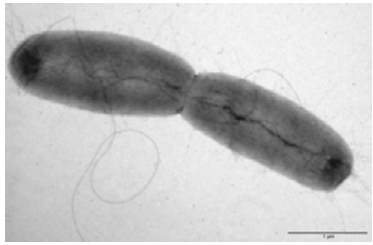
GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

Microbiological threats: how have Germany and France responded to the crisis with enterohemorrhagic *E. coli* (EHEC) and what about the future?

Dr. Elisabeth Hauser (BfR) / Dr. Patrick Fach (ANSES)

Enterohemorrhagic *Escherichia coli* (EHEC)



Enterohemorrhagic *E. coli* (EHEC):

„Shiga toxin-producing *E. coli* (STEC syn. VTEC), capable to cause symptoms of illness in humans and are therefore pathogenic for humans“

Shiga toxin-producing *E. coli:*

Diverse group of *E. coli* with more than 500 described serotyps

- common feature: production of Shiga toxin (Stx)

Reservoir: intestinal tract of cattle, other ruminants (goat, deer …)

- **Infection by contaminated food like undercooked meat, raw milk, sprouts …**

Most important virulence factors and serogroups

Shiga toxin (Stx)

Two different types Stx1 and Stx2

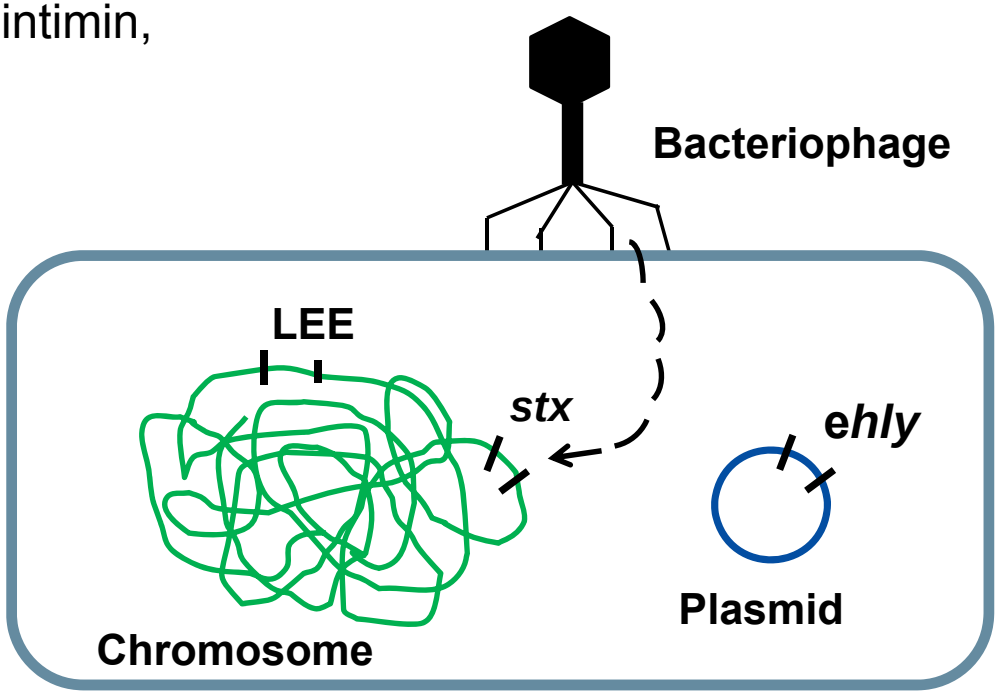
Several subtypes (e.g. Stx2a)

Locus of Enterocyte Effacement (LEE)

Pathogenicity island, *eae* marker gene, intimin, adhesion factors

Important serogroups

O157
O26
O103
O111
O145
O45
O91
...



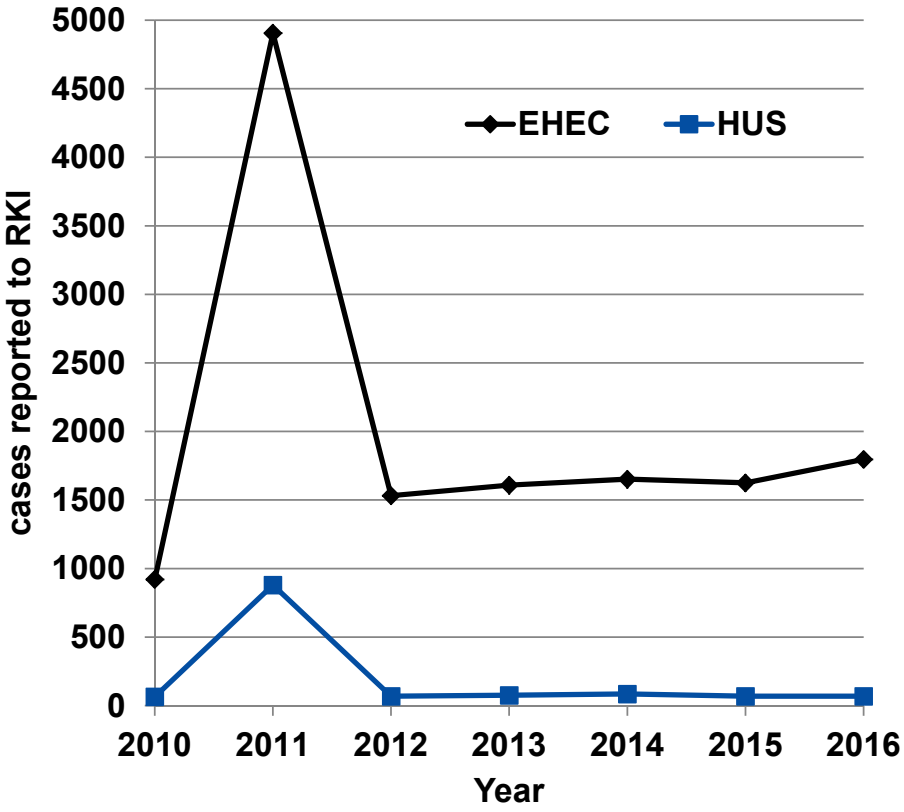
Symptoms and Epidemiology

Infectious dose: 10-100 bacteria

Symptoms of EHEC infection:

- Diarrhoea / Bloody diarrhoea
- Hemolytic uremic syndrome (HUS) including renal failure
- neurological disorders

Reported cases 2016: EHEC 1797, HUS 69



EHEC outbreak 2011

To the RKI reported EHEC/ HUS cases	**2.987 EHEC** **855 HUS**
To the RKI reported fatal cases due to EHEC/ HUS	**18 EHEC** **35 HUS**

Source: RKI, „Abschließende Darstellung und Bewertung der epidemiologischen Erkenntnisse im EHEC O104:H4 Ausbruch Deutschland 2011“

Source: RKI, Epidemiologisches Bulletin 03/2011, 03/2012, 03/2013, 03/2014, 03/2015, 03/2016, 03/2017

HUS incidence and epidemiological curve of the outbreak

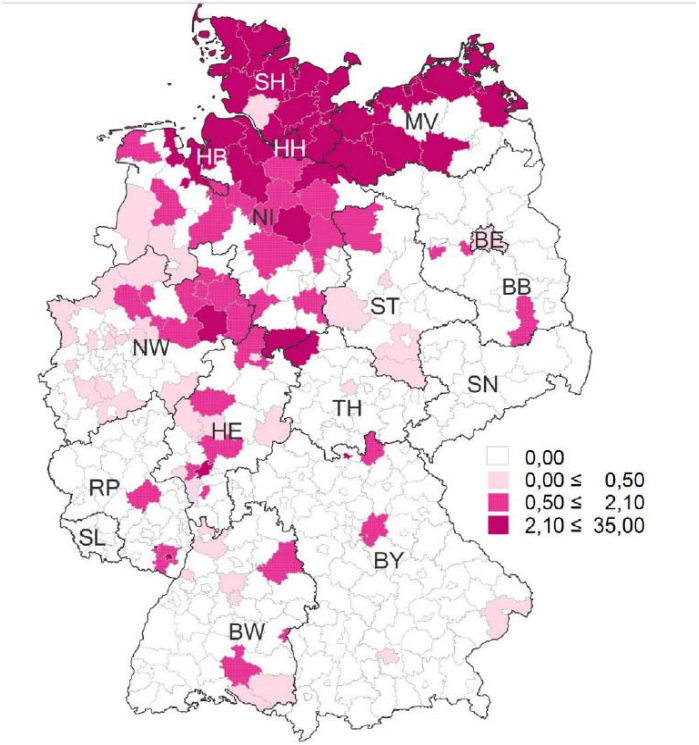
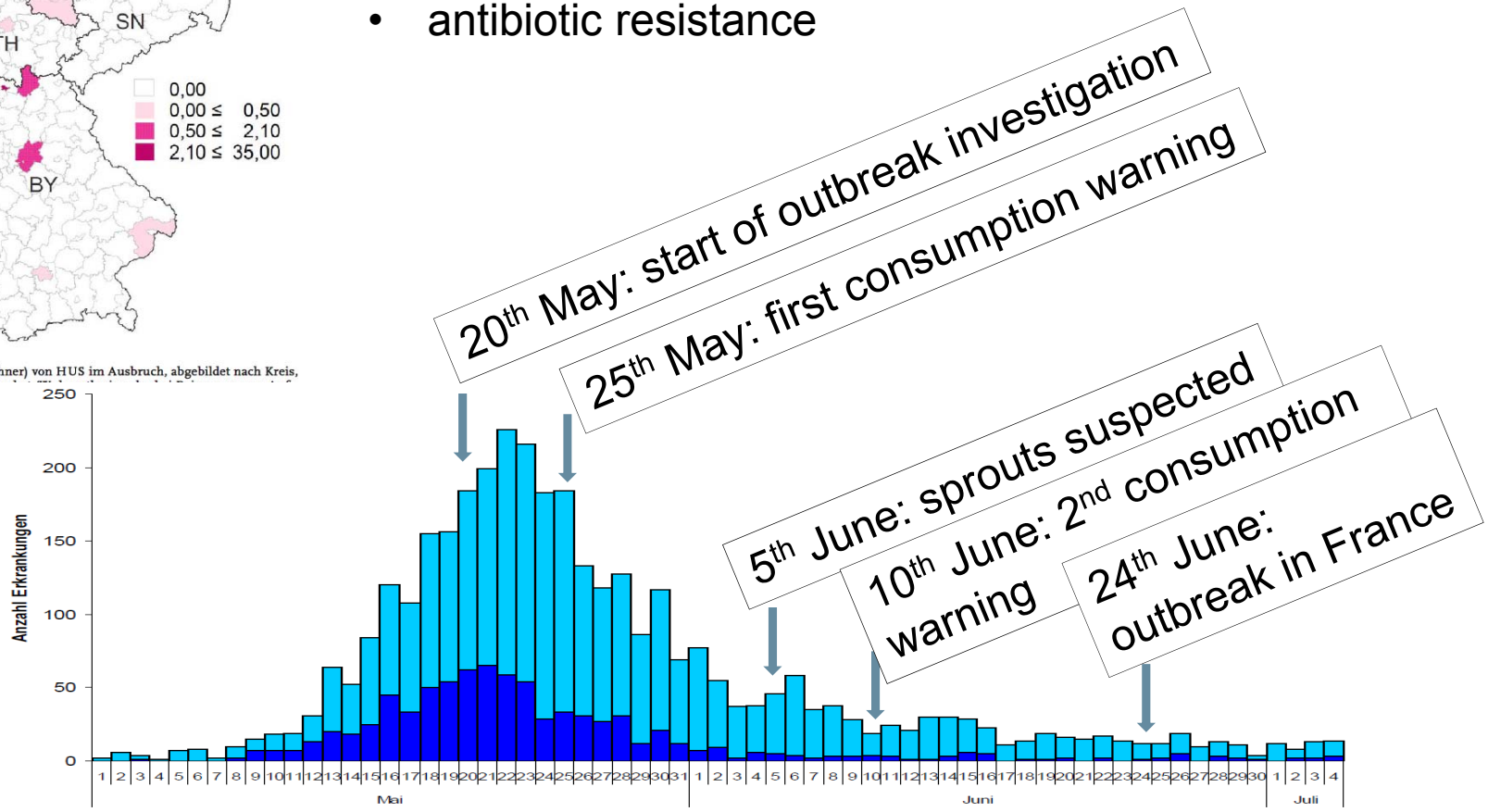


Abbildung 3: Inzidenz (Fälle pro 100.000 Einwohner) von HUS im Ausbruch, abgebildet nach Kreis, in dem die Infektion wahrscheinlich stattgefunde enthaltskreis zum Zeitpunkt der Infektion).

unusual characteristics:

- adults
- woman
- high HUS rate
- rare serotype O104:H4
- antibiotic resistance



Source: RKI, „Abschließende Darstellung und Bewertung der epidemiologischen Erkenntnisse im EHEC O104:H4 Ausbruch Deutschland 2011“

Characteristics of the EHEC O104:H4 outbreak strain

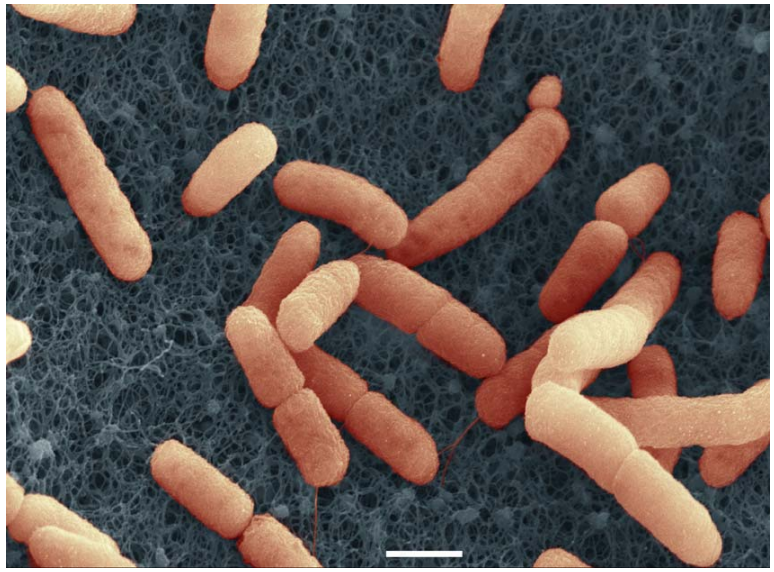
Major characteristics of the outbreak strain

Hybrid pathogenicity characteristics

EHEC/Enteroaggregative *E. coli* ► „EAHEC“

- ***stx*2a (Shiga toxin 2)-positive**
- **enteroaggregative (AAF/I fimbrial cluster)**
- **ESBL (CTX-M-15)**

- ***eae* (intimin)-negative**
- **and e*hly* (enterohemolysin)-negative**

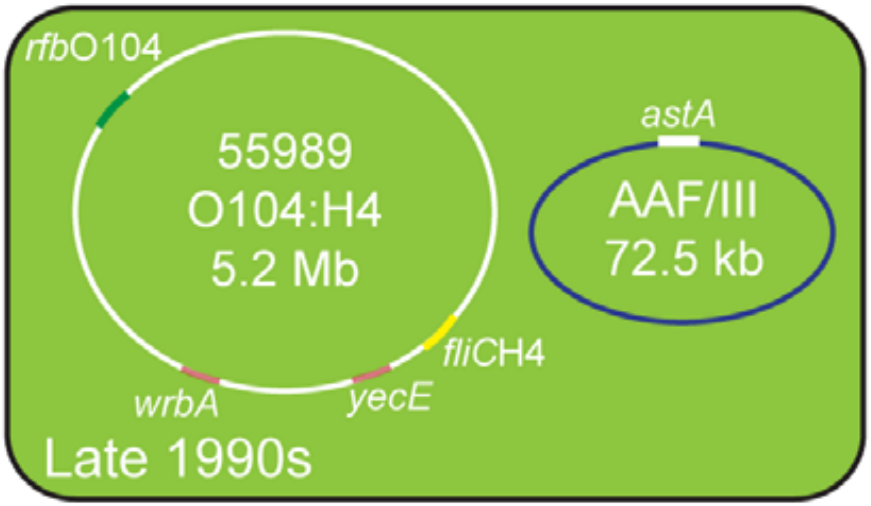


EHEC-Bacteria of the outbreak strain O104:H4. Raster-Electronmicroscopy. Scale: 1 µm
Source: *Gudrun Holland, Michael Laue/RKI*

► Aggregative adherence (AA) means effective and long colonization of humans

► Production of Stx2a associated with an effective and long intestinal colonization could explain the high virulence of this organism!

Comparison of EAEC and German outbreak strain

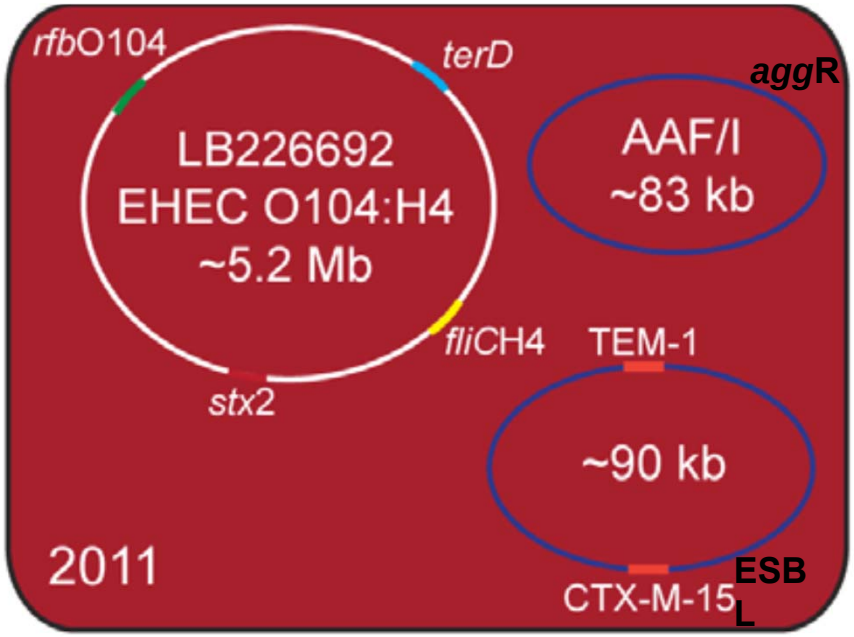


AAF/III aggregative adherence fimbriae type III
intact *stx* integration site at *wrbA*

EAEC 1995/96

Entero-Aggregative *E. coli*

outbreak isolate



AAF/I aggregative adherence fimbriae type I

EAHEC 2011

Entero-Aggregative-Haemorrhagic *E. coli*

Brzuszkiewicz et al. Arch Microbiol 2011 June 29

Mellmann et al. PLoS One. 2011;6(7):e22751

Searching for the outbreak source

652 samples of food and environment were investigated

→→ analysis of 980 subsamples (27. May - 24. July)

SampleType	Sample Number
DNA	14
Isolates	27
Vegetables*	73
Seeds	58
Sprouts	329
Swaps/environment	77
Water	41
Other	33

***Vegetables including cucumbers, tomatoes and lettuce**

All samples were screened with an in-house developed real-time PCR for specific marker genes (stx2a, wzxO104)

Pilot study: optimization of enrichment by acid treatment

Buffered peptone water

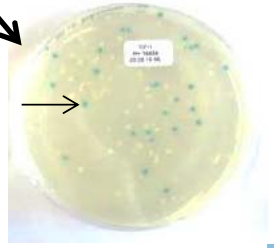
TSB pH2

TBX agar

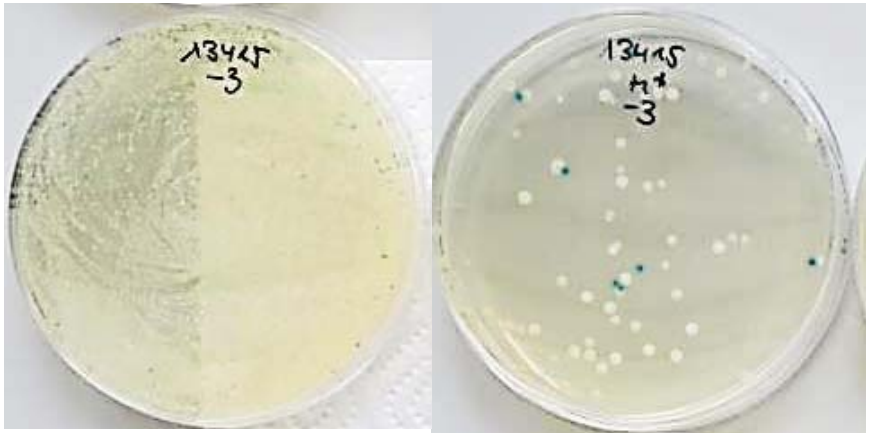
DNA isolation

Real-Time PCR (*stx1*, *stx2*, *eae*)

Dilution for isolation



acid treatment

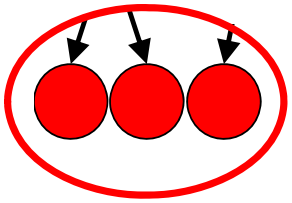


STEC detection	In house method	Acid treatment
Sensitivity	70.2%	92.2%
Specificity	95.2%	96.8%

STEC isolation	In house method	Acid treatment
Sensitivity	32.1%	87.5%
Specificity	100%	100%

Outbreak Scenario: Affecting Multiple Locations or Countries

- Contamination during production/processing
- Diffuse distribution of cases
- Low dose
- Low infection rate
- Complex investigation



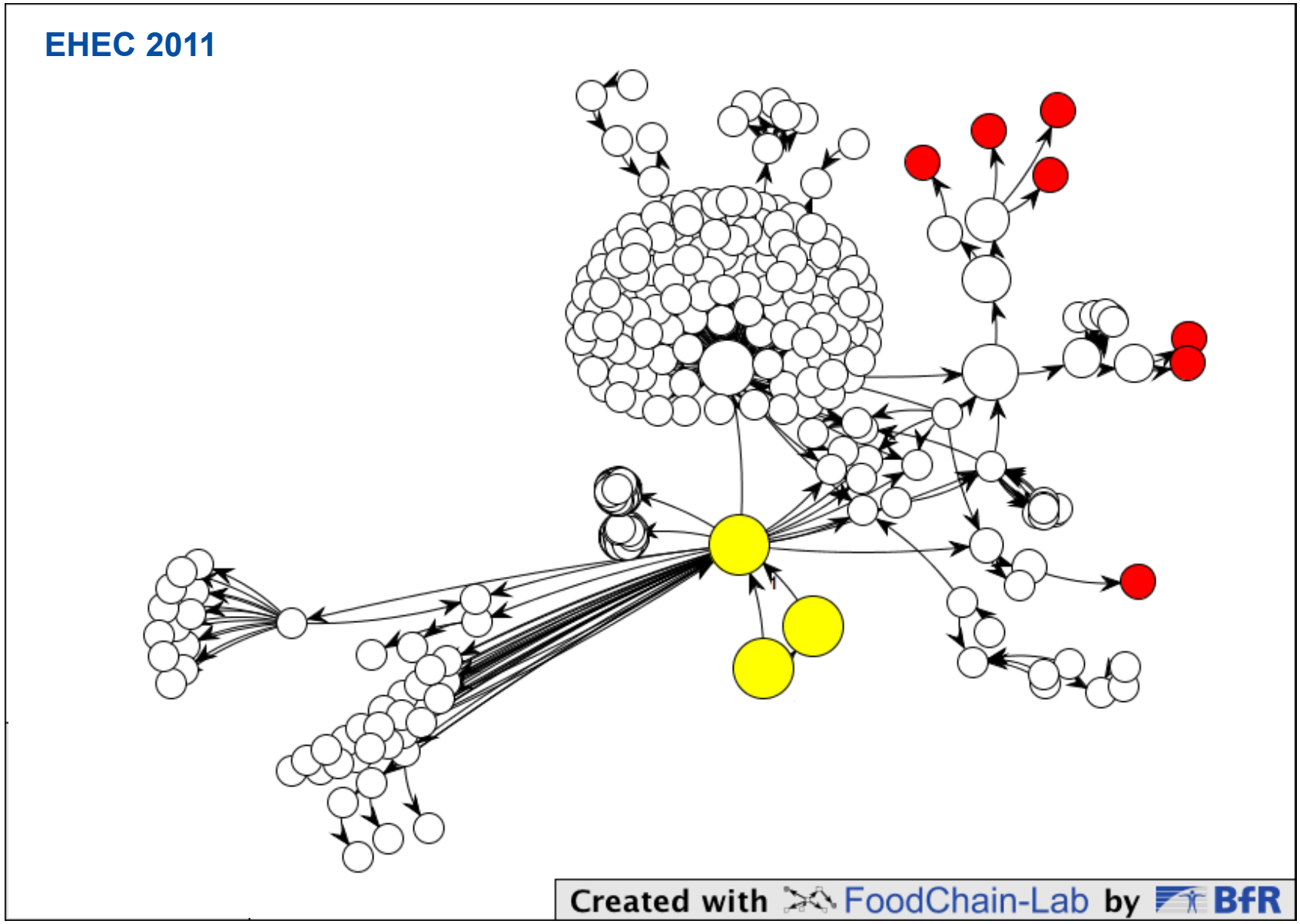
The outbreak investigation teams can only see Cases

Food Chain Lab
Powered by BfR

FOODChain-Lab

- Open source software

https://foodrisklabs.bfr.bund.de



Other cases:

DE:
Norovirus 2012
Salm M. 2015

EU:
HAV 2013/14

UK:
EHEC 2016

BfR