

# **Application of whole genome sequencing for foodborne outbreak investigations and epidemiological studies in Germany**

Burkhard Malorny

# European salmonellosis outbreak

## Update:

- 60 cases in six European countries
- Last cases august 22/23
- No source identified yet

## Rare Salmonella strain sickens 50 in five EU countries

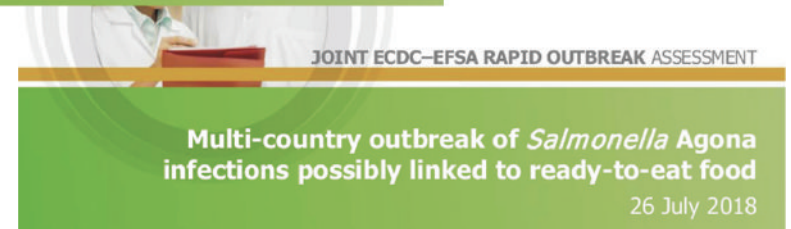
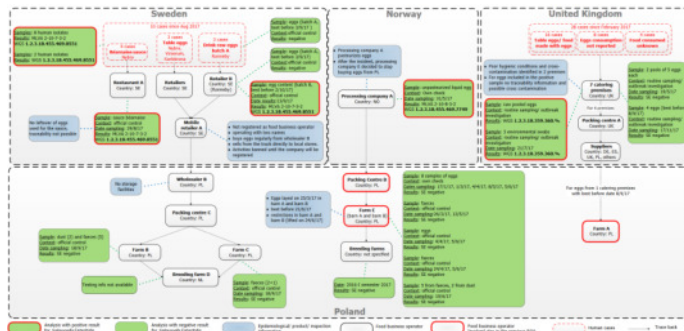
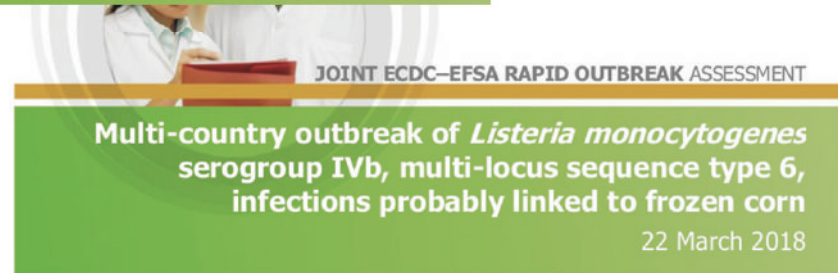
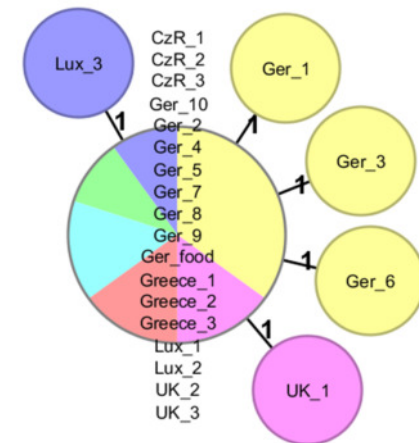
By **Joe Whitworth** on September 1, 2018

Around 50 people in five European countries have been struck down with a rare strain of Salmonella.

Salmonella Mikawasima has infected 15 people in Germany, 13 in Sweden, eight in both Denmark and the Czech Republic and six in Austria.

Source: Food Safety News. <https://www.foodsafetynews.com/2018/09/rare-salmonella-strain-sickens-50-in-five-eu-countries/>

# EFSA-ECDC Rapid outbreak assessments, Scientific opinions



# Outbreaks in Germany

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Campylobacter and raw milk was the most frequent food and pathogen combination

## Campylobacter and Salmonella behind most outbreaks in Germany

By **Joe Whitworth** on October 3, 2018

Almost 400 foodborne outbreaks occurred in Germany last year, according to a report. Most outbreaks with high evidence were caused by raw milk.

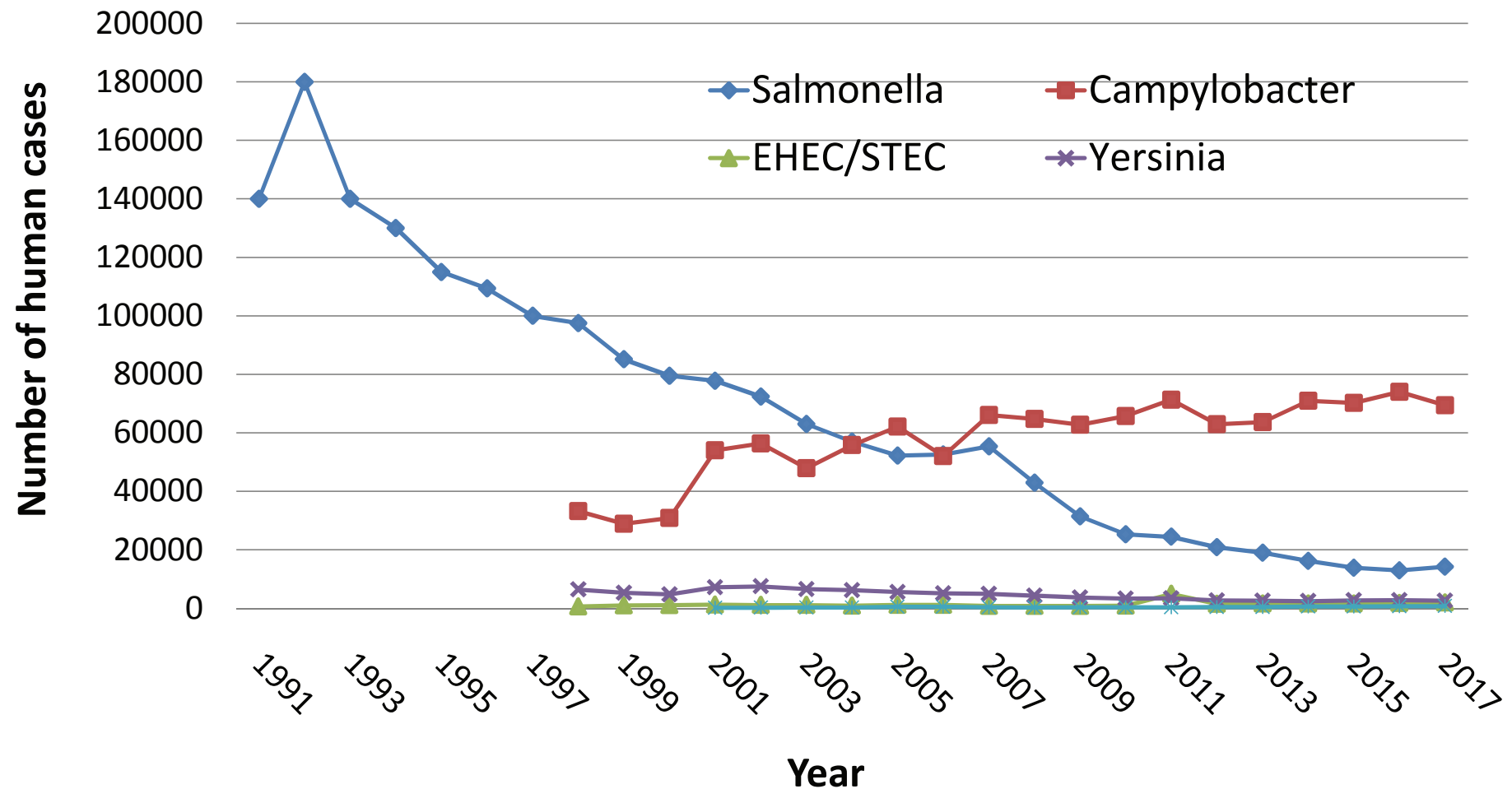
A total of 389 outbreaks involved at least 2,277 illnesses, 412 hospitalizations and four deaths. Salmonella was implicated in two deaths and Verotoxin-producing E. coli (VTEC) and Hepatitis A virus in one each.



Quelle: Fotolia, ddbase

Source: Food Safety News. <https://www.foodsafetynews.com/2018/10/campylobacter-and-salmonella-behind-most-outbreaks-in-germany/>

# Reported human cases in Germany



Source: RKI SurvStat, <http://www3.rki.de/SurvStat/>

# Reported foodborne outbreaks in Germany 2016/2017 with low evidence

	Number of outbreaks		Number of cases		Number of hospitalisation		Number of fatal cases	
	2016	2017	2016	2017	2016	2017	2016	2017
<i>Campylobacter</i> spp.	198	131	552	331	71	61	0	0
<i>Salmonella</i> Enteritidis	53	72	169	265	35	62	0	0
<i>Salmonella</i> Typhimurium	11	22	36	96	10	23	0	0
Other <i>Salmonella</i> spp.	18	25	65	122	18	51	2	0
VTEC (EHEC)	5	10	20	22	7	1	0	0
<i>Shigella</i> spp.	3	1	8	2	1	0	0	0
<i>Listeria monocytogenes</i>	2	1	4	2	4	2	1	0
<i>Francisella tularensis</i>	1	-	6	-	2	-	0	-
<i>Clostridium botulinum</i>	1	-	2	-	2	-	0	-
<i>Bacillus cereus</i>	1	3	3	53	0	0	0	0
Norovirus	29	17	627	134	21	10	0	0
Hepatitis Virus (A or E)	10	12	25	31	14	12	0	0
<i>Giardia</i>	6	7	13	15	0	1	0	0
<i>Cryptosporidium</i> spp.	2	3	4	8	0	0	0	0
Unknown pathogen	16	28	190	161	n.k.	7	0	0
<b>Total</b>	<b>356</b>	<b>332</b>	<b>1724</b>	<b>1242</b>	<b>185</b>	<b>230</b>	<b>3</b>	<b>0</b>

Source: Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL). 2017. Gemeinsamer nationaler Bericht des BVL und RKI zu lebensmittelbedingten Krankheitsausbrüchen in Deutschland 2017. [https://www.bvl.bund.de/DE/01\\_Lebensmittel/01\\_Aufgaben/9\\_BELA/BELA\\_node.html](https://www.bvl.bund.de/DE/01_Lebensmittel/01_Aufgaben/9_BELA/BELA_node.html)

# Reported foodborne outbreaks in Germany 2016/2017 with high evidence

	Number of outbreaks		Number of cases		Number of hospitalisation		Number of fatal cases	
	2016	2017	2016	2017	2016	2017	2016	2017
<i>Campylobacter</i> spp.	11	16	113	221	29	26	0	0
<i>Salmonella</i> spp.	9	14	81	334	13	106	0	2
Norovirus	6	3	462	173	14	2	0	0
<i>Bacillus cereus</i>	5	2	73	33	3	n.k.	0	0
Histamin	3	2	9	9	0	n.k.	0	0
VTEC (EHEC)	1	2	3	27	3	17	0	1
<i>Staphylococcus aureus</i>	1	3	21	43	n.k.	11	0	0
<i>Listeria monocytogenes</i>	1	-	12	-	10	-	1	0
<i>Clostridium botulinum</i>	1	-	4	-	4	-	0	0
<i>Clostridium perfringens</i>	1	4	3	114	0	n.k.	0	0
<i>Cryptosporidium</i> spp.	1	-	2	-	0	-	0	-
Hepatitis A	-	1	-	5	-	4	-	1
Flavivirus	1	1	2	13	2	1	0	0
<b>Total</b>	<b>41</b>	<b>48</b>	<b>785</b>	<b>972</b>	<b>78</b>	<b>167</b>	<b>1</b>	<b>4</b>

Source: Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL). 2017. Gemeinsamer nationaler Bericht des BVL und RKI zu lebensmittelbedingten Krankheitsausbrüchen in Deutschland 2017. [https://www.bvl.bund.de/DE/01\\_Lebensmittel/01\\_Aufgaben/9\\_BELA/BELA\\_node.html](https://www.bvl.bund.de/DE/01_Lebensmittel/01_Aufgaben/9_BELA/BELA_node.html)



# Listeriose outbreak in Germany 2012 – 2016

## caused by pork products

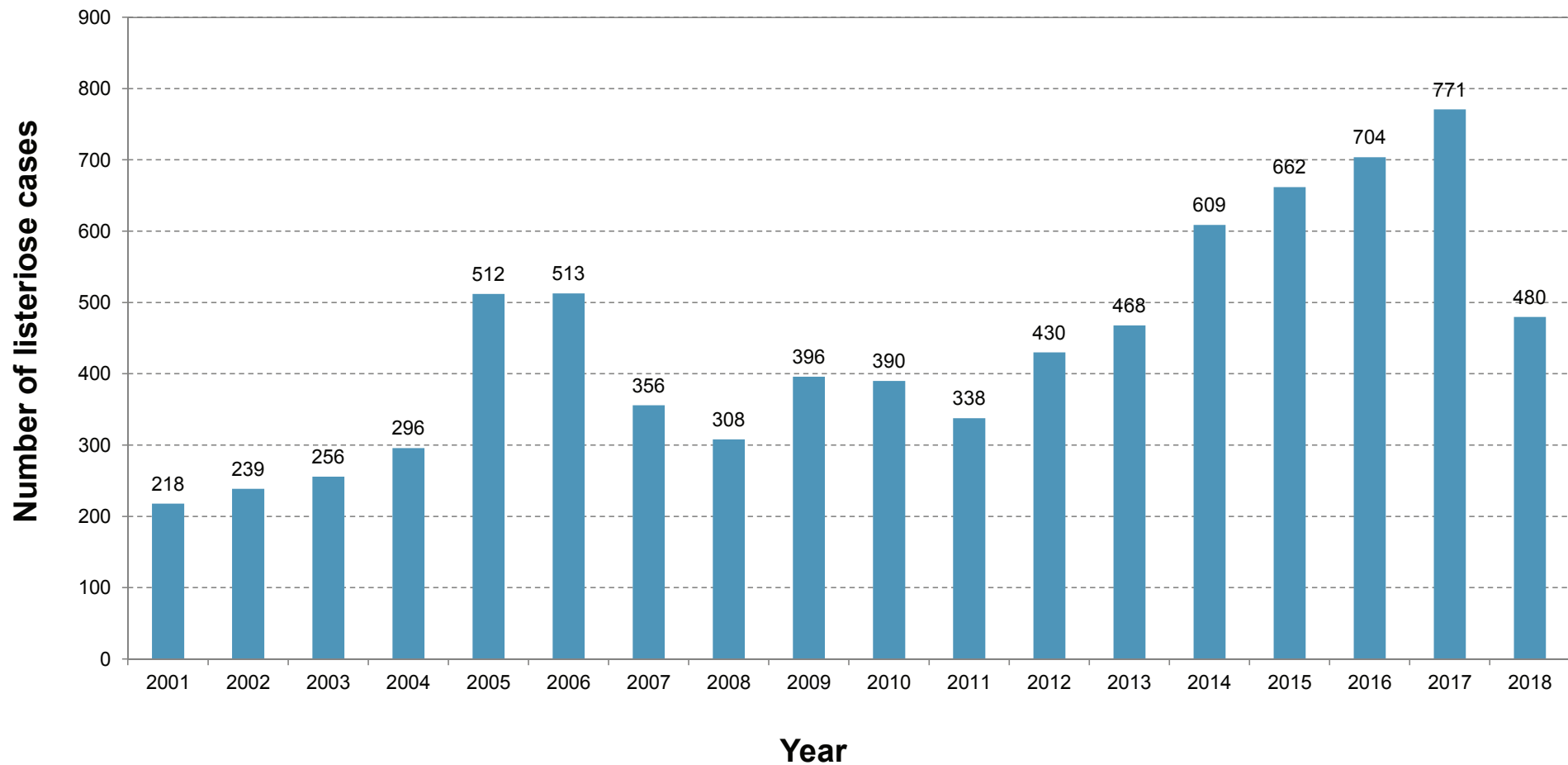
Publication: Kleta et al. (2017). Molecular tracing to find source of protected invasive listeriosis outbreak, southern Germany, 2012-2016. Emerging Infect. Dis. 23: 1680-1683





# Listeriose cases in Germany

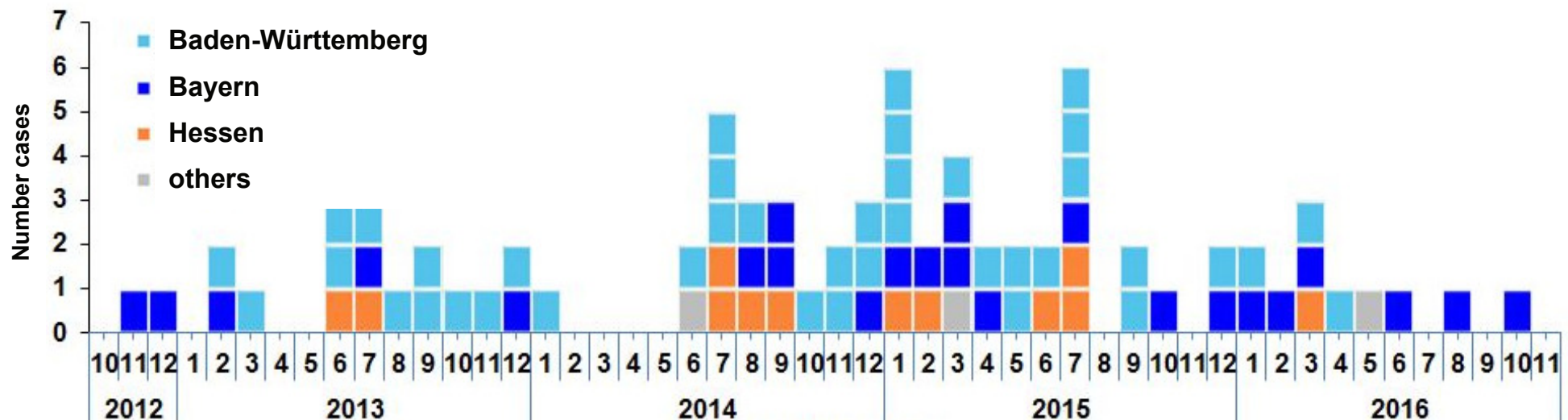
Source: SURVSTAT@RKI 2.0, data up to 04.10.2018



# Listeriosis outbreak, Germany, 2012-2016

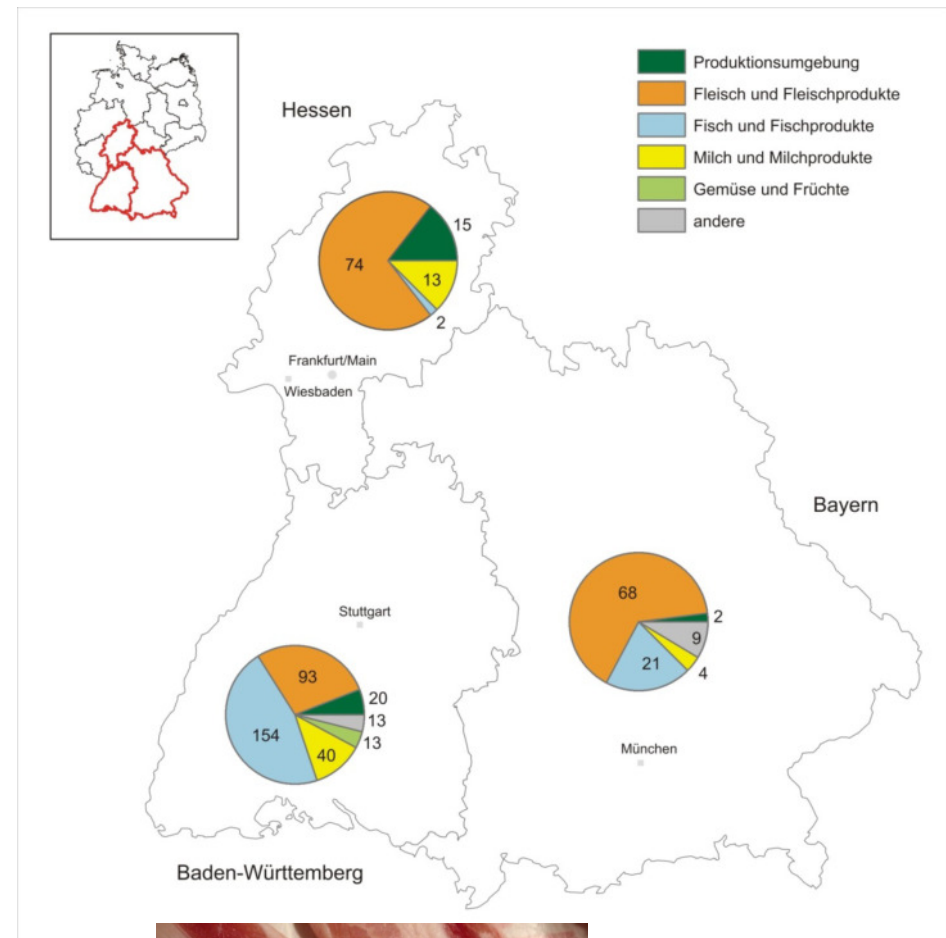
- 78 cases Nov 2012 – Oct 2016 in Germany
- 8 fatal cases, 4 confirmed by listeriosis
- explorative interviews without clear reference to causative food
- Several PFGE profile matches with NRL and EURL-Listeria database but no match of isolates with outbreak strain using whole genome sequencing

Epicurve [Source: Robert Koch-Institut]



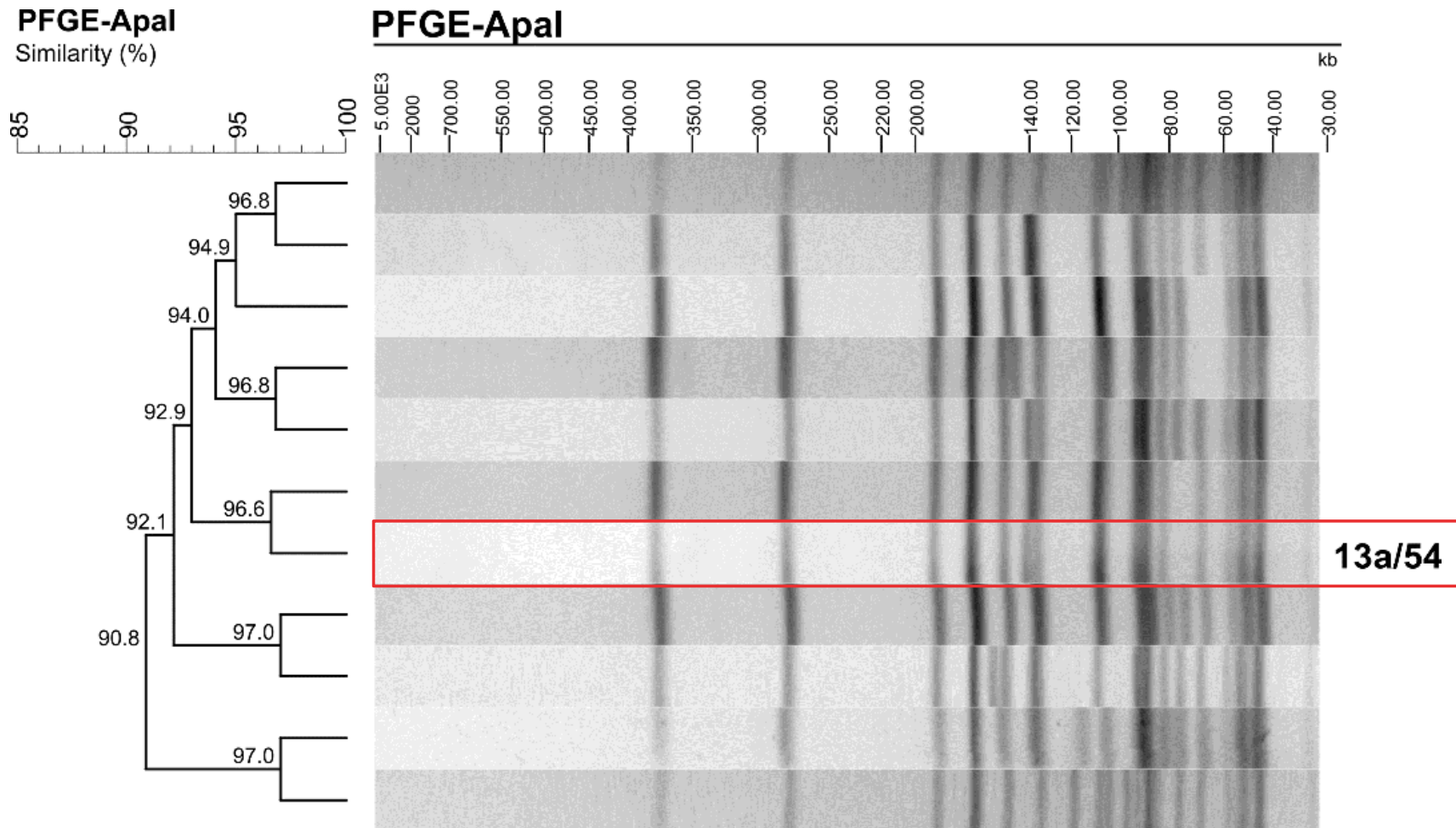
# Molecular typing of *L. monocytogenes* isolated from food

- Further epidemiological surveys point to pork products from supermarkets in southern Germany
- Intensified official sampling of food and production environments in BY, BW and HE
- Altogether 543 isolates were typed
- Smoked pork belly positive sampled by food inspectors positive (May 2016),  $1.9 \times 10^5$  CFU/g
  - ⇒ Match with human outbreak strain:  
**PFGE 13a/54, CT1248**
- 4 other products of company positive related to the outbreak strain



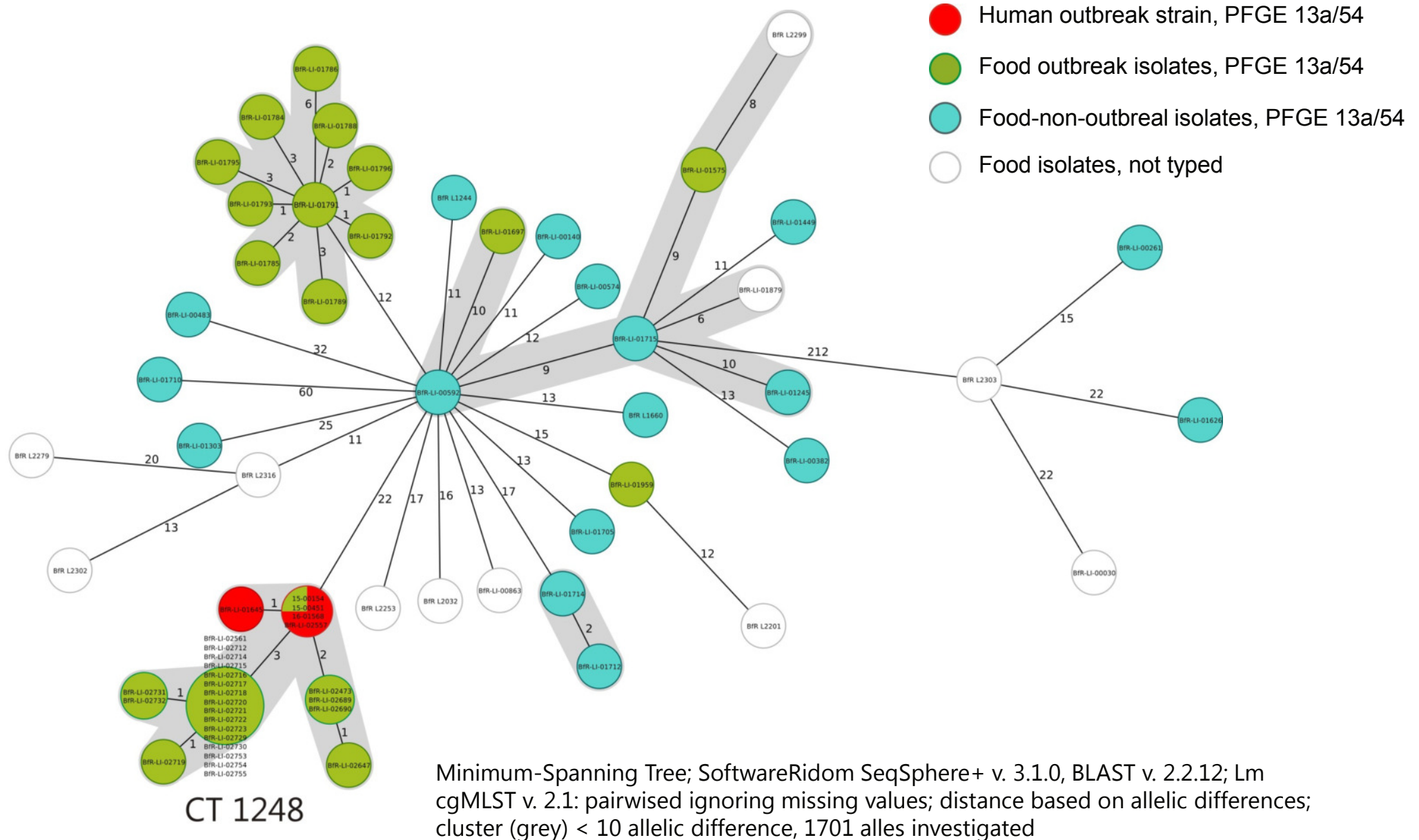
# PFGE-cluster-analysis (ApaI)

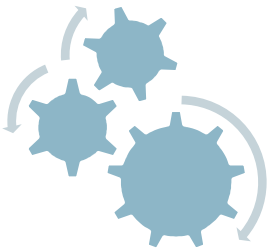
## human outbreak strain + 90% cluster similarity in food



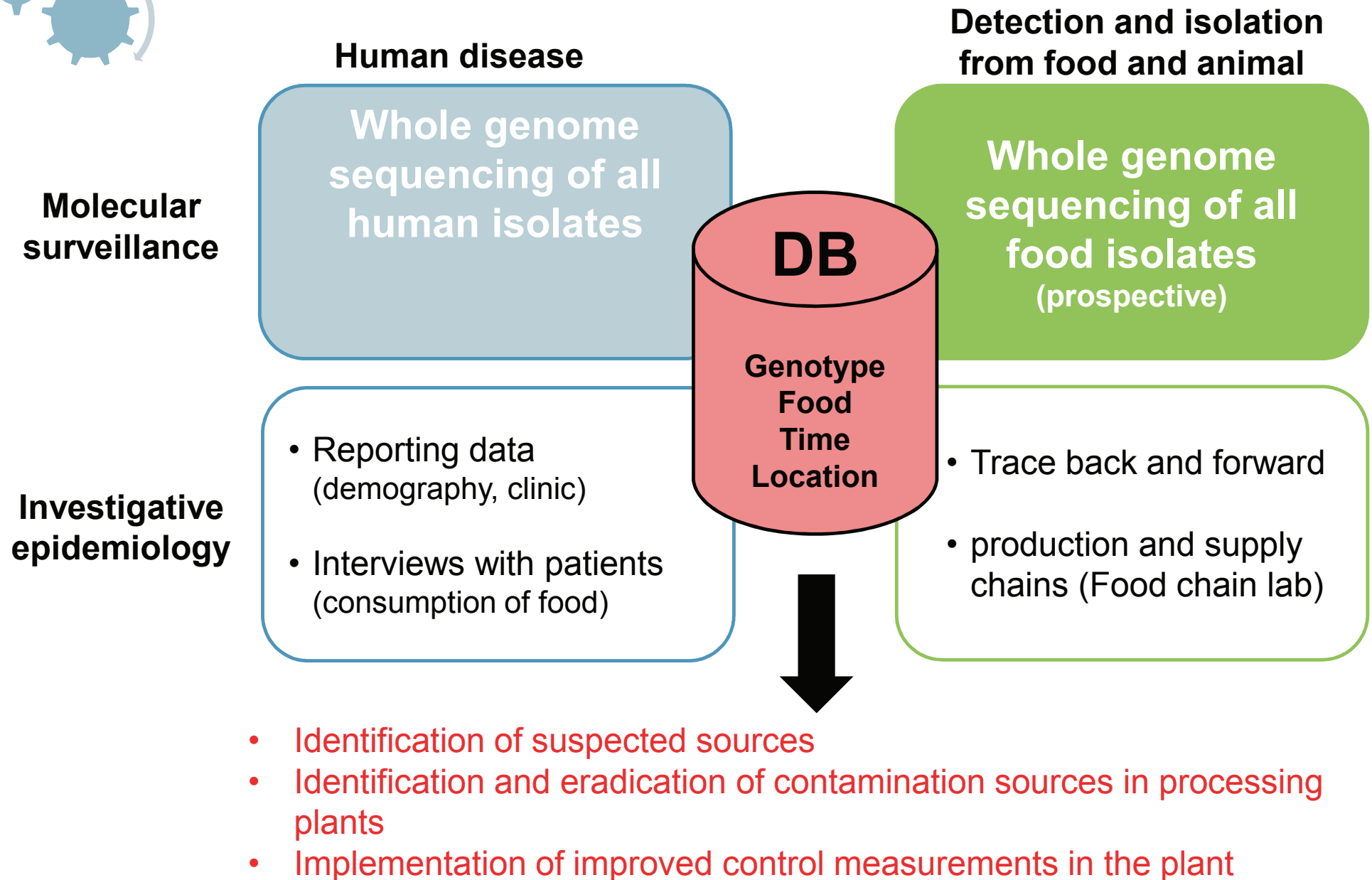
Bionumerics software v. 7.5 , similarity matrix using band based Dice coefficient with optimization 1% and band matching tolerance 1%, UPGMA method

# Whole-genome sequencing, cgMLST-analysis





# Efficient surveillance of foodborne pathogens



# Conclusions

- There is a transition in Europe to investigate outbreaks using WGS technologies
- Efforts are under way to establish a common database system for improved investigation of European outbreaks (EFSA and ECDC)
- Nomenclature of WGS data needs to be harmonized to easier comparison of data (worldwide problem!)
- Global Microbial Identifier initiative should be supported to harmonize WGS analyses and to create WGS repositories on global level





**Thank you for your attention**

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