

Article

# The benefits of whole genome sequencing for foodborne outbreak investigation from a National Reference Laboratory perspective in a smaller country

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**Abstract:** Gradually, conventional methods for foodborne pathogen typing are replaced by whole genome sequencing (WGS). Despite studies describing the overall benefits, National Reference Laboratories of smaller countries often show slower uptake of WGS, mainly because of significant investments required to generate and analyze data of a limited amount of samples. To facilitate this process and incite policy makers to support its implementation, a Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 (*stx1+*, *stx2+*, *eae+*) outbreak (2012) and a STEC O157:H7 (*stx2+*, *eae+*) outbreak (2013) were retrospectively analyzed using WGS and compared with their conventional investigations. Corresponding results were obtained, with WGS delivering even more information, e.g., on virulence and antimicrobial resistance genotypes. Besides an universal, all-in-one workflow with less hands-on-time (five versus seven actual working days for WGS versus conventional), WGS-based cgMLST-typing demonstrated increased resolution. This enabled accurate cluster definition which remained unsolved for the 2013 outbreak, partly due to scarce epidemiological linking with the suspect source. Moreover, it allowed detecting two and one earlier circulating STEC O157:H7 (*stx1+*, *stx2+*, *eae+*) and STEC O157:H7 (*stx2+*, *eae+*) strains as closely related to the 2012 and 2013 outbreaks, respectively, which might have further directed epidemiological investigation initially. Although some bottlenecks concerning centralized data-sharing, sampling strategies, and perceived costs should be considered, we delivered a proof-of-concept that even in smaller countries, WGS offers benefits for outbreak investigation, if sufficient budget is available to ensure

its implementation in surveillance. Indeed, applying a database with background isolates is critical in interpreting isolate relationships to outbreaks, and leveraging the true benefit of WGS in outbreak investigation and/or prevention.

**Keywords:** whole genome sequencing, WGS, foodborne outbreak investigation, Shiga Toxin-producing *Escherichia coli*, STEC, food safety, surveillance

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Supplementary Tables

1. Table S1: Characteristics of all background isolates determined with conventional methods and WGS

Table S1. The characteristics of all background isolates determined with conventional methods and WGS.

| Strain ref. | Isolation date (dd/mm/yyyy) | Origin | Conventional methods |                 |      |     |      | WGS  |     |                         |                 |      |      |     |      |      |      |      |      |      |      |      |     |     |     |           |     |      |     |      |           |       |     |     |     |       |      |      |
|-------------|-----------------------------|--------|----------------------|-----------------|------|-----|------|------|-----|-------------------------|-----------------|------|------|-----|------|------|------|------|------|------|------|------|-----|-----|-----|-----------|-----|------|-----|------|-----------|-------|-----|-----|-----|-------|------|------|
|             |                             |        | Serotype             | Virulence genes |      |     |      |      | AMR | Serotype                | Virulence genes |      |      |     |      |      |      |      |      |      |      |      |     |     |     | AMR genes |     |      |     |      |           |       |     |     |     |       |      |      |
|             |                             |        |                      | stx1            | stx2 | eae | ehxA | aggR |     |                         | aaiC            | stx1 | stx2 | eae | ehxA | aggR | aaiC | katP | toxB | astA | cdtB | celB | esp | gad | nle | tir       | iss | etpD | iha | tccP | blaTEM-1B | catA1 | str | sul | tet | aadA1 | dfrA | ermB |
| EH1819      | 28/7/2009                   | Human  | O157:H7              | -               | +    | +   | +    | -    | -   | S                       | O157:H7         | -    | c    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | -   | -    | -         | -     | -   | -   | -   | -     | +    |      |
| EH1829      | 11/8/2009                   | Human  | O157:H7              | +               | +    | +   | +    | -    | -   | AMP, CHL, STR, SUL, TET | O157:H7         | a    | c    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | +   | +    | +         | +     | +   | +   | +   | +     | +    |      |
| EH2012      | 18/1/2011                   | Human  | O157:H7              | -               | a    | +   | +    | -    | -   | S                       | O157:H7         | -    | a    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | -   | -    | -         | -     | -   | -   | -   | -     | +    |      |
| EH2130      | 13/3/2012                   | Human  | O157:H7              | a               | a    | +   | +    | -    | -   | STR, SXT, SUL, TET      | O157:H7         | a    | a    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | -   | -    | -         | -     | 1   | A   | +   | 1     | +    | +    |
| EH2131      | 13/3/2012                   | Human  | O157:H7              | a               | a    | +   | +    | -    | -   | STR, SXT, SUL, TET      | O157:H7         | a    | a    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | -   | -    | -         | 1     | A   | +   | 1   | +     | +    |      |
| EH2216      | 12/9/2012                   | Human  | O157:H7              | -               | a    | +   | +    | -    | -   | S                       | O157:H7         | -    | a    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | -   | -    | -         | -     | -   | -   | -   | -     | +    |      |
| EH2217      | 12/9/2012                   | Human  | O157:H7              | -               | a    | +   | +    | -    | -   | S                       | O157:H7         | -    | a    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | -   | -    | -         | -     | -   | -   | -   | -     | +    |      |
| EH2220      | 20/9/2012                   | Human  | O157:H7              | -               | a    | +   | +    | -    | -   | S                       | O157:H7         | -    | a    | +   | +    | -    | -    | +    | +    | +    | -    | -    | +   | +   | +   | +         | +   | +    | -   | -    | -         | -     | -   | -   | -   | -     | +    |      |





|                 |           |                |         |   |   |   |    |    |    |    |         |   |   |   |   |   |   |   |   |   |   |   |           |   |       |   |   |   |   |   |   |   |       |   |   |   |   |   |
|-----------------|-----------|----------------|---------|---|---|---|----|----|----|----|---------|---|---|---|---|---|---|---|---|---|---|---|-----------|---|-------|---|---|---|---|---|---|---|-------|---|---|---|---|---|
| <b>TIAC1653</b> | 16/5/2007 | Bovine carcass | O157:H7 | + | + | + | NA | NA | NA | NA | O157:H7 | a | c | + | + | - | - | + | + | + | - | - | A B F J P | + | A B C | + | + | + | + | + | - | - | A B 2 | - | - | - | - | + |
| <b>TIAC1664</b> | 17/7/2007 | Meat           | O157:H7 | - | + | + | NA | NA | NA | NA | O157:H7 | - | c | + | + | - | - | + | + | + | - | - | A B F J P | + | A B C | + | + | + | + | + | - | - | -     | - | - | - | - | + |
| <b>TIAC1888</b> | 9/6/2011  | Bovine carcass | O157:H7 | + | + | + | NA | NA | NA | NA | O157:H7 | a | c | + | + | - | - | + | + | + | - | - | A B F J P | + | A B C | + | + | + | + | + | - | - | -     | - | - | - | - | + |

All background isolates originating from food matrices or swabs are indicated with a TIAC-number, and isolates originating from human stools are indicated with a EH-number.

The genotypic characteristics of all isolates with the conventional method (serotyping and virulence gene detection) and WGS (serotyping, virulence and AMR genotyping; determined with read mapping-based gene detection (SRST2) using the SerotypeFinder, VirulenceFinder, and ResFinder databases, respectively) are visually indicated in green (+; present), red (-; absent) and grey (NA ; not tested according to the ISO/TS12126:2012, and ISO 16654:2001). The detected subtypes of a gene (e.g. *stx*, *esp*, *nle*, etc.) are specified. Food isolates are not routinely phenotypically tested for AMR (indicated as not applicable; NA). Phenotypic AMR susceptibility is indicated as S, whereas resistance is indicated with the abbreviation of the respective antibiotic (Ampicillin (AMP), Chloramphenicol (CHL), Streptomycin (STR), Sulfonamides (SUL), Tetracycline (TET)). Isolates whose reference number is shown in red had a similar (EH2216, EH2217, and EH2220) or identical (EH2012, EH2130, and EH2131) IS629 fingerprints compared to those of the Limburg 2012 (EH2130 and EH2131) or Flanders 2013 (EH2012, EH2216, EH2217, and EH2220) outbreak isolates.

2. Table S2: WGS-based serotype determination

Table S2. WGS-based serotype determination

| Isolate reference | Outbreak | O-determining allele |            | O-type | H-determining allele | H-type |
|-------------------|----------|----------------------|------------|--------|----------------------|--------|
|                   |          | <i>wzx</i>           | <i>wzy</i> |        | <i>fliC</i>          |        |
| EH2165            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2169            | 2012     | 199                  | 201        | O157   | 15                   | H7     |
| EH2170            | 2012     | 201                  | 201        | O157   | 15                   | H7     |
| EH2171            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2174            | 2012     | 201                  | 200        | O157   | 15                   | H7     |
| EH2176            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2177            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2178            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2179            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2180            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2181            | 2012     | 201                  | 201        | O157   | 15                   | H7     |
| EH2182            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2183            | 2012     | 201                  | 200        | O157   | 15                   | H7     |
| EH2189            | 2012     | 201                  | 201        | O157   | 15                   | H7     |
| EH2192            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2193            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2194            | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| TIAC1150          | 2012     | 199                  | 201        | O157   | 15                   | H7     |
| TIAC1151          | 2012     | 201                  | 201        | O157   | 15                   | H7     |
| TIAC1152          | 2012     | 199                  | 201        | O157   | 15                   | H7     |
| TIAC1153          | 2012     | 199                  | 200        | O157   | 15                   | H7     |
| EH2264            | 2013     | 201                  | 200        | O157   | 15                   | H7     |
| EH2268            | 2013     | 201                  | 200        | O157   | 15                   | H7     |
| EH2272            | 2013     | 199                  | 200        | O157   | 15                   | H7     |
| EH2274            | 2013     | 201                  | 201        | O157   | 15                   | H7     |
| EH2275            | 2013     | 201                  | 201        | O157   | 15                   | H7     |
| EH2277            | 2013     | 199                  | 200        | O157   | 15                   | H7     |
| EH2278            | 2013     | 199                  | 200        | O157   | 15                   | H7     |
| EH2279            | 2013     | 199                  | 200        | O157   | 15                   | H7     |
| EH2280            | 2013     | 199                  | 200        | O157   | 15                   | H7     |
| EH2281            | 2013     | 199                  | 201        | O157   | 15                   | H7     |
| EH2285            | 2013     | 199                  | 200        | O157   | 15                   | H7     |
| EH2295            | 2013     | 199                  | 201        | O157   | 15                   | H7     |
| EH2297            | 2013     | 201                  | 201        | O157   | 15                   | H7     |
| EH2299            | 2013     | 201                  | 200        | O157   | 15                   | H7     |
| EH2308            | 2013     | 199                  | 200        | O157   | 15                   | H7     |
| TIAC1903          | 2013     | 201                  | 200        | O157   | 15                   | H7     |

Serotyping results per isolate of both outbreaks (O), i.e. Limburg 2012 and Flanders 2013, are indicated through the detected O- and H- serotype determining alleles. Results were obtained from WGS data analysis using SRST2 and the SerotypeFinder database. Presence of the respective gene allele is indicated with “+” in a dark green box in case the reference allele was detected with 100.00% coverage and 0.00% sequence divergence, “+” in a light green box in case the reference allele was detected with 100.00% coverage but < 10.00% sequence divergence, or “+” in a grey box in case the reference allele was detected with > 60.00% coverage and < 10.00% sequence divergence. When the reference allele was not detected within these criteria, a “-” in a red box is shown. The SerotypeFinder database determined the O- and H-types from the detected serotyping alleles, based on decision rules [1].

3. Table S3: WGS-based virulence allele typing

Table S3. WGS-based virulence allele typing results

| Isolate reference | Outbreak | <i>stx1</i> | <i>stx2</i> | <i>elxA</i> | <i>eae</i> | <i>nleA</i> | <i>nleB</i> | <i>nleC</i> | <i>toxB</i> | <i>astA</i> | <i>etpD</i> | <i>katP</i> | <i>iha</i> | <i>espA</i> | <i>espP</i> | <i>espB</i> | <i>espF</i> | <i>espJ</i> | <i>tccP</i> | <i>iss</i> | <i>gad</i> | <i>tir</i> |
|-------------------|----------|-------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|------------|------------|
| EH2165            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2169            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2170            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| EH2171            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2174            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| EH2176            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2177            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2178            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2179            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| EH2180            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| EH2181            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| EH2182            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2183            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| EH2189            | 2012     | 7           | 31          | 3           | 42         | -           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| EH2192            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2193            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |
| EH2194            | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | -          | 26         |
| TIAC1150          | 2012     | 7           | 31          | 3           | 42         | 8           | -           | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | 10          | 2           | -           | -          | 6          | 26         |
| TIAC1151          | 2012     | 7           | 31          | 3           | 42         | 8           | -           | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | 6          | 26         |
| TIAC1152          | 2012     | 7           | 31          | 3           | 42         | 8           | 13          | 4           | -           | -           | 1           | 1           | 14         | 19          | 4           | 6           | -           | 2           | -           | -          | -          | 26         |

|                 |      |    |    |   |    |   |    |   |   |   |   |   |    |    |   |   |    |   |   |   |   |    |
|-----------------|------|----|----|---|----|---|----|---|---|---|---|---|----|----|---|---|----|---|---|---|---|----|
| <b>TIAC1153</b> | 2012 | 12 | 52 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | - | 6 | 10 | 2 | - | - | - | 26 |
| <b>EH2264</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | -  | 2 | - | - | - | -  |
| <b>EH2268</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2272</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | -  | 2 | - | - | - | -  |
| <b>EH2274</b>   | 2013 | /  | 24 | 3 | 42 | 8 | -  | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | -  | 2 | - | - | - | -  |
| <b>EH2275</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | -  | 2 | - | - | - | -  |
| <b>EH2277</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2278</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2279</b>   | 2013 | /  | 24 | 3 | 42 | 8 | -  | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2280</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2281</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2285</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2295</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | -  | 2 | - | - | - | -  |
| <b>EH2297</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2299</b>   | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |
| <b>EH2308</b>   | 2013 | 12 | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | -  | 2 | - | - | - | -  |
| <b>TIAC1903</b> | 2013 | /  | 24 | 3 | 42 | 8 | 13 | 4 | - | - | 1 | 1 | 14 | 19 | 4 | 6 | 10 | 2 | - | - | - | -  |

Virulence allele typing results determined with WGS using direct read mapping with SRST2 are indicated for all O157 isolates with similar characteristics, collected during the Limburg 2012 and Flanders 2013 outbreak. The VirulenceFinder database was used. Only alleles for which exact matches with the gene alleles in the VirulenceFinder database were obtained are shown (i.e. 100% query coverage and 0% sequence divergence). When multiple exact matches were obtained, the largest hit is retained. The partial detection of a gene allele (here indicated as “-”) are likely new alleles of the gene not present in the database. Detection of *stx1* alleles 7 and 12 encode for the *stx1a* subtype. Detection of *stx2* alleles 24 and 31 encode for the *stx2a* subtype, whereas *stx2* allele 52 encodes for the *stx2c* subtype.

## 4. Table S4: NCBI SRA accession numbers of WGS data of all isolates

Table S4. NCBI SRA accession numbers of WGS data of all isolates

| Bioproject accession | Accession    | Isolate reference | Reference                          |
|----------------------|--------------|-------------------|------------------------------------|
| PRJNA574887          | SRR10201483  | TIAC1151          | Nouws and Bogaerts et al. 2020 [2] |
|                      | SRR10201465  | TIAC1152          |                                    |
|                      | SRR10201452  | TIAC1153          |                                    |
|                      | SRR10201427  | EH2165            |                                    |
|                      | SRR10201416  | EH2174            |                                    |
| PRJNA645975          | SAMN15521963 | EH2169            | This study                         |
|                      | SAMN15521964 | EH2170            |                                    |
|                      | SAMN15521965 | EH2171            |                                    |
|                      | SAMN15521966 | EH2176            |                                    |
|                      | SAMN15521967 | EH2177            |                                    |
|                      | SAMN15521968 | EH2178            |                                    |
|                      | SAMN15521969 | EH2179            |                                    |
|                      | SAMN15521970 | EH2180            |                                    |
|                      | SAMN15521971 | EH2181            |                                    |
|                      | SAMN15521972 | EH2182            |                                    |
|                      | SAMN15521973 | EH2183            |                                    |
|                      | SAMN15521974 | EH2189            |                                    |
|                      | SAMN15521975 | EH2192            |                                    |
|                      | SAMN15521976 | EH2193            |                                    |
|                      | SAMN15521977 | EH2194            |                                    |
|                      | SAMN15521978 | TIAC1150          |                                    |
|                      | SAMN15521979 | EH2264            |                                    |
|                      | SAMN15521980 | EH2268            |                                    |
|                      | SAMN15521981 | EH2272            |                                    |
|                      | SAMN15521982 | EH2274            |                                    |
|                      | SAMN15521983 | EH2275            |                                    |
|                      | SAMN15521984 | EH2277            |                                    |
|                      | SAMN15521985 | EH2278            |                                    |
|                      | SAMN15521986 | EH2279            |                                    |
| SAMN15521987         | EH2280       |                   |                                    |
| SAMN15521988         | EH2281       |                   |                                    |
| SAMN15521989         | EH2285       |                   |                                    |
| SAMN15521990         | EH2295       |                   |                                    |
| SAMN15521991         | EH2297       |                   |                                    |
| SAMN15521992         | EH2299       |                   |                                    |

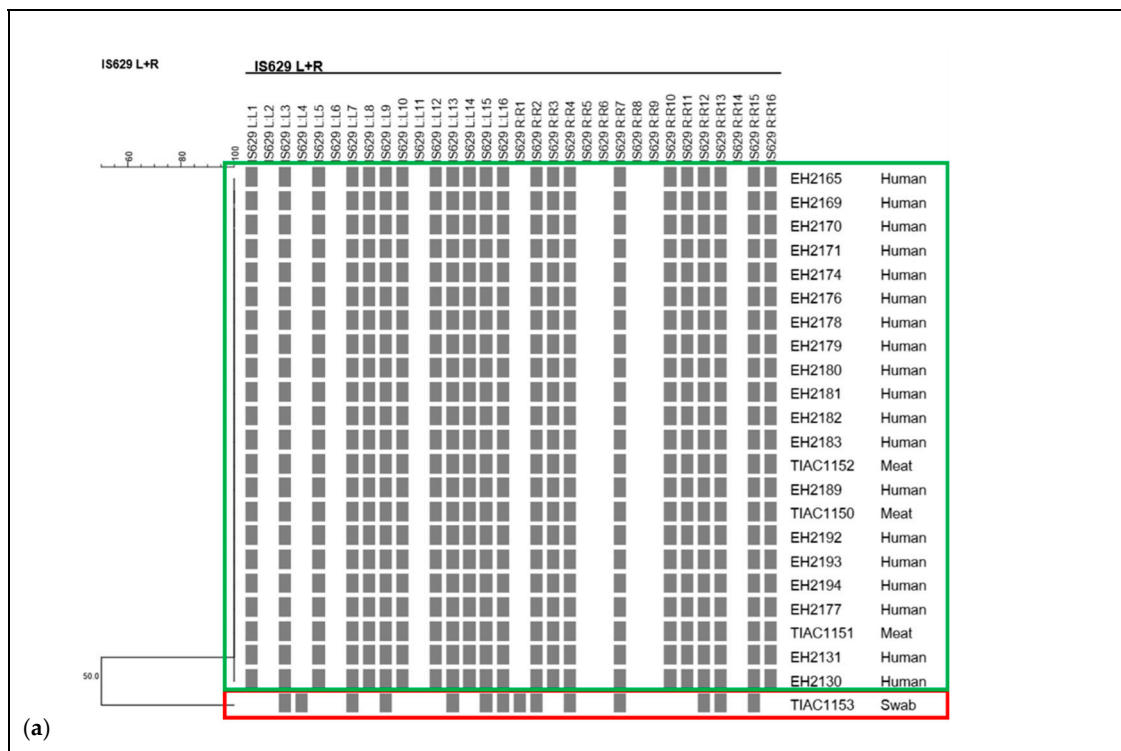
|             |              |          |                                  |
|-------------|--------------|----------|----------------------------------|
|             | SAMN15521993 | EH2308   |                                  |
|             | SAMN15521994 | TIAC1903 |                                  |
|             | SAMN15521995 | EH2012   |                                  |
|             | SAMN15521996 | EH2130   |                                  |
|             | SAMN15521997 | EH2131   |                                  |
|             | SAMN15521998 | EH2216   |                                  |
|             | SAMN15521999 | EH2217   |                                  |
|             | SAMN15522000 | EH2220   |                                  |
|             | SRR11816078  | EH1819   |                                  |
|             | SRR11816076  | EH1829   |                                  |
|             | SRR11816023  | TIAC1218 |                                  |
|             | SRR11816054  | TIAC1354 |                                  |
|             | SRR11816049  | TIAC1356 |                                  |
|             | SRR11816048  | TIAC1369 |                                  |
|             | SRR11816047  | TIAC1372 |                                  |
|             | SRR11816046  | TIAC1382 |                                  |
|             | SRR11816044  | TIAC1398 |                                  |
|             | SRR11816043  | TIAC1399 |                                  |
|             | SRR11816042  | TIAC1400 |                                  |
|             | SRR11816031  | TIAC1402 |                                  |
|             | SRR11816053  | TIAC1408 |                                  |
|             | SRR11816030  | TIAC1411 |                                  |
|             | SRR11816000  | TIAC1419 |                                  |
| PRJNA633966 | SRR11815999  | TIAC1420 | Bogaerts and Nouws et al.<br>[1] |
|             | SRR11816041  | TIAC1426 |                                  |
|             | SRR11816029  | TIAC1428 |                                  |
|             | SRR11816028  | TIAC1435 |                                  |
|             | SRR11816027  | TIAC1440 |                                  |
|             | SRR11815998  | TIAC1442 |                                  |
|             | SRR11816040  | TIAC1448 |                                  |
|             | SRR11816026  | TIAC1449 |                                  |
|             | SRR11816025  | TIAC1454 |                                  |
|             | SRR11816039  | TIAC1460 |                                  |
|             | SRR11816024  | TIAC1463 |                                  |
|             | SRR11816022  | TIAC1464 |                                  |
|             | SRR11816021  | TIAC1472 |                                  |
|             | SRR11816020  | TIAC1475 |                                  |
|             | SRR11816019  | TIAC1477 |                                  |
|             | SRR11816018  | TIAC1478 |                                  |
|             | SRR11816017  | TIAC1479 |                                  |

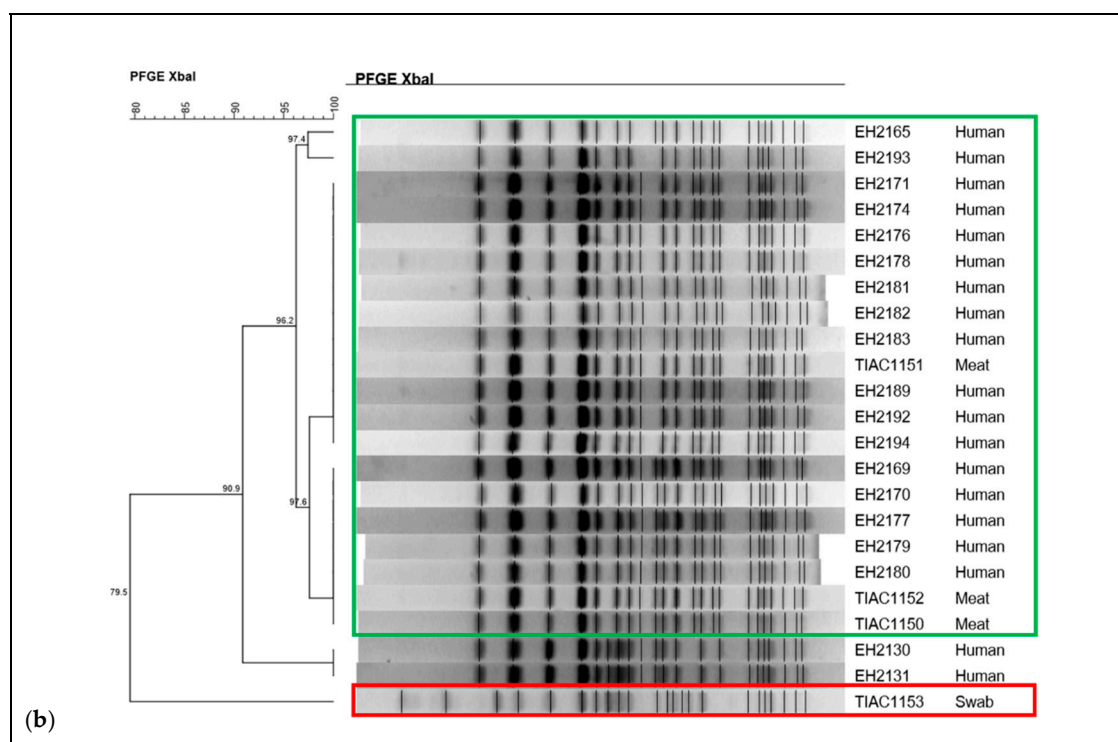
|  |             |          |  |
|--|-------------|----------|--|
|  | SRR11815977 | TIAC1653 |  |
|  | SRR11815976 | TIAC1664 |  |
|  | SRR11816002 | TIAC1888 |  |

The datasets supporting the conclusions of this study have been deposited in the NCBI SRA under the bioproject accession numbers PRJNA574887, PRJNA645975, and PRJNA633966 (all in-house sequenced data) as listed in the first column. The accession numbers of each isolate is shown.

### Supplementary Figures

1. Figure S1: Conventional typing results of the Limbug 2012 outbreak





**Figure S1.** The IS629 fingerprints (a) and PFGE patterns (b) of all suspected outbreak isolates involved in the Limburg 2012 outbreak are depicted. All isolates that are clustering together are encircled in green. The carcass isolate that had a complete different IS629 fingerprint and PFGE pattern compared to all other outbreak isolates is encircled in red. Two background isolates were identified with identical IS629-types as the isolates collected during the outbreak period.

## Supplementary Methods

### 1. Conventional ISO workflows for STEC detection, isolation and identification from food samples, as performed during the initial outbreak investigation

The initial outbreak investigation was performed according to the conventional methods. The detection, isolation and identification of *E. coli* O157 STEC from food and carcass swab samples was performed at the NRL-STECC according to the ISO/TS 13136:2012 and ISO 16654 workflows, as part of the initial investigation. For the Limburg 2012 outbreak, these procedures were also described by Braeye *et al.* (2014) [3].

Detection of STEC in food was performed according to the ISO/TS 13136:2012 standard. Shortly, 25mg or 25ml of the susceptible matrix or 1 swab was solubilized in 225ml or 90ml of Buffered Peptone Water (BPW; Lab M Limited, Lancashire, UK), respectively, mixed for 1 min in a stomacher bag using a Bagmixer 400 CC (Interscience, Louvain-La-Neuve, Belgium) and incubated at 37±1°C for 18±2h (food samples) or at 41.5±1°C for 6h (carcass swabs). Fifty microliters of the enriched samples was used for 10min of heat lysis DNA extraction at 100°C using the Pall Food Extraction Pack (Pall Corporation, New York, USA) and subsequent qPCR reaction using the GSTEHEC106006 GeneDisc (Pall Corporation, New York, USA) for the detection of major STEC virulence genes (*stx1*, *stx2*, and *eae*) and *rfbE*<sub>O157</sub>, according to the manufacturer's protocol. When *stx* and *eae* were detected, the Pall Food Extraction Pack and GEHECID106006 GeneDisc were applied for detection of *wzx*<sub>O26</sub>, *wzx*<sub>O103</sub>, *wbd1*<sub>O111</sub>, *fliC*<sub>H7</sub> and *thp1*<sub>O145</sub>, according to the manufacturer's protocol.

Isolation of the *E. coli* O157 STEC was performed according to ISO 16654:2001 for detection and isolation of *E. coli* O157 STEC. Shortly, 25g food matrix or 1 swab was mixed in 225ml or 90ml modified Tryptic Soy Broth with 20mg/l Novobiocin (mTSB + n; BioMérieux, Marcy-l'Étoile, France), respectively, using a Bagmixer 400 CC. The samples were incubated at 42±1°C for 6±1h, and again for

18±2h. Separation and concentration of the *E. coli* O157 was executed by immunocapturing, for which 20µl Dynabeads *E. coli* anti-O157 immunomagnetic beads (Invitrogen, California, USA) were added to 1ml enriched sample and processed according to the manufacturer's protocol. Fifty microliters of washed beads were spread on CT-SMAC (BioMérieux, Marcy-l'Étoile, France) and incubated for 18–24h at 37°C for growth. *E. coli* O157 isolates appear as sorbitol negative, colorless colonies. To confirm the presence of these *E. coli* O157, the Oxoid *E. coli* O157 Latex Test (Oxoid Limited, Hampshire, UK) was performed using anti-O157 immunoglobulins, according to the manufacturer's instructions. Confirmation of *E. coli/Shigella* was obtained using the MALDI-TOF MS through adding 1µl of HCCA (Bruker, Massachusetts, USA) to a bacterial colony before reading the plate. Confirmation of STEC was performed using qPCR with the Pall Food Extraction Pack and GSTEHEC106006 GeneDisc for *stx1*, *stx2*, and *eae* detection. The obtained isolates from food and carcass swabs were then transferred to the NRC-STECC for further characterization and study of the relatedness with the human isolates (see main manuscript).

## References

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