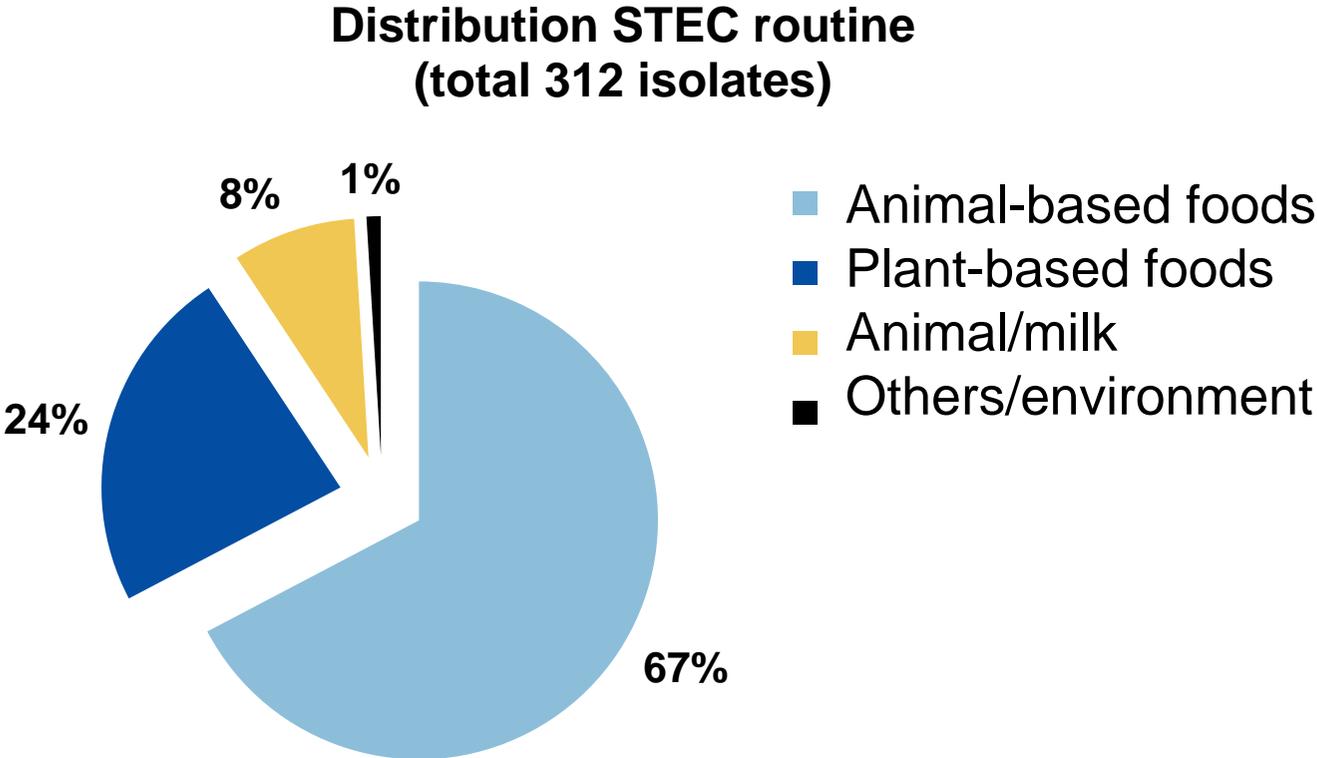
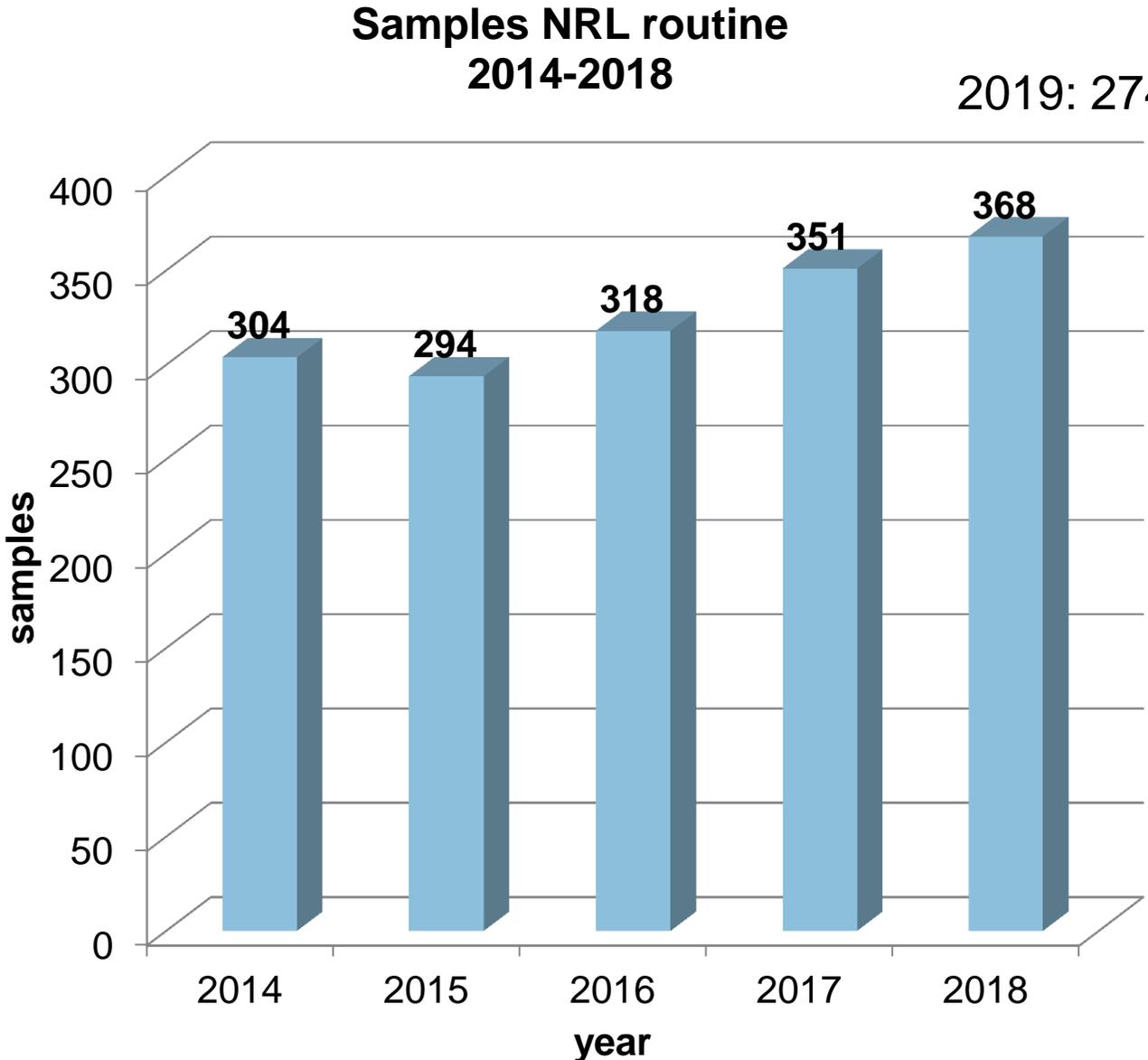


STEC in flour – data from Germany

Elisabeth Schuh and Marina Lamparter

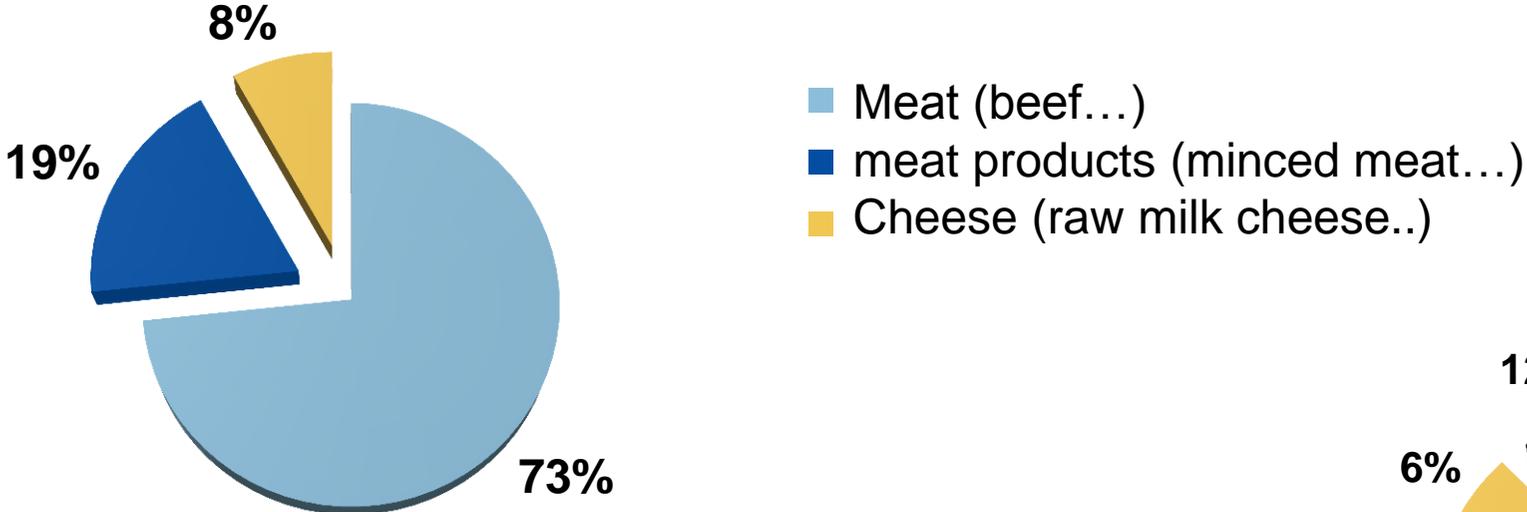


Overview samples routine NRL *E. coli* Germany

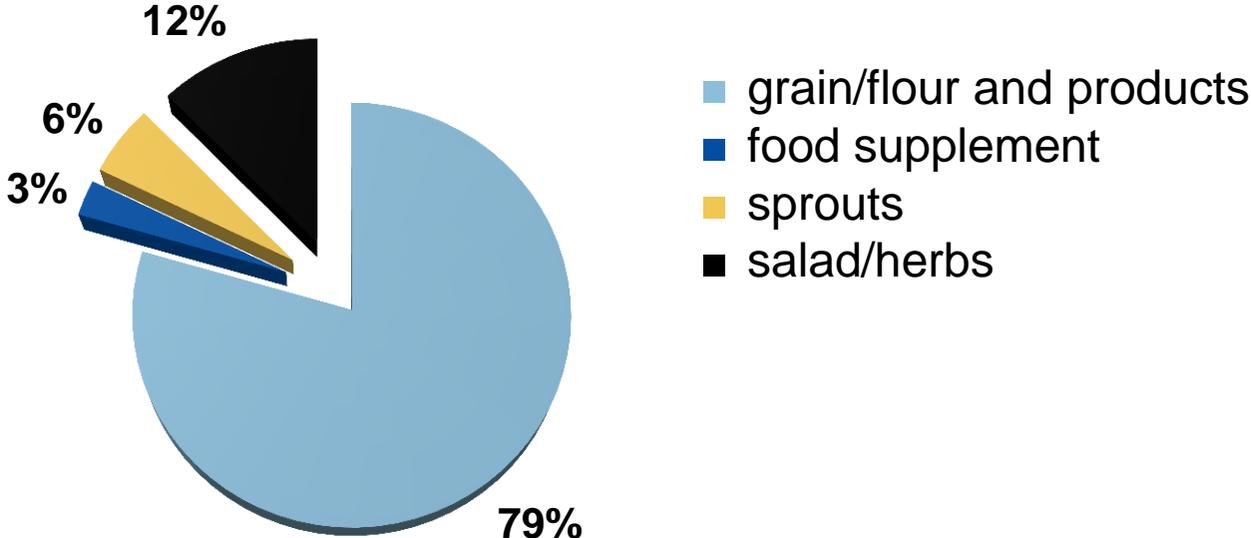


Distribution of isolates

**Animal-based foods
(total 210 isolates)**



**Plant-based foods
(total 73 isolates)**



STEC in flour - background

- Several outbreaks linked to flour or dough reported from USA and Canada (2009 – 2019)
- In Germany: Search for STEC in flour by Prof. Mäde and colleagues at state laboratory (Mäde et al. 2017)
 - 51 Samples (25 g) of wheat or rye flour from mills in Saxony-Anhalt between 2014 and 2017
 - Analysis of 98 Subsamples
 - Enrichment in buffered peptone water and plating on TBX agar
 - 39% positive by molecular detection with *stx* real-time PCR (38 samples)
 - Isolation from 17 positive flour samples (19%)
 - Modification of standard methods (ISO/TS 13136:2012) necessary for isolation
 - No statistical differences for STEC detection for different grain species
 - Increasing number of subsamples increased detection rate
 - Correlation between specific mills and STEC detection rate (Mäde et al. 2018)

Isolates from flour and flour products at the NRL

Samples received between 2015 and 2019
Sent by nine different federal states
Federal control plan 2018
133 (sub-)samples resulting in 105 singular STEC isolates

Samples from

Wheat (62)

Type 550

Type 405

Type 812

Type 1050

Others

Rye (27)

Type 1150

Type 997

Others

Spelt (5)

Mixed flour (6)

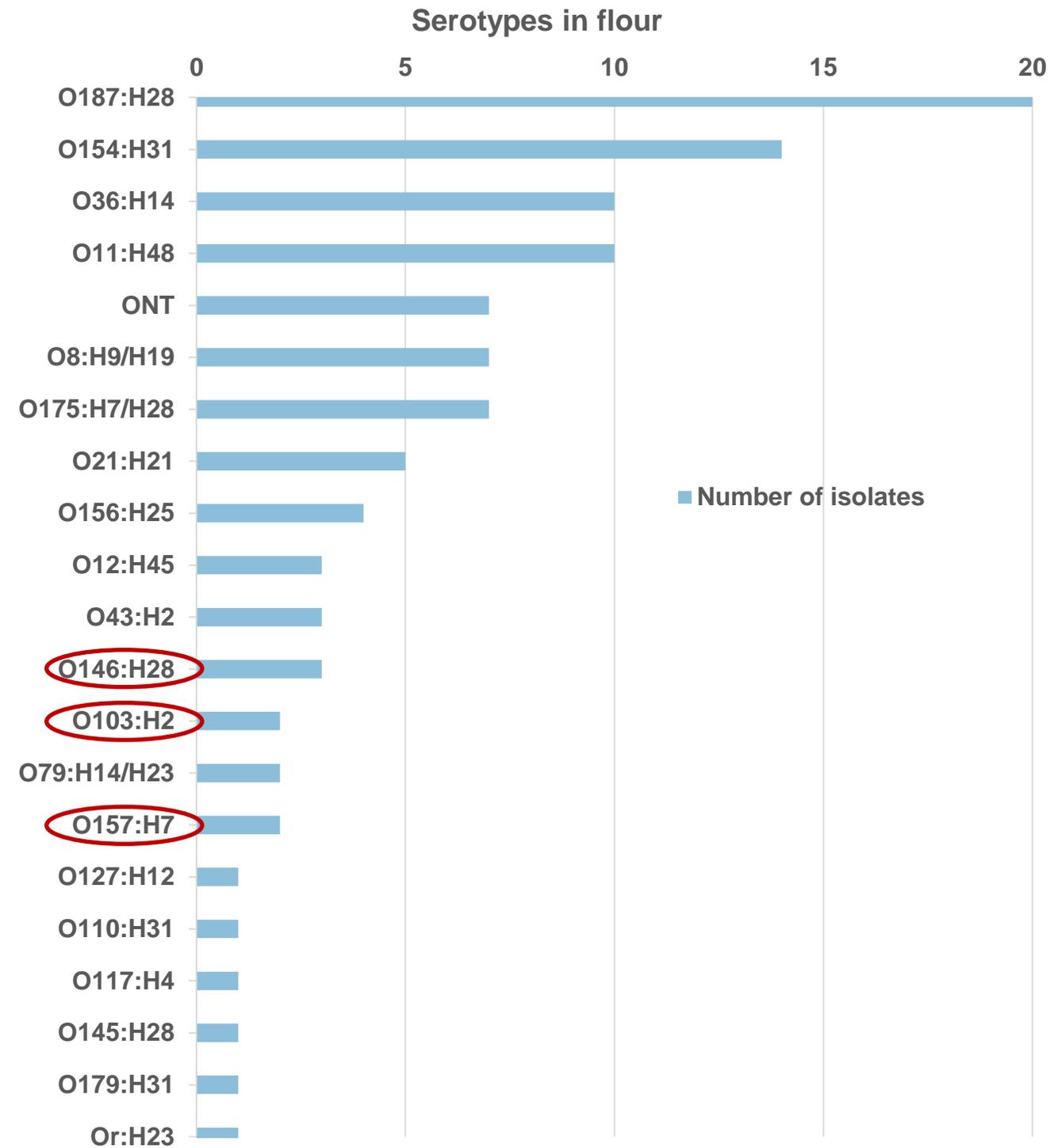
Baking mixture (4)

Waste grains (1)



Typing results

stx-subtype	Number of isolates
stx1a	7
stx1c	1
stx1d	37
stx2a	-
stx2b	15
stx2c	2
stx2d	-
stx2e	3
stx2f	-
stx2g	35
stx1 + stx2	-
eae (stx1a, stx2c)	9

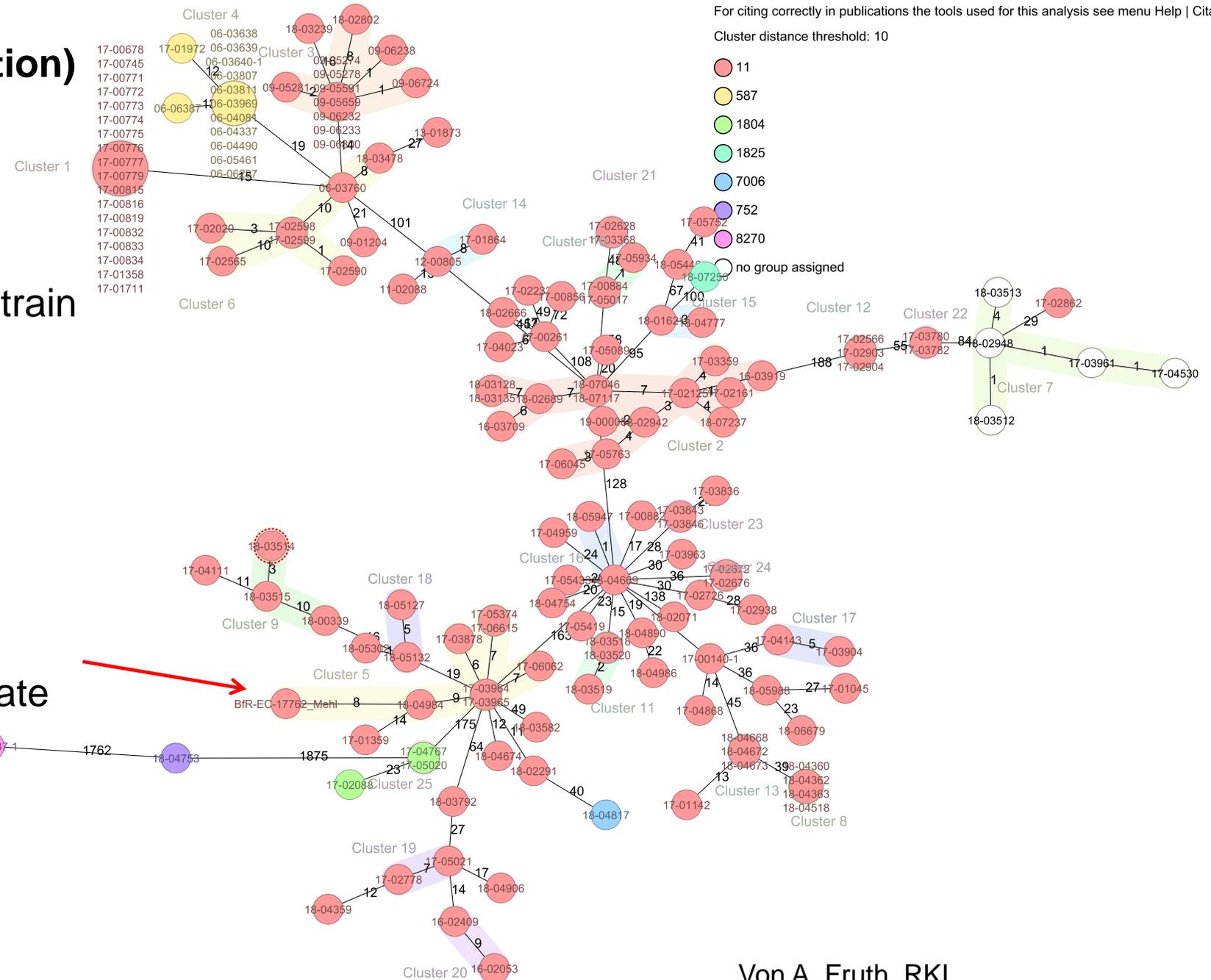


Comparison to human strains by cgMLST

Task Templates: E. coli cgMLST v1.0, E. coli MLST Warwick v1.0
 E. coli cgMLST Complex Type / Cluster-Alert distance: 10
 Comparison Table Retrieval: E. coli_cgMLST_CL [unstored]
 Comparison Table created: 04.03.2019 17:01 (v5.9.0-rc65_(2019-02))
 Ridom SeqSphere+ MST for 165 Samples based on 2055 columns, no missing values
 Distance based on columns from E. coli cgMLST (2050), E. coli MLST Warwick (5)
 For citing correctly in publications the tools used for this analysis see menu Help | Citations.
 Cluster distance threshold: 10

Ridom Seqsphere+, 2055 loci/alleles (RKI cooperation)

- O103 and O146 isolates: no match
- O157 flour isolate clustered to human O157 STEC strain by 8 alleles (threshold of 10)
 - Geographical distance of samples about 100 km



ARIES/Galaxy, 2360 loci/alleles

10 allele distance between O157 human and flour isolate

-> close genetic relation indication for high pathogenic STEC in flour also in Germany

Von A. Fruth, RKI

A glance at the literature

Source and survival of STEC in flour

- Contamination in crop production by organic fertilizer, irrigation, feces from wild ruminants¹
- STEC after more than 100 days reisolated from contaminated soil²
- Into the plant: damage, roots or leaf stomata, seed germination³
- Entry in the mills: dust encrusted grains, sediments in transportation bins, pests, tempering/conditioning step⁴
- Viability of STEC in flour:
 - Strain dependent
 - Protective effect against thermal inactivation
 - Detection (O26, O103, O111, O157, O145, O121, O45) at room temperature for at least nine month^{5,6}

1 Allende und Monaghan, 2015; Barak und Liang, 2008; Beuchat, 2002; Jacobsen und Bech, 2012; Li et al., 2015; Miles et al., 2009; Olaimat und Holley, 2012; Persad 2014, Laidler 2013, Mäde 2018; 2 Ma, Ibekwe et al. 2011; 3 Zhang, Ma et al. 2009, Zhang, Ma et al. 2009, Berger, Sodha et al. 2010, Saldana, Sanchez et al. 2011; 4 Gilbert 2010, Mäde et al 2017; 5 Forghani 2018, 6 Forghani 2019, Liu 2014



Summary & Outlook

- Flour is a minimal processed agricultural product, which could be contaminated
- It has normally not undergone an inactivation procedure. This could be a risk in the case of raw dough consume or insufficient heat treatment
- STEC is detectable in flour / methodical challenging
- Outbreaks are described (USA, Canada)
- STEC in flour in Europe? (Germany, Austria, Switzerland)
- Prevalence in Germany -> Zoonosis Monitoring 2020
- More research/ data needed



Brian deWitt / Flickr.com

Cookie dough

Aknowledgements

Federal State Laboratories / Prof. Dietrich Mäde

Robert Koch Institut / Dr. Angelika Fruth

Colleagues of the NRL E. coli

Sequencing unit of the BfR



Thank you for your attention

Elisabeth Schuh

German Federal Institute for Risk Assessment

Max-Dohrn-Str. 8-10 • 10589 Berlin, GERMANY

Phone +49 30 - 184 12 - 24204 • Fax +49 30 - 184 12 – 99 0 99

elisabeth.schuh@bfr.bund.de • www.bfr.bund.de/en

Task Templates: E. coli cgMLST v1.0, E. coli MLST Warwick v1.0

E. coli cgMLST Complex Type / Cluster-Alert distance: 10

Comparison Table Retrieval: E. coli_cgMLST_CL [unstored]

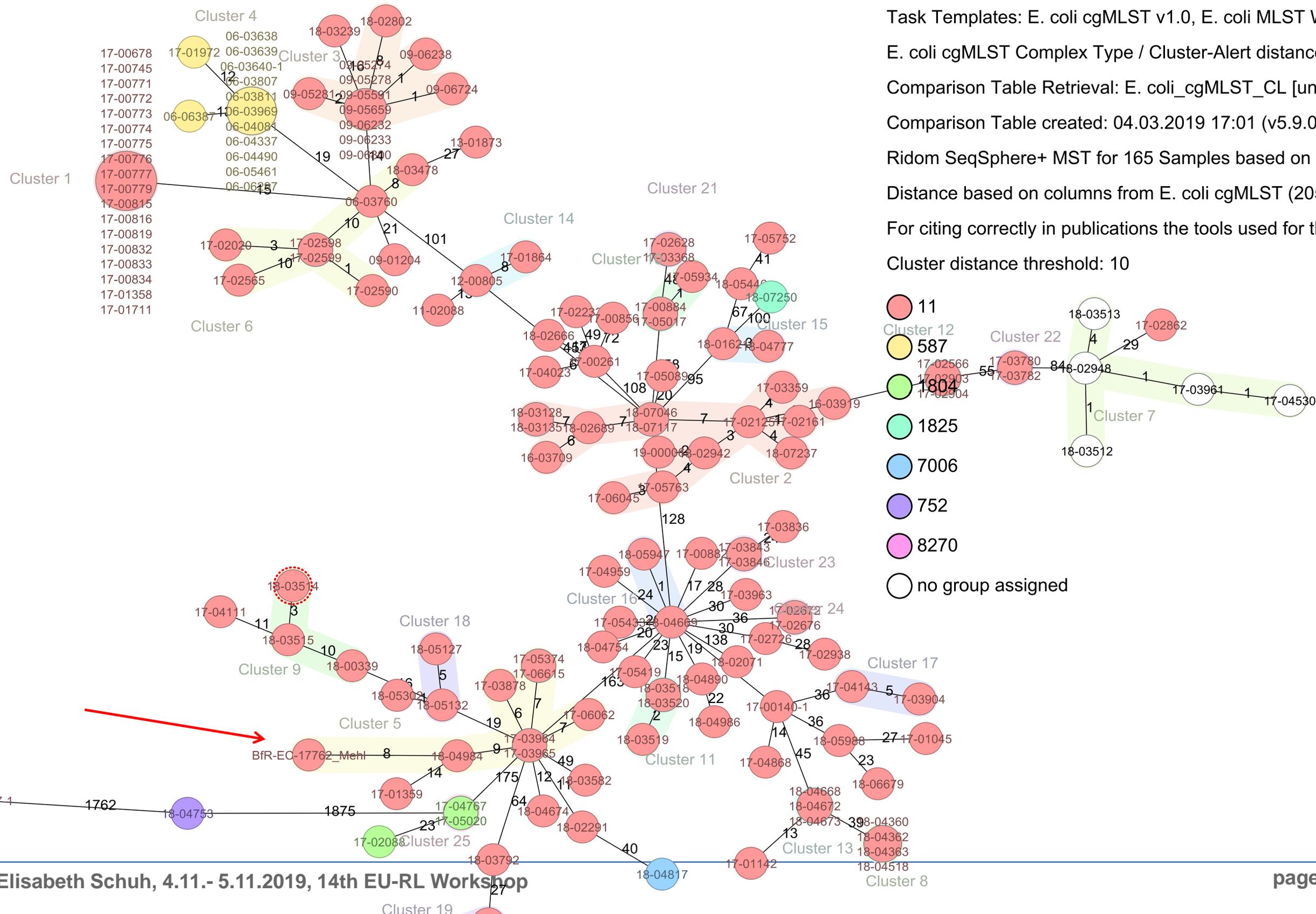
Comparison Table created: 04.03.2019 17:01 (v5.9.0-rc65_(2019-02))

Ridom SeqSphere+ MST for 165 Samples based on 2055 columns, no missing values

Distance based on columns from E. coli cgMLST (2050), E. coli MLST Warwick (5)

For citing correctly in publications the tools used for this analysis see menu Help | Cita

Cluster distance threshold: 10



Sequenzabgleich ARIES/Galaxy cgMLST I: Human - Mehl

cgMLST: 2360 Gene, kuratiert von innuendoweb.org

Workflow in Anlehnung an EU-RL VTEC (**modifiziert**):

Fastq positional and quality trimming –

SPADES (contigs) –

filter SPADES repeats – **Map with BWA-MEM – SAM-to-BAM** – pilon

(fasta) –

chewBBACA (Allele) – MentaLIST Distance Matrix

O157: 10 allele distance (8 Ridom)

Weiterer Abgleich von O187, O146, etc. Mehl-Wild Isolaten



ARIES

STEC in flour in Europe?

Country	Time frame	Samples	Detection	Isolation	Discussion	Literature
Germany (Saxony-Anhalt)	2014 - 2017	98	39 %	19 %	“Our findings show the frequent presence of STEC in flour in Germany.”	Mäde et al. 2017
Germany	May – December 2018	85* + 153	21,2 % bzw. 10,5 %**		Preliminary data from the federal control plan	
Austria	July 2017	31	19,4 %	3,2 % (9,6 %)	“Our findings indicate that flour may pose a risk for STEC infections also in Austria. “	Schlager et al. 2018
Switzerland	Oktober 2017 – March 2018	93	10,8 %	8,6 %	“Our results highlight [...] the fact that flour is a potentially and probably underestimated source of STEC infections in humans.”	Boss et al. 2019
Switzerland	March 2018	70	12,9 %	11,4 %	“it is important that STEC is recognized as a potential biological contaminant of raw flour, placing consumers of raw or inadequately cooked flour at risk of infection.”	Kindle et al. 2019

* 2 Subsamples were analyzed; **STEC detection