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AN EHEC OUT BREAK SITUATION IN GERMANY – THE ROLE OF LAB DIAGNOSTIC

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The story of the E. coli out break in Germany

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- A novel strain of *Escherichia coli* O104.H4 (the "O" in the serological classification identifies the cell wall lipopolysaccharide antigen, and the "H" identifies the flagella antigen) bacteria caused a serious outbreak of foodborne illness focused in northern Germany in May through June 2011.
- The illness was characterized by bloody diarrhea, with a high frequency of serious complications, including hemulytic uremic syndrome (HUS), a condition that requires urgent treatment.
- The outbreak was originally thought to have been caused by an enterohemorrhagic (EHEC) strain of *E. coli*, but it was later shown to have been caused by an enteroaggregative verocytotoxin-producing *E. coli* (EAggEC VTEC) O104:H4 strain that had acquired the genes to produce Shiga toxins.

http://en.wikipedia.org

The story of the E. coli out break in Germany

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- On 30 June 2011, the Institut de veille sanitaire announced that the results of the analyses performed by the French national reference centre for *E. coli* and Shigella showed that the *E. coli* O104:H4 strain that caused the outbreak in Germany was also responsible for a small epidemic episode in France .
- The *E. coli* causing the outbreak in France in June is genetically related to the German outbreak strain. This strengthens the evidence pointing to a common source behind these outbreaks.
- Epidemiological fieldwork suggested fresh vegetables were the source of infection. The agriculture minister of Lower Saxony identified an organic farm in Bienenbüttel, Lower Saxony, Germany, which produces a variety of sprouted foods, as the likely source of the *E. coli* outbreak

http://en.wikipedia.org

The story of the E. coli out break in Germany

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- The same time the German *Bundesinstitut für Risikobewertung (BfR)* (*Federal Institute for Risk Assessment*), announced that seeds of fenugreek imported from Egypt were likely the source of the outbreak.
- In addition to Germany, where 3,785 cases and 45 deaths had been reported as of 27 July, a handful of cases were reported in several countries including Switzerland, Poland, the Netherlands, the UK, Canada and the USA.
- Essentially all affected people had been in Germany or France shortly before becoming ill.

http://en.wikipedia.org

The story of the E. coli out break in Germany

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- Initially German officials made incorrect statements on the likely origin and strain of E. coli.
- The German health authorities, without results of ongoing tests, incorrectly linked the O104 serotype to cucumbers imported from Spain.
- Later, they recognized that Spanish greenhouses were not the source of the *E. coli* and cucumber samples did not contain the specific *E. coli* variant causing the outbreak.

http://en.wikipedia.org

The story of the E. coli out break in Germany

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- Spain consequently expressed anger about having its produce linked with the deadly *E. coli* outbreak, which cost Spanish exporters 200M € per week.
- Russia banned the import of all fresh vegetables from the European Union until June 22.

http://en.wikipedia.org

Global situation of E. coli O104:H4 infections, 30. June 2011

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Country	HUS		EHEC	
	Cases	Deaths	Cases	Deaths
Austria	1	0	4	0
Canada	0	0	1	0
Czech Republic	0	0	1	0
Denmark	9	0	14	0
France	8	0	10	0
Germany	845	31	3154	17
Greece	0	0	1	0
Luxembourg	1	0	1	0
Netherlands	4	0	7	0
Norway	0	0	1	0
Poland	2	0	1	0
Spain	1	0	1	0
Sweden	18	1	35	0
Switzerland	0	0	5	0
United Kingdom	3	0	3	0
United States of America	4	1	2	0
Total	**896**	**33**	**3241**	**17**

The table below shows the total number of globally reported cases and deaths from *E. coli* (EAggEC VTEC) O104:H4 infection since the beginning of the outbreak in Germany on 1 May 2011.
In total, 16 countries in Europe and North America have reported 4137 cases of *E. coli* O104:H4 infection, including 50 fatalities.

http://www.euro.who.

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- *Escherichia coli* O104:H4 is a rare enterohemorrhagic strain of the E. coli.
- Analysis of genomic sequences obtained by BGI (http://bgiamericas.com) Shenzhen show that the O104:H4 outbreak strain is an EAEC or EAggEC type E. coli that has acquired Shiga toxin genes, presumably by horizontal gene transfer.

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- Genome assembly and copy number analysis both confirmed that two copies of the Shiga toxin stx2 prophage gene cluster are a distinctive characteristic of the out break strain.
- The O104:H4 strain is characterized by the following genetic markers:
  - Shiga toxin stx2 positive;
  - terE positive (tellurite resistance gene cluster) ;
  - eae negative (intimin adherence gene) ;
  - ß-lactamases ampC, ampD, ampE, ampG, ampH are present;

The relation of age and sex of HUS affected persons

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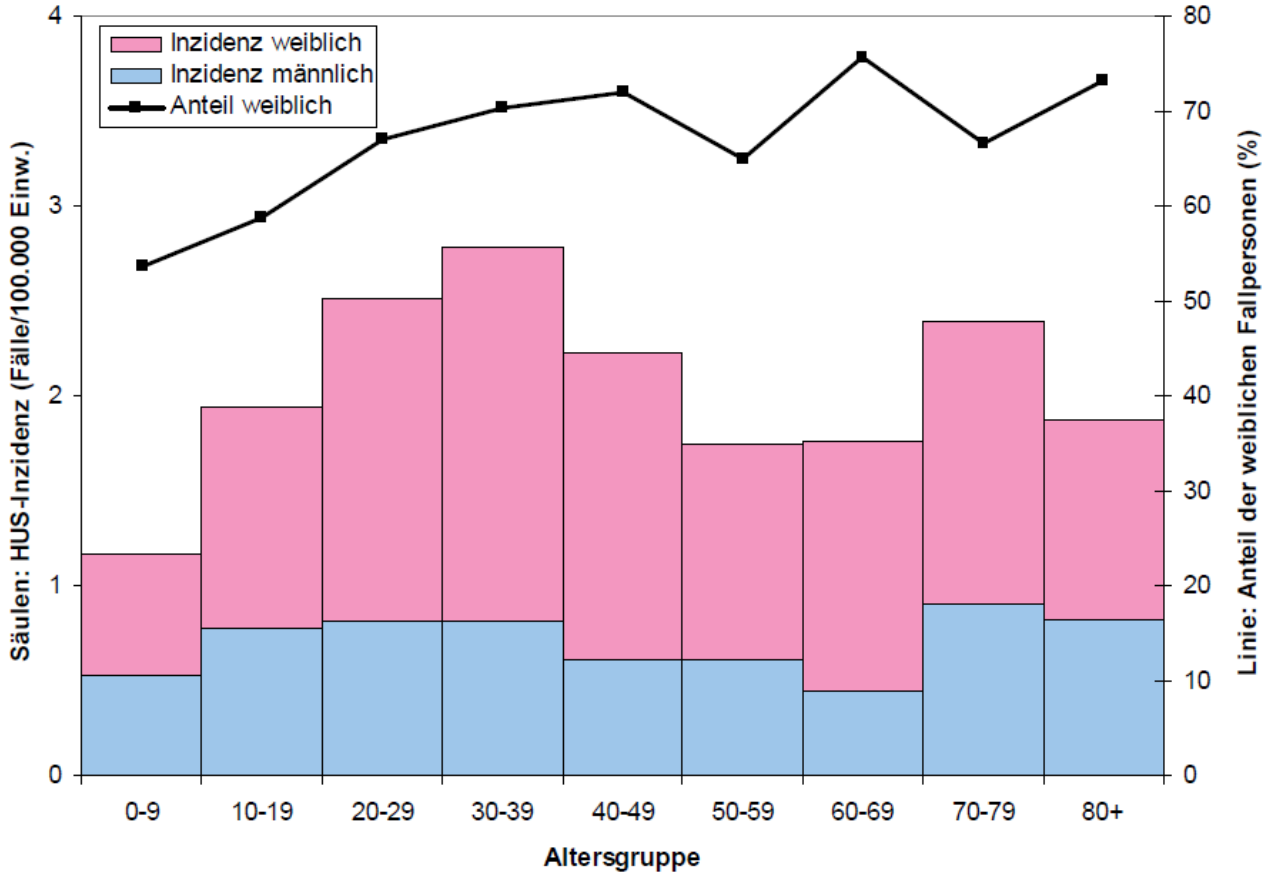

Abbildung 1: Inzidenz von HUS nach Altersgruppe und Geschlecht (linke Y-Achse) sowie Anteil der weiblichen Fallpersonen (rechte Y-Achse) pro Altersgruppe (n=855 HUS-Fälle).

http://www.rki.de

The relation between HUS and confirmed EHEC outbrake cases

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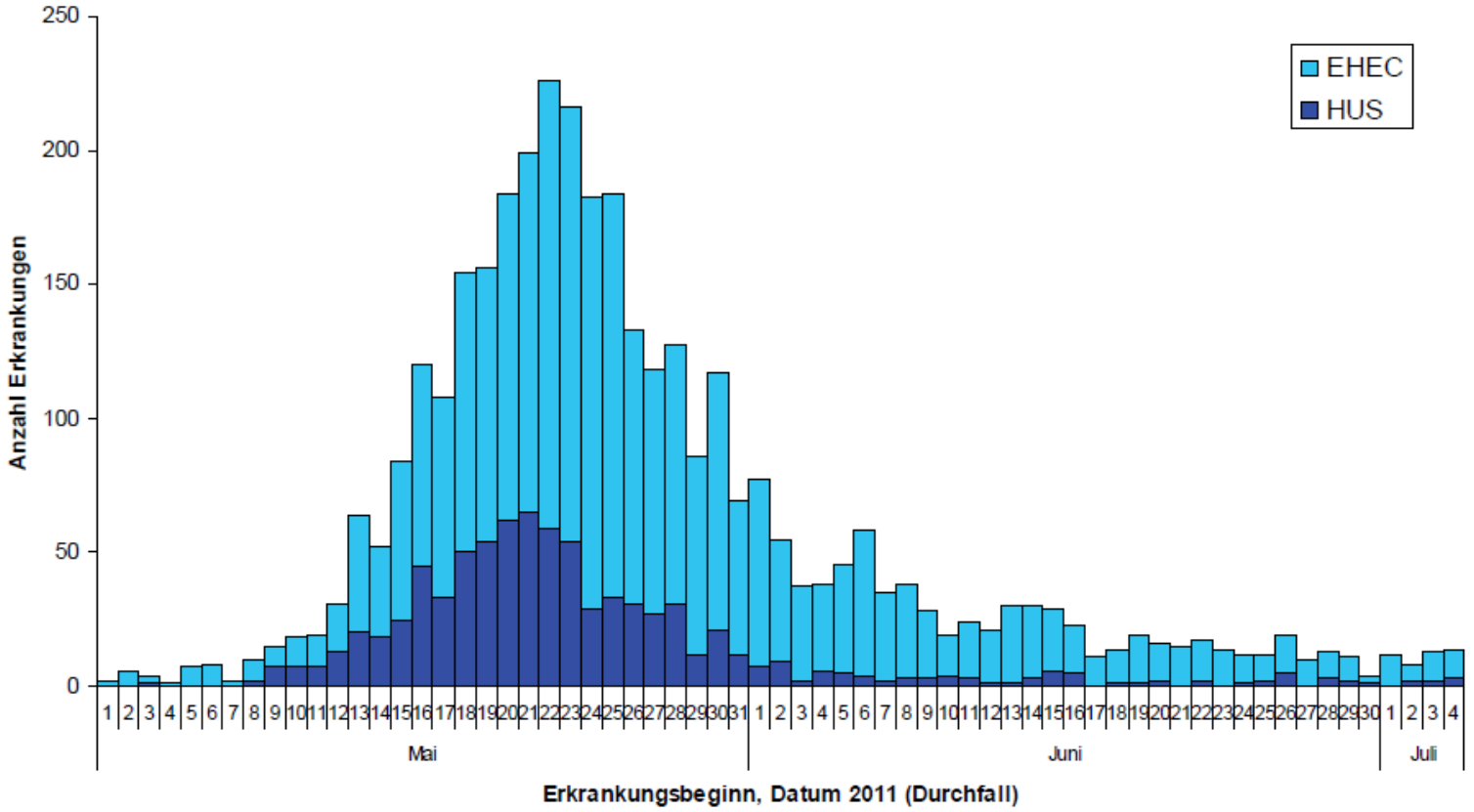

Abbildung 2: Epidemiologische Kurve der HUS- und EHEC-Ausbruchsfälle (809 HUS- und 2.717 EHEC-Fälle mit bekanntem Erkrankungsbeginn an Durchfall im Ausbruchszeitraum).

http://www.rki.de

The relation between incidence of HUS and the location the infection occurred

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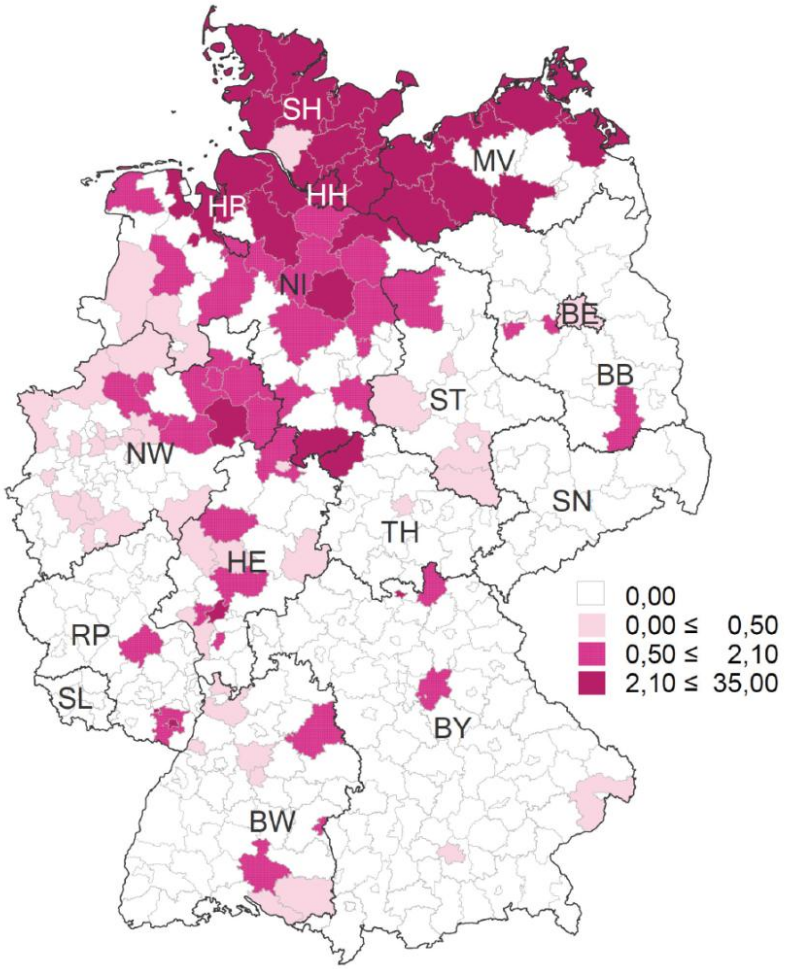

Abbildung 3: Inzidenz (Fälle pro 100.000 Einwohner) von HUS im Ausbruch, abgebildet nach Kreis, in dem die Infektion wahrscheinlich stattgefunden hat (Wohnortkreis, oder bei Reiseanamnese Aufenthaltskreis zum Zeitpunkt der Infektion).

http://www.rki.de

Pathogenic *E. coli*

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E.coli-Pathotype	Clinical syndrom
Enterotoxic E.coli (ETEC)	Travel diarrhoea
Enteroinvasive E.coli (EIEC)	*Shigellose*-like illness
Enteropathogenic E.coli (EPEC)	Diarrhoea escpecially in children
Enterohämorrhagic E.coli (EHEC)	Hemorrhagic colitis, HUS
Enteroaggregative E.coli (EAEC)	Diarrhoea escpecially in children
Diffuse adhaerent E.coli (DAEC)	Diarrhoea escpecially in children

Pathways for infection

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- Smear infection from fecal contaminated food e.g. meet, salad, sausage, milk, sprouted food, fruit juice and tap water.
- Smear infections from human to human or animal to human.
- Low infection dose of 10 –100 germs.

Virulence and pathogenicy factors

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- Shigatoxin genes are a common signature for all EHECs.
- Production of shigatoxin is the main cause of HUS.
- We distinguish between Stx1 und and variants of Stx2: Stx2c, Stx2d, Stx2e und Stx2f
- In EHEC-strains Stx1 and/or Stx2 are found.

GenoType® EHEC, Hain Lifescience

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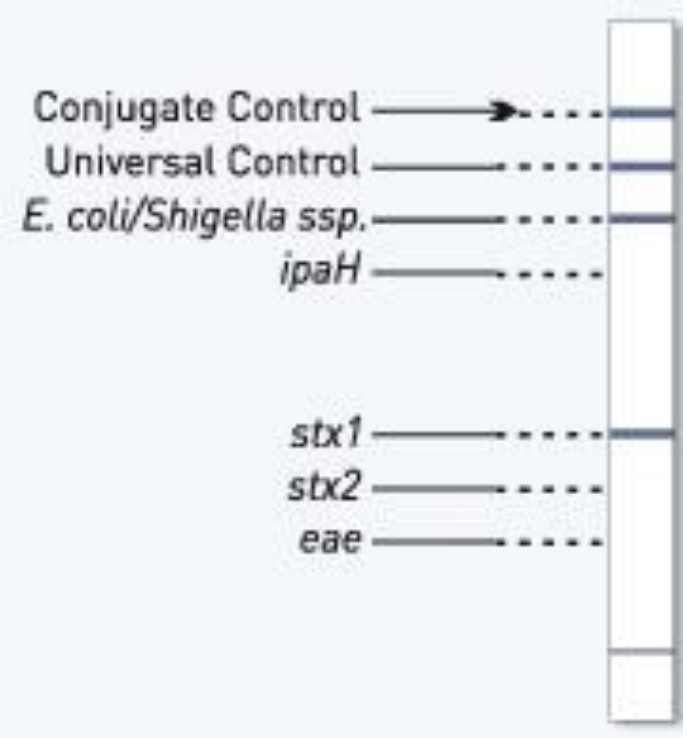

GenoType® EHEC, Hain Lifescience

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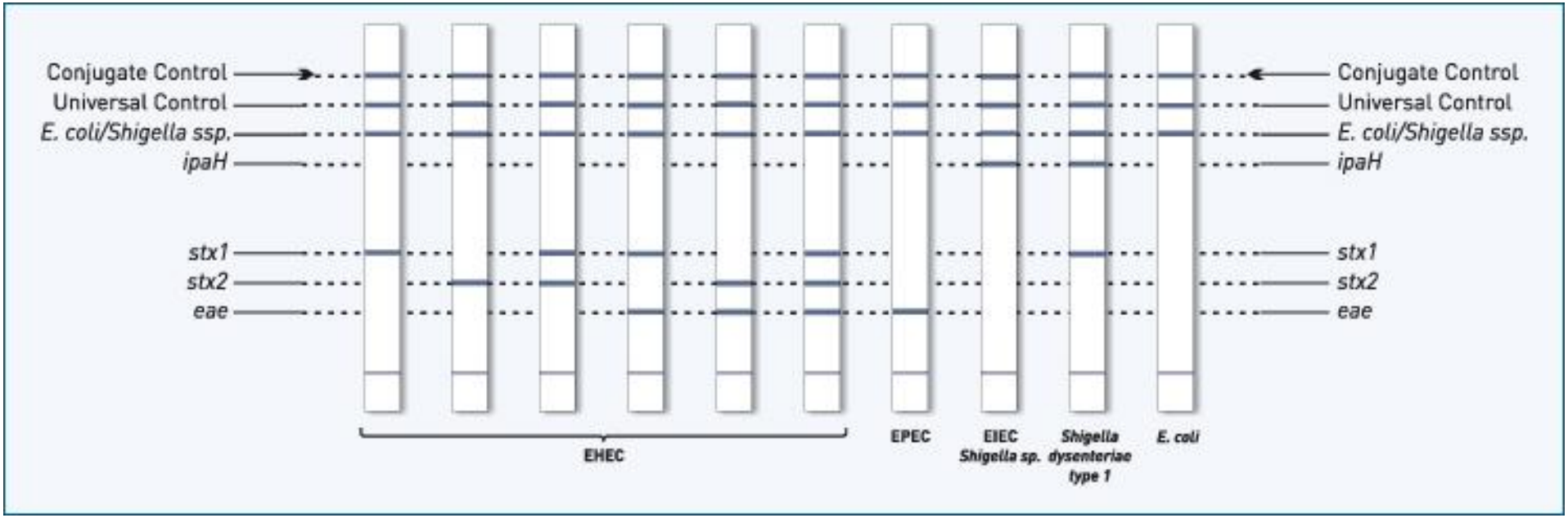

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- Laboratory Limbach investigated between 21. May to 26. July 2011, 2407 stool specimens. This was ten times more compared to previous months.
- In 122 (5%) samples pathogenic E. coli was detected
- In a first period all positive samples containing stx 2 were send to the German Reference Center (RKI), Wernigerode.
- Later on an in house PCR based on published primer for specific detection of O104:H4 were additional implemented to the Hain GenoType EHEC PCR.
  (http://www.ehec.org/pdf/LaborInfo_30052011.pdf).

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- The RKI investigated during the outbreak-periode 3244 specimens, sent with a first diagnosis of an pathogenic E. coli to the reference center.
- 1023 contained strain O104:H4
- 702 were pathogenic E. coli, not related to the outbrake strain.
- In 209 cases strain typing failed for unknown reasons.
- 590 specimens were Stx negative.

The diagnostic pattern for diagnosing EHEC, laboratory Limbach

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Stool

↓

Inoculation of a Mac Conkey-bouillon

↓

Inoculation of 2 MC-agar plates
1x confluent pattern,
1x striking pattern

↓

Cell wash from the confluent pattern after incubation

↓

PCR, GenoType EHEC, Hain

↓

Sxt1/eae

↓

EHEC /EPEC

Stx2 or Stx1 + 2
(independent eae+ oder Ø)

Inoculation of a swab with cultured cells

To the RKI, Wernigerode

* Contains two specimens of one patient ;
** 5 specimens not confirmed by the RKI were repeatedly send to the RKE. Two samples could be confirmed as O104:H4, 3 were repeated not confirmed.

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61x EHEC stx 2	50xO104:H4*	O104:H4 →32 ♀ and 18 ♂ Stx2+, eaeØ, ESBL+	49/50 comfirmed RKI
	1x0157:H7	0157:H7, Stx2+	RKI
	1xEHEC 2	Stx2+, not related to the out brake strain, no typing result	RKI
	1xEHEC 2	Stx2+, no typing result	RKI
	1xEHEC 2	Stx2+, eae+, ESBLØ, not related to the out brake strain	Laboratory Limbach
	7xEHEC 2**	Not confirmed by the RKI	RKI
11x EHEC stx 1+2	1xEHEC 1+2	O157:H-, Stx1+2 +	RKI
	1xEHEC 1+2	O5:H-, Stx1+2 +	RKI
	4xEHEC 1+2	Stx1+2+, no typing result	RKI
	5xEHEC 1+2	Not confirmed by the RKI	RKI

Case reports

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- A couple was having dinner in a Northgerman restaurant. The husband ordered a beef steak which was decorated with sprouts.
- He passed over the sprouts to his wife.
- The lady acquired a O104:H4 infection.

Case reports

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- Six persons with an outbreak strain infection had lunch in a factory canteen, which offered sprouts delivered by the company Sodexo from Lower Saxonia contaminated with O104:H4 .

Case reports

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- A family including two kids (4 and 7 years) were all infected with O104:H4.
- In one kid O104:H4 was detectable for 55 days, the other for 49 days.

What is the diagnostic situation today?

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- We see a slight increase in EHEC requests, but EHEC is still underdiagnosted.
- We still lack reimburment figures for EHEC diagnosis.
- As PCR has to be part of EHEC diagnosis methods, we have increased lab costs.

What is the diagnostic situation today?

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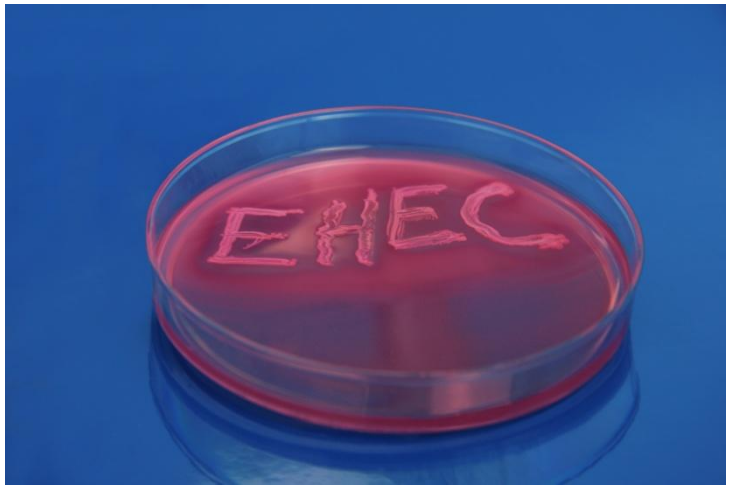
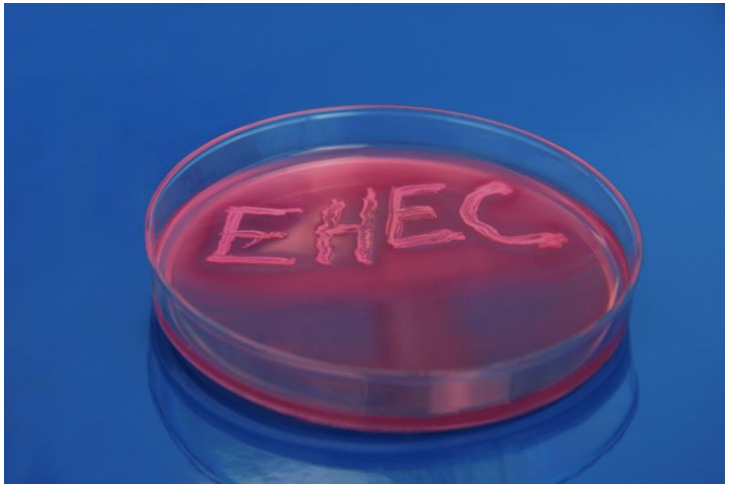
- EHEC PCR from stool specimen is possible, but when compared to PCR from pre-cultured cells it is discrepant in some cases (like with pre-cultured specimens).
- Value of speed is yet not clearly quantified.
- Mostly, sensitivity for bacteria detection from stool specimens is improved, when more than one stool aliquot is tested.

Conclusions



- This was the most severe EHEC outbreak in Germany and regarding the numbers of HUS the most prevalent world wide.
- We have to notice that after the identification of the contaminated sprouts the number of cases declaimed to normal levels.
- Epidemiological investigations were the most important weapon to stop the infection chain.
- The outbreak strain was not able to establish itself in Germany.

Conclusions



- The RKI strengthens the efforts to improve hygiene procedures especially in the public food sector (canteens, restaurants etc.)
- All identified cases of EHEC gastroenteritis and/or HUS has to be reported to the RKI [Infektionsmeldegesetz(IfSG) §6 and §7]
- A laboratory has to report a EHEC case after **molecular** detection of toxin genes. Serological typing is optional.

Conclusions

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- The good thing what we learned from this dramatic outbreak is, that we learned how to act against similar situations in the future.
- The most effective weapon in a outbreak situation is epidemiology and diagnostic.
- But we also learned, that we can never be 100% save from epidemic bacterial outbreaks also in countries with an excellent hygiene standard.

Thank you for attention!

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Thank you to the colleagues from the Microbiology department laboratory Limbach:
Dr. Marion Rolhfs
Dr. Ulrich Eigner
Dr. Martin Holfelder