figure 2). The *eaeA* gene codes for the virulence factor intimin which is involved in the adherence to the gastrointestinal epithelium and the production of attaching and effacing (A/E) lesions. The *hlyA* gene codes for enterohaemolysin and is located on the EHEC virulence plasmid. Nineteen isolates were atypical STEC; lacking one or both of these virulence determinants. In 2016, no STEC strain was found positive for EaggEC virulence genes *aaiC* and *aggR*.

**Figure 1:** Number of STEC strains annually isolated at the NRC STEC/VTEC (1994-2016)



**Figure 2:** Annual number of typical and atypical STEC isolates (1994-2016)



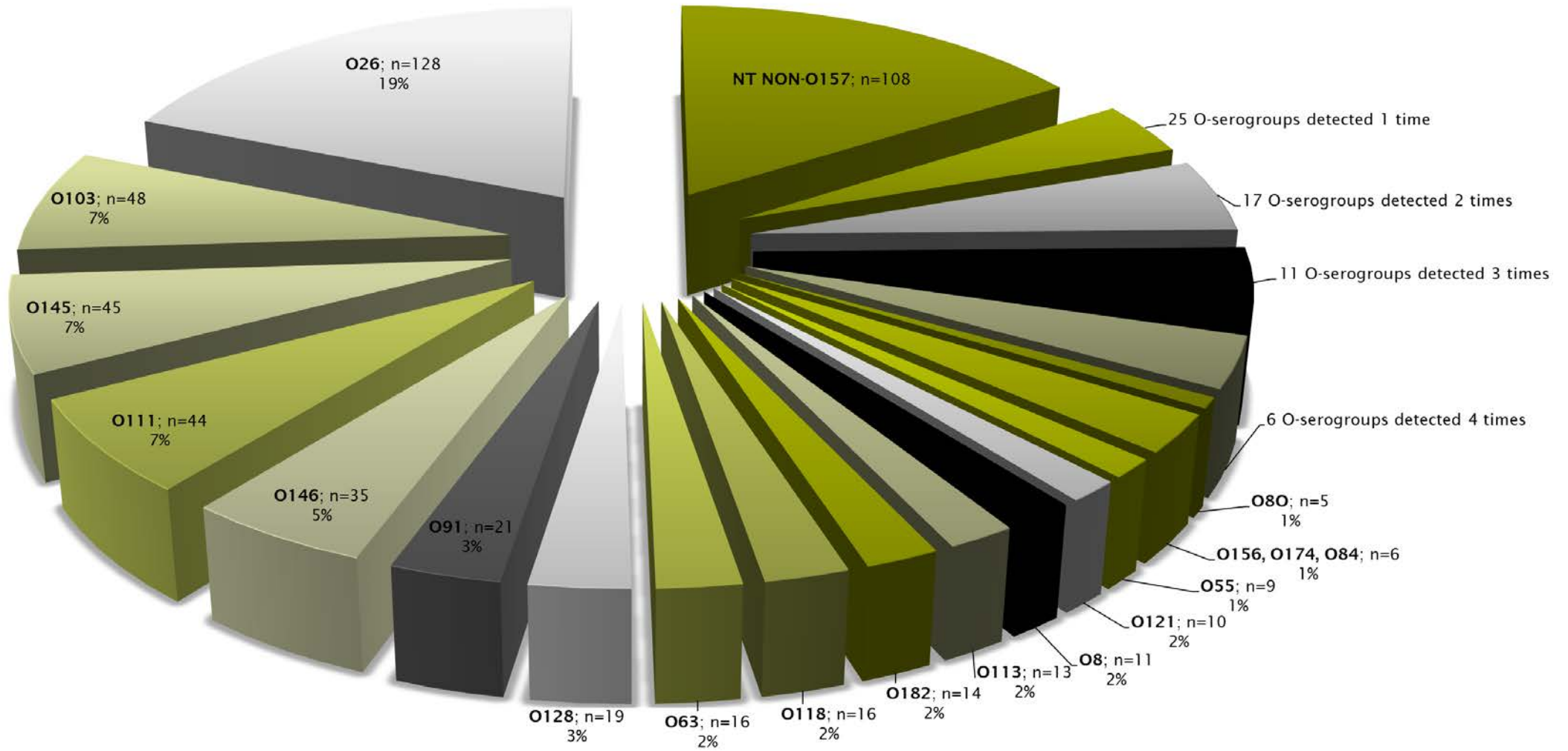
## STEC SEROTYPES

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. Figure 3 gives an overview of all O-serogroups represented in the Belgium STEC strains isolated between 1994 and 2016. Sixty percent of the STEC strains typed at the Belgian NRC STEC between 1994 and 2016 were of the O157:H7:H- serotype (n=841). The O-serogroup of 480 of the 588 STEC non-O157 could be determined, classifying them in 77 different O-serogroups (Figure 3).

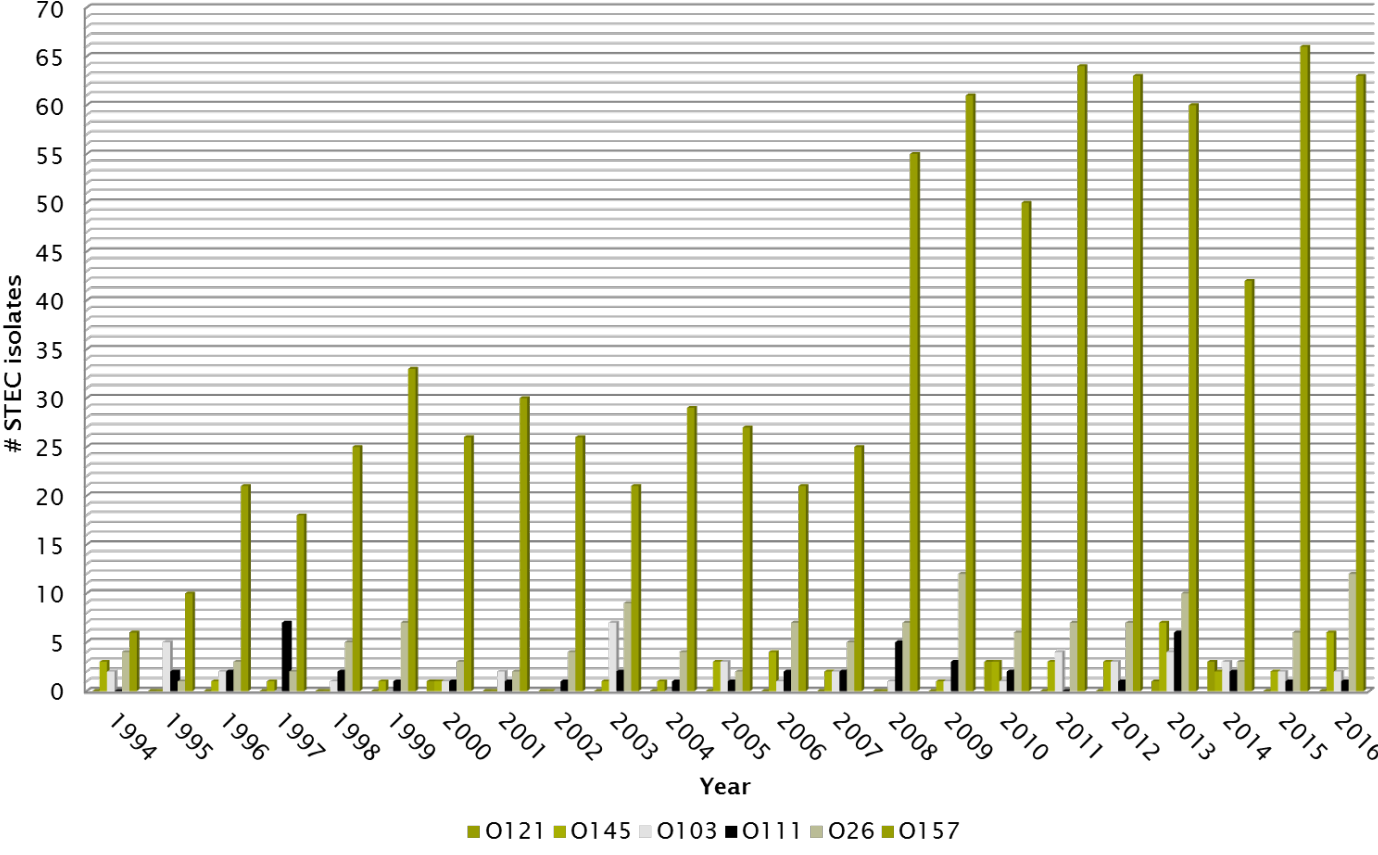
Figure 4 gives an overview of the evolution of the 6 most important serogroups, also called the "pathogenic gang of 6": O26, O103, O111, O121, O145 and O157. Additionally 6 other non-O157 serogroups have been isolated frequently over the years: O146, O91, O128, O118, O63 and O182. Interestingly, O118 was detected regularly in the early years of screening but has not been found since 2009; while O182 and O63 have only been isolated since 2003 and 2008, 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.

In 2016 the majority (63/106; 59,4%) of the STEC strains belonged to serogroup O157 (serotype O157:H7 or O157:H-), all of them were typical EHEC (*eaeA+*, *hlyA+*). Four of the 'top 5' non-O157 serogroups were represented: 12 O26 strains, 2 O103 strains, 6 O145 isolates and 1 STEC O111 (Figure 4).

**Figure 3:** Occurrence of the STEC non-O-serogroups (1994-2016)



**Figure 4:** Annual distribution of the 'pathogenic gang of six' (1994-2016)



## SHIGA TOXIN SUBTYPES

STEC's main pathogenic mechanism is 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. Besides the Stx produced by *Shigella* species, 3 subtypes for Stx1 (Stx1a, stx1c and Stx1d) and 7 subtypes for Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g) have been described in STEC. 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 be easily lost or acquired by horizontal transfer (Scheutz et al. 2012; Tozoli and Scheutz 2014). 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; EFSA Panel on Biological Hazards (BIOHAZ) 2013; Ethelberg et al. 2004; Persson et al. 2007).

In 2016, more than half of the isolates were stx2 positive (55/106, 51,9%), 17.9% (19/106) were stx1 positive and 30.2% were stx1 and stx2 positive (32/106). They belonged to various stx subtypes: stx1a (13), stx1c (6), stx1a+stx2a (9), stx1a+stx2a+stx2c (1), stx1a+stx2b (3), stx1a+stx2c (17), stx1a+stx2d (1), stx1a+stx2? (1) (stx2 gene lost in the lab), stx2a (33), stx2a+stx2c (8), stx2b+stx2d (1), stx2c (7), stx2d (1), and stx2f (5).



## HAEMOLYTIC UREMIC SYNDROME

Shiga toxin- or verocytotoxin-producing *E. coli* (STEC/VTEC) infections are the cause of sporadic and epidemic watery or bloody diarrhoea worldwide. In some individuals it may progress to more severe disease such as the life-threatening haemolytic uremic syndrome (HUS). Table 2 gives an overview of the STEC serotypes detected in Belgian patients suffering from HUS from 2011 until 2016. Every year the majority of HUS-cases were associated with STEC O157.

In 2016, STEC strains were isolated from the fecal samples of 17 HUS cases: 9 STEC O157, 3 STEC O26:Hunk, 1 O111:H-, 2 O145, 1 O80:H-, and 1 O174:Hunk. Additionally, STEC infection could be confirmed in 9 HUS patients by detection of antibodies against STEC O lipopolysaccharide in their serum samples (2 times O26 and 7 times O157).

**Table 2:** Number of HUS-associated STEC serotypes

Serotype	2011	2012	2013	2014	2015	2016
O157:H- /H7	10	9	6	4	15	9
O157 serology <sup>a</sup>	1	1	3	0	0	7
O26:Hunk	0	1	0	0	1	3
O26 serology <sup>a</sup>	0	1	2	0	0	2
O55:H7	0	1	0	0	0	0
O61:H-	0	0	1	0	0	0
O63:H6	0	1	0	0	0	0
O80:H-	0	0	0	0	0	1
O103:Hunk	0	0	1	0	0	0
O104:H4	0	0	1	0	0	0
O111:H-	0	0	1	0	0	1
O111 serology <sup>a</sup>	0	0	0	0	1 <sup>d</sup>	0
O145:Hunk/H-	1	1	4	0	0	2
O148:H8	0	1	0	0	0	0
O174:Hunk	0	0	0	0	0	1
O182:H25	0	0	1	0	0	0
Orough:H- <sup>b</sup>	1	0	0	0	0	0
NON- O157 <sup>b</sup>	0	0	0	2	2	0
<b>TOTAL</b>	<b>13</b>	<b>16</b>	<b>20</b>	<b>6</b>	<b>18</b>	<b>26</b>

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

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

<sup>c</sup> The sample was found positive for stx genes, but it was not possible to isolate a STEC strain.

<sup>d</sup> A STEC O157 was isolated from the stool from the same patient.

## OUTBREAKS

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

### **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).

### **AGG- STEC O104:H4 – 2011, GERMANY**

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-STEC 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 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).

### **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)

### **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.

## **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.

## **STEC O157 – 2016, BELGIUM, SEVERAL SMALL CLUSTERS**

In 2016 several small clusters of EHEC O157 infection were detected in Belgium:

### ***May 2016, Flemish Brabant***

In May 2016 an outbreak of gastroenteritis occurred in a nursery home in Averbode in the province of Flemish Brabant. The same *stx2a* positive typical STEC O157:H7 could be isolated from the fecal samples of six patients. The patients were between 79 and 91 years old. Two patients had uncomplicated diarrhoea, two patients suffered from bloody diarrhoea, and two patients developed HUS. No source was found.

### ***July 2016, Namur***

A two-year-old girl with HUS and her five-year old sister living in the province of Namur were infected with a *stx1a* and *stx2a* positive typical STEC O157:H7 strain. The girls were presumably infected by the consumption of cheese as an identical strain was found in this food item.

### ***September 2016, Liège and Namur***

In September 2016 a *stx2c* positive typical STEC O157:H7 was found in the stool samples of two brothers and their nephew living in the province of Liège and Namur, respectively. The boys were only 2 months, 20 months and 21 months of age. The youngest boy had uncomplicated diarrhoea, while the other two were asymptomatic. All had attended a family meeting at a carnival at the end of August. All family members had eaten at a stand selling

French fries and several adult members of the family also suffered from an episode of diarrhoea in the week following the gathering.

### ***September – October 2016, Hainaut and Luxembourg***

In October 2016 a three-year-old boy and his twenty-months-old sister who attended a children's day-care centre in Tournai in the province of Hainaut were infected with a *stx2a* positive typical STEC O157:H7. The boy had bloody diarrhoea and the girl uncomplicated diarrhoea. Other children attending the day-care as well as some of the personnel were screened for the presence of STEC, but no other cases were detected. Two weeks earlier the same strain was found in the fecal sample of a girl with diarrhoea in a children's day-care centre located in Marche-en-Famenne in the province of Luxembourg. This specific type is commonly seen in the Belgium STEC O157 isolates. During the screening of the day-care in Marche-of-Famenne a closely related, though not exactly the same, typical *stx2a* positive STEC O157:H7 was found in another asymptomatic girl. No source was found in any of the cases.

### ***October 2016, Brussels Capital Region***

A *stx2c* positive typical STEC O157:H7 was isolated from a eight-month-old girl who suffered from uncomplicated diarrhoea. The girl attended a children's day-care centre in Anderlecht in the Brussels capital region and stool samples from other children attending the day-care as well as care-takers were screened for the presence of STEC. An identical strain could be isolated from the sample of child-care workers, who was asymptomatic. No other cases were identified.

### ***October 2016, Limburg***

Two young patients, a girl and a boy, attending the same day-care centre in Diepenbeek in the province of Limburg were infected with the same *stx1a* and *stx2c* positive typical STEC O157:H- strain. The girl had uncomplicated diarrhoea, the symptoms of the boy were not communicated to the NRC. The parents, other children and personnel working at the day-care centre were screened, but no other STEC infections were identified. The parents were thoroughly questioned, but no source could be identified.

## CONCLUSIONS 2016

The number of STEC infections identified at the NRC in 2016 stayed stable in comparison to the years before. As seen in previous years approximately 60 % of the strains were typical STEC O157:H7/H-. Most of the infections were sporadic, but six small clusters of infection with EHEC O157:H7/H- in families and day-care centres were detected.

## REFERENCES

1. Braeye T, Denayer S, De Rauw K, Forier A, Verluyten J, *et al.* Lessons learned from a textbook outbreak: EHEC-O157:H7 infections associated with the consumption of raw meat products, June 2012, Limburg, Belgium. *Arch Public Health* 2014;72(1):44.
2. Buvens G, De Gheldre Y, Dediste A, de Moreau AI, Mascart G, *et al.* Incidence and virulence determinants of verocytotoxin-producing *Escherichia coli* infections in the Brussels-Capital Region, Belgium, in 2008-2010. *J Clin Microbiol* 2012;50(4):1336-45.
3. De Rauw K, Vincken S, Garabedian L, Levtchenko E, Hubloue I, *et al.* Enteroaggregative Shiga toxin-producing *Escherichia coli* of serotype O104:H4 in Belgium and Luxembourg. *New Microbes New Infect* 2014;2(5):138-43.
4. De Schrijver K, Buvens G, Possé B, Van den Branden D, Oosterlynck O, *et al.* Outbreak of verocytotoxin-producing *E. coli* O145 and O26 infections associated with the consumption of ice cream produced at a farm, Belgium, 2007. *Euro Surveill* 2008; 3(7).
5. European Food Safety Authority (EFSA). Tracing seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in relation to the Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 Outbreaks in Germany and France. 2011.
6. Frank C, Werber D, Cramer JP, Askar M, Faber M, *et al.* Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *N Engl J Med* 2011;365(19):1771-80.
7. Gould LH, Bopp C, Strockbine N, Atkinson R, Baselski V, *et al.* Recommendations for diagnosis of shiga toxin-producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep* 2009;58(RR-12):1-14.