EHEC O104:H4 in Germany 2011:
Large outbreak of bloody diarrhoea and haemolytic uraemic syndrome by Shiga toxin-producing E.coli via contaminated food

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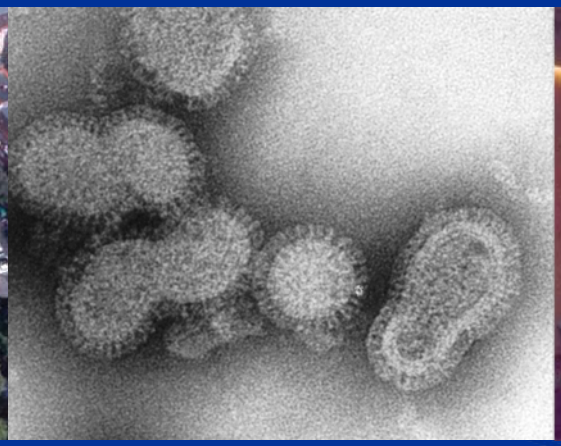



USAMRIID
Frederick, MD, July 19, 2011

PP F 211

Current situation in Germany (July 13, 2011)

	Cases	Deaths
EHEC	3396	17
HUS	862	34
Total	4258	51

EHEC/HUS: International situation (July 11, 2011)

	EHEC	HUS	Deaths
Sweden	35	18	1
Denmark	15	10	0
France	10	8	0

Single cases in 12 additional European countries
in US (2/4/1) and Canada (1/0/0)

First days of the epidemic: course of events

First days: course of events I

- Thursday 19.05:
  - Call from local Public Health Department: invitation from Hamburg
  - **RKI informs BfR (Federal Institute for Risk Assessment) and BMG (Federal Ministry of Health)**
- Friday 20.05:
  - 1st team goes to Hamburg
  - Discussion of the situation with local public health authorities,

    first patient interviews
- Saturday 21.05:
  - First qualitative evidence on the role of **vegetables** is passed on to Food Safety Authorities
  - **1st case-control study**

Source: Robert Koch Institute, 20.6.2011

First days: course of events II

- Sunday 22.05:
  - Analysis of the 1st case-control study
  - **First European Early Warning EWRS+ information submitted to the WHO**
  - Warning to local public health authorities
  - Interview with dpa (German Press Agency), possible role for uncooked vegetables
- Monday 23.05:
  - **Activation of the Situation Room**
  - Information on website
  - Preparations for 2nd case-control study
- Tuesday 24.05:
  - Press conference
  - **First official IHR notification**
  - Start of **2nd case-control study**

First days: course of events III

- Wednesday 25.05:
  - **Identification of the pathogen by RKI**
  - Interdisciplinary teleconference organised by the RKI
  - Teleconference with state public health authorities
  - Ministerial press conference
  - Press conference BfR + RKI: results of the 2nd case-control study, **Advice on food consumption:**

    No raw tomato, cucumber, salad IN Northern Germany
- Thursday 26.05:
  - Information on website in English
  - Epidemiological Bulletin
  - Article in „Eurosurveillance“

Source: Robert Koch Institute, 20.6.2011

Export of Spanish cucumbers into Germany

Reduced by 80 - 200 Mio Euro due to ban of import

(after announcement of Hamburg Senator that EHEC demonstrated)

Surveillance

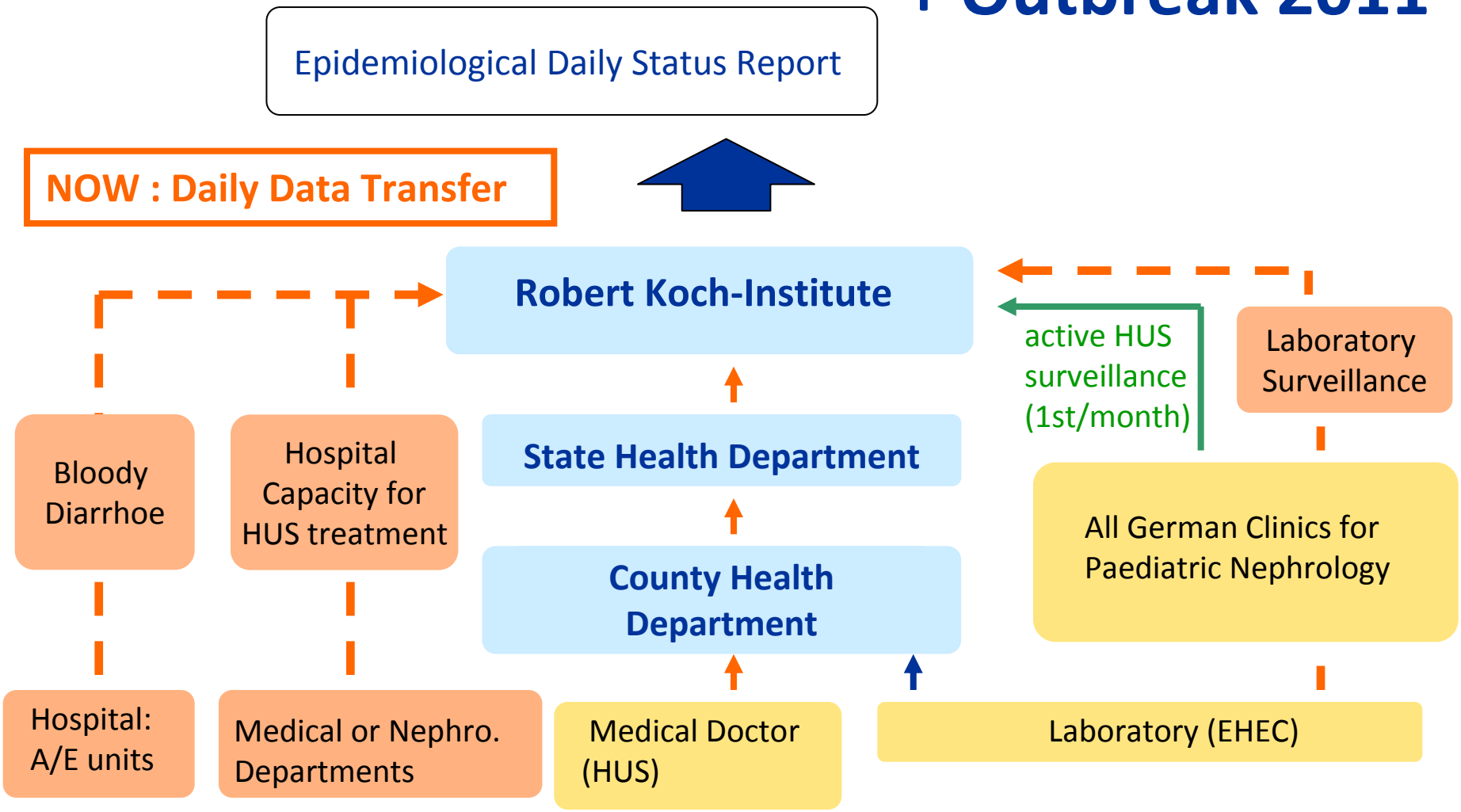
Surveillance

- Ongoing **regular** (routine) EHEC/HUS Surveillance
  - Reporting of EHEC/HUS cases according to Protection Against Infection Law (IfSG), accelerated by daily data transfer
  - **Active** surveillance (individual requests) of the number of HUS cases in cooperation with clinics and the German Society of Nephrology
- Additional EHEC/HUS surveillance
  - Active Surveillance (RKI request) of emergency cases with **bloody diarrhoea** admitted to hospitals
  - **Laboratory**-based surveillance of positive EHEC test results
  - Enquiry of the hospital treatment capacity

Source: Robert Koch Institute, 20.6.2011

Routine-Surveillance EHEC/HUS

+ Outbreak 2011



Quelle: Robert Koch Institut, 20.6.2011

Epidemiological studies

Initial exploratory survey

- Detailed interview of the HUS and EHEC patients

  hospitalised in Hamburg, Lübeck, Bremen and Bremerhaven

  on Friday May 20, 2011 regarding possible source of infection

Source: Robert Koch Institute, 20.6.2011

Case-control studies

- **Case-control studies**
  - **1st case-control study** (14 patients, 16 controls) in Hamburg: to identify possible risk factors based on explorative interviews
  - **2nd case-control study** (25 patients from Hamburg and 96 controls matched by age group, gender and place of residence), results published on May 26, 2011
  - **3nd case-control study** (46 new cases from Lübeck, Hamburg, Bremen and control group comprised of 2100 healthy individuals from northern Germany, interviewed via an on-line survey on food consumption), results published on June 3, 2011
  - **4th case-control study** (~20 new cases from Bremerhaven, Lübeck and Cuxhaven and 60 healthy controls of similar age, gender and place of residence)
  - **In hospitals**, that were not affected before observed current increases in the number of HUS cases
  - **Among foreign cases**, in cooperation with ECDC
- **Detailed analysis** of the case-control studies to identify associated food supply chains
- **Meta-analysis of all case-control studies** (pooled analysis of the results of individual studies)

Source: Robert Koch Institute, 20.6.2011

Cohort studies in disease clusters

- Over 30 cohorts investigated since June 1, 2011 to identify the vehicle of infection and further cases, e.g.
  - ⇨ Cohort studies of **travel groups** (in cooperation with foreign authorities)
  - ⇨ Cluster analysis of different **restaurant-associated outbreaks**
  - ⇨ Analysis of **billing data of guests** at an affected canteen; results published on June 3, 2011 (press release RKI-BfR)
  - ⇨ **„Recipe-based restaurant cohort study“**

Source: Robert Koch Institute, 20.6.2011

Recipe-based Restaurant Cohort Study

- Goal: independent of patient memory
  - 10 Groups (total 168) identified
  - Dinner in same restaurant (May 12-16)
  - 18 % with bloody diarrhoea or EHEC/HUS (31)
  - Questioned: which meals ordered (photographs as reminder)
  - Booking/billing and group photos used for confirmation
  - Chef of restaurant interviewed for detailed ingredients of dish
- Relative risk of disease (RR):

  14.2 times higher (univariate analysis) compared to people not served sprouts – All 31 patients had sprouts!

→ **It is the sprouts !**

The question of sprout consumption

- In the 4th case-control study 18/26 (69%) of the cases and 73/80 (91%) of the controls denied consumption of sprouts.
- 7 (39%) of 18 cases and 37 (51%) of 73 controls who denied consumption of sprouts were questioned again in more detail
  - 4 out of 7 cases who originally denied sprouts consumption remembered eating sprouts during the relevant time-period
  - None of 37 controls who denied sprout consumption changed their mind
- Among other cases who were interviewed earlier (not within the 4th CCS)
  - 5 (62%) of 8 cases who denied sprout consumption remembered eating spouts upon further questioning (95% CI: 24-91%)
  - None of the controls changed their mind

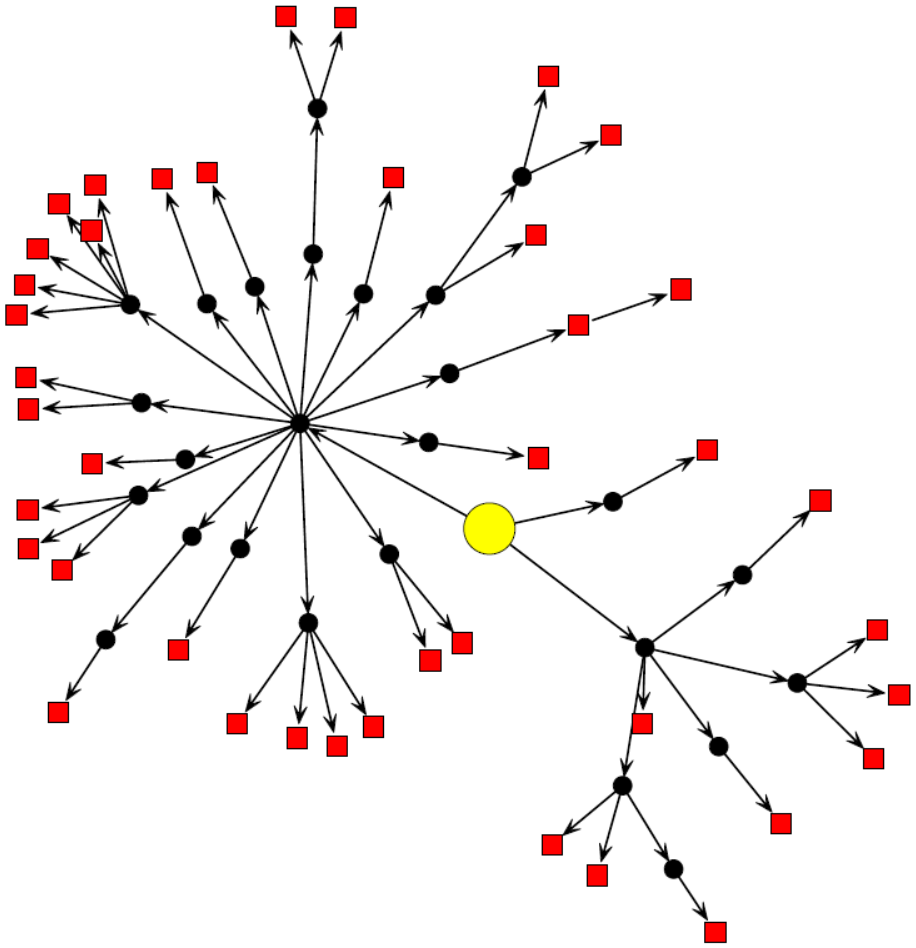
➢ Questioning of controls from the first CCS on sprout consumption
  - unclear whether significant association would have been identified
  - Would have explained only a small proportion of cases
  - And therefore probably would have not have generated evidence for sprouts as a source for the outbreak

➢ It is not that RKI failed to investigate sprout consumption, but rather, study participants failed to remember consumption correctly

Source: Robert Koch Institute, 20.6.2011

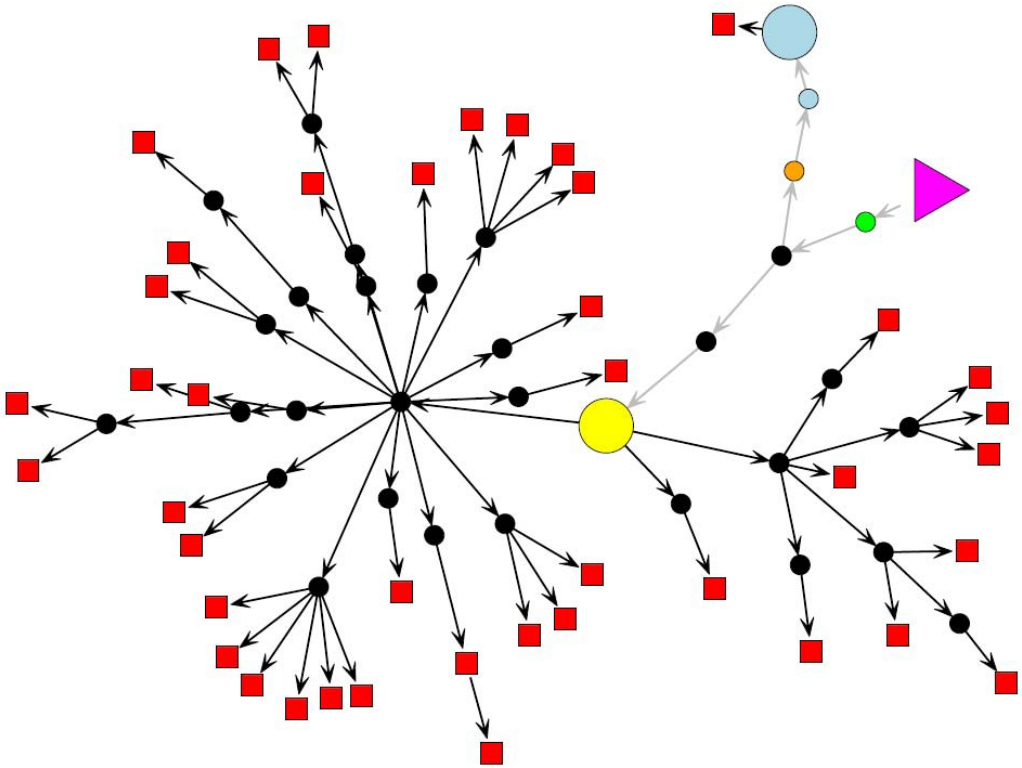
Forward-/Backward-Tracing Strategy: Links of clusters to distribution from one single farm

Fed. Inst. for Risk Assessment (Food Safety), July 5, 2011



Link between German and French EHEC-outbreak

Fed. Inst. for Risk Assessment (Food Safety), July 5, 2011



Blue: French distributer

Magenta: Common Source

Yellow: German sprout producer (NS)

Red: Clusters of EHEC-cases

EU-Withdrawal of Egyptian seeds from market July 5, 2011 (Eur. Food Safety Authority-report)

EFSA: One lot of fenugreek seed from one Egyptian export „the most likely common link“ between outbreak in Germany and France

Other lots?

Contamination with fecal material of human/animal origin? Where?

- Withdrawal of fenugreek seeds from market
- Temporary ban of the import of Egyptian seeds/beans

Common link between German and French outbreak

Fenugreek seeds (Trigonella foenum-graecum)
(including seed mixes and homegrown, risk of cross-contamination)

(Source Galster's Kräuter, Fürth)

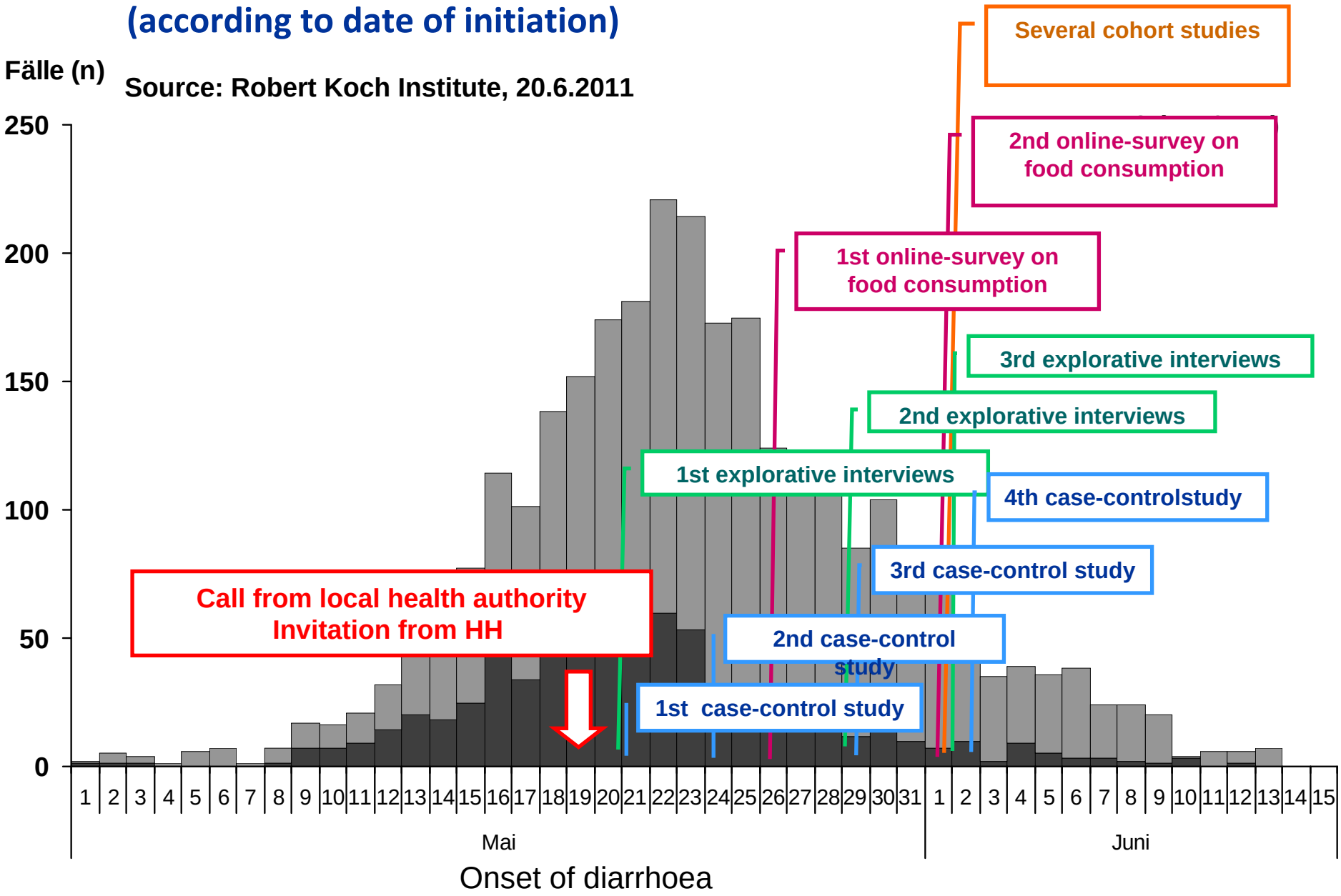
Further epidemiological studies

- Studies to investigate the shedding period and household transmission to estimate the extent of human-to-human transmission
- Online-survey of dietary habits of the general population since June 26, 2011
- Representative country-wide online survey of the disease burden

Source: Robert Koch Institute, 20.6.2011

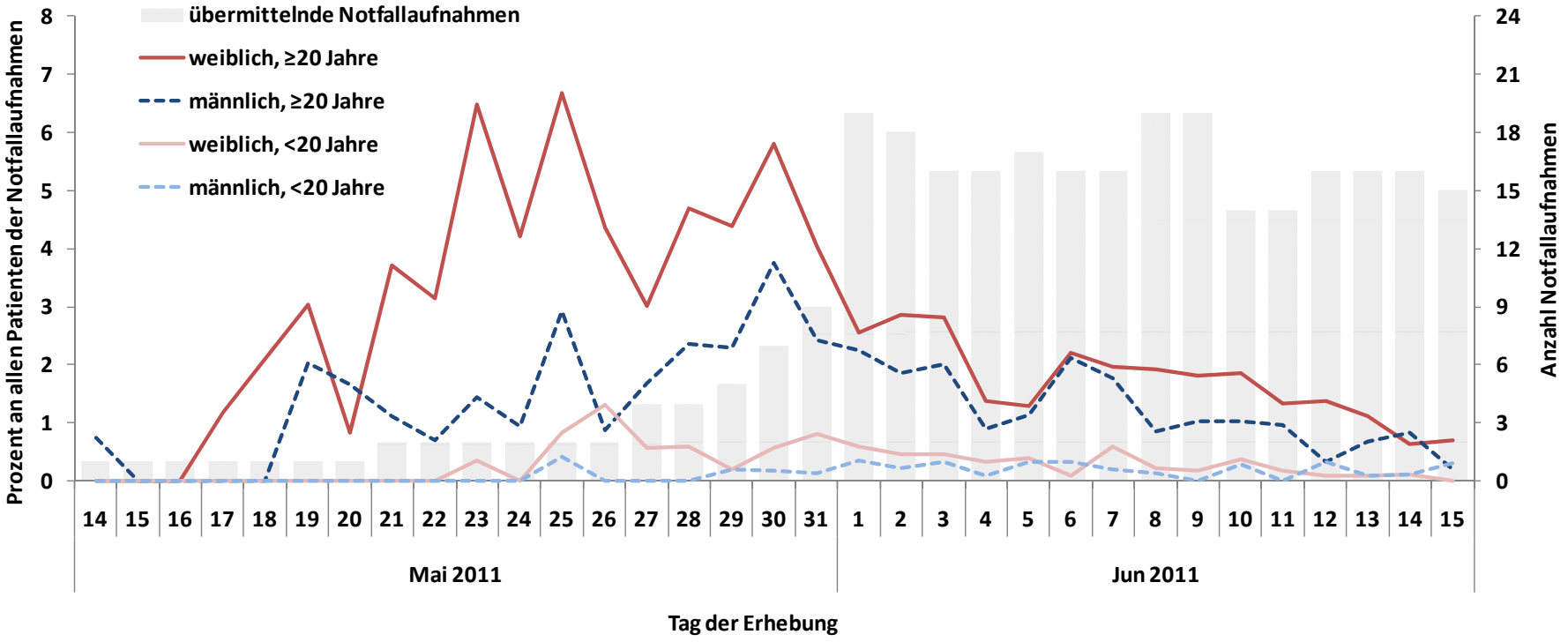
Time line of epidemiological studies

(according to date of initiation)



Surveillance of cases of bloody diarrhoea presenting to emergency departments (ED) in affected areas (June 16, 2011)

Proportion (%) of patients with bloody diarrhoea among all ED patients by age group and gender

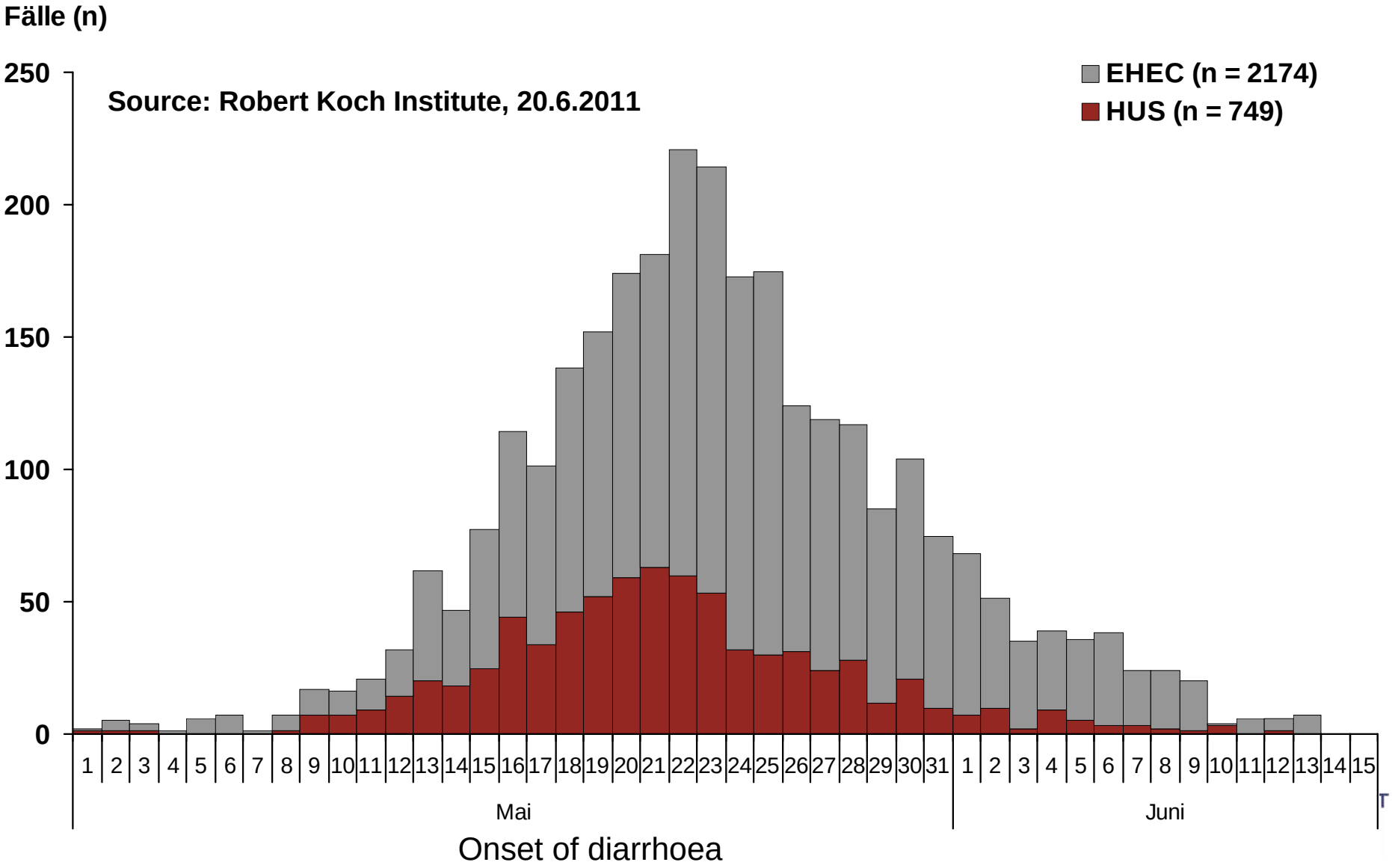


→ *first signs of decline in case numbers?*

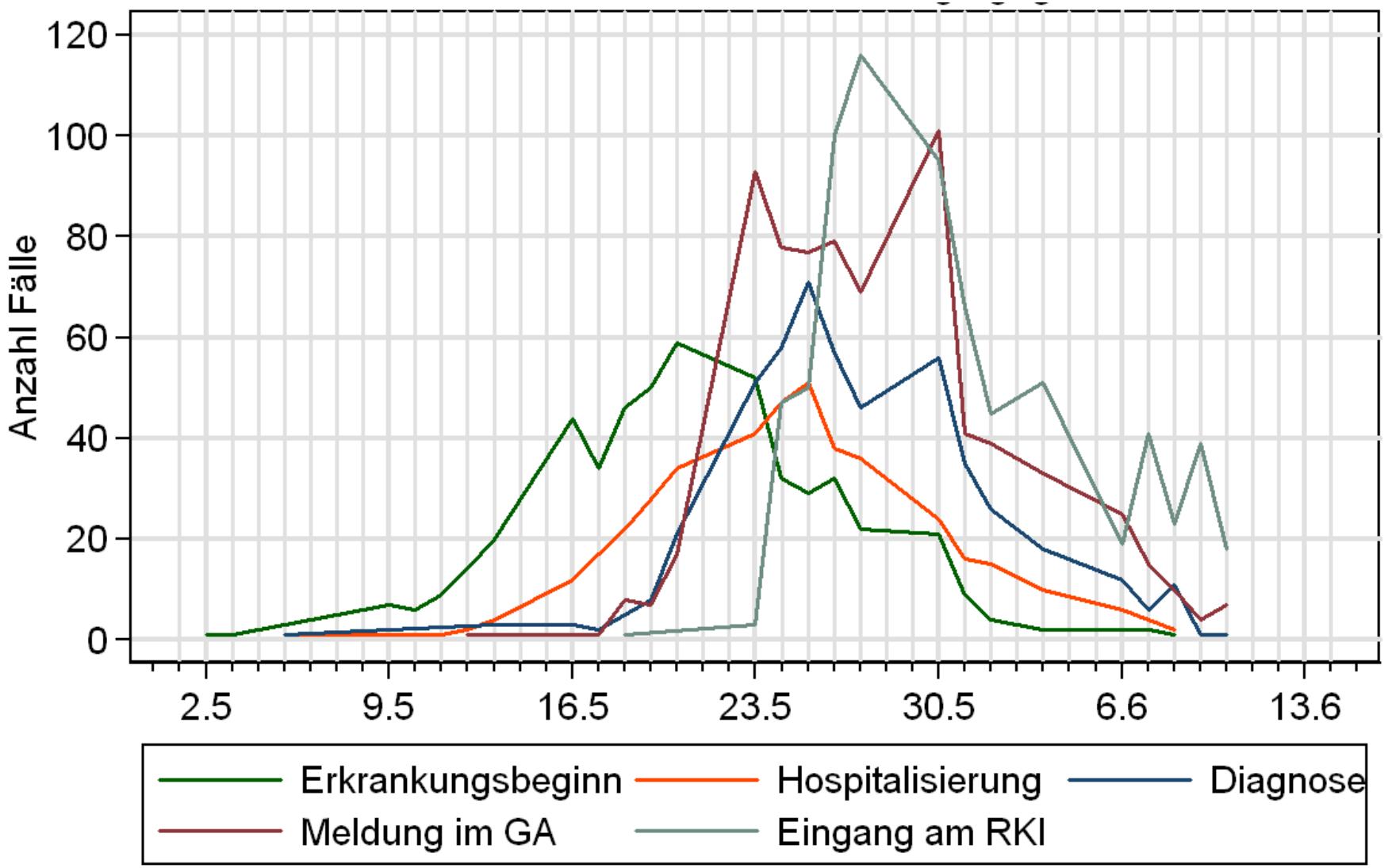
Source: Robert Koch Institute, 20.6.2011

Description of EHEC/HUS outbreak

Epidemiological curve of the EHEC and HUS cases with known date of illness onset (June 17, 2011)

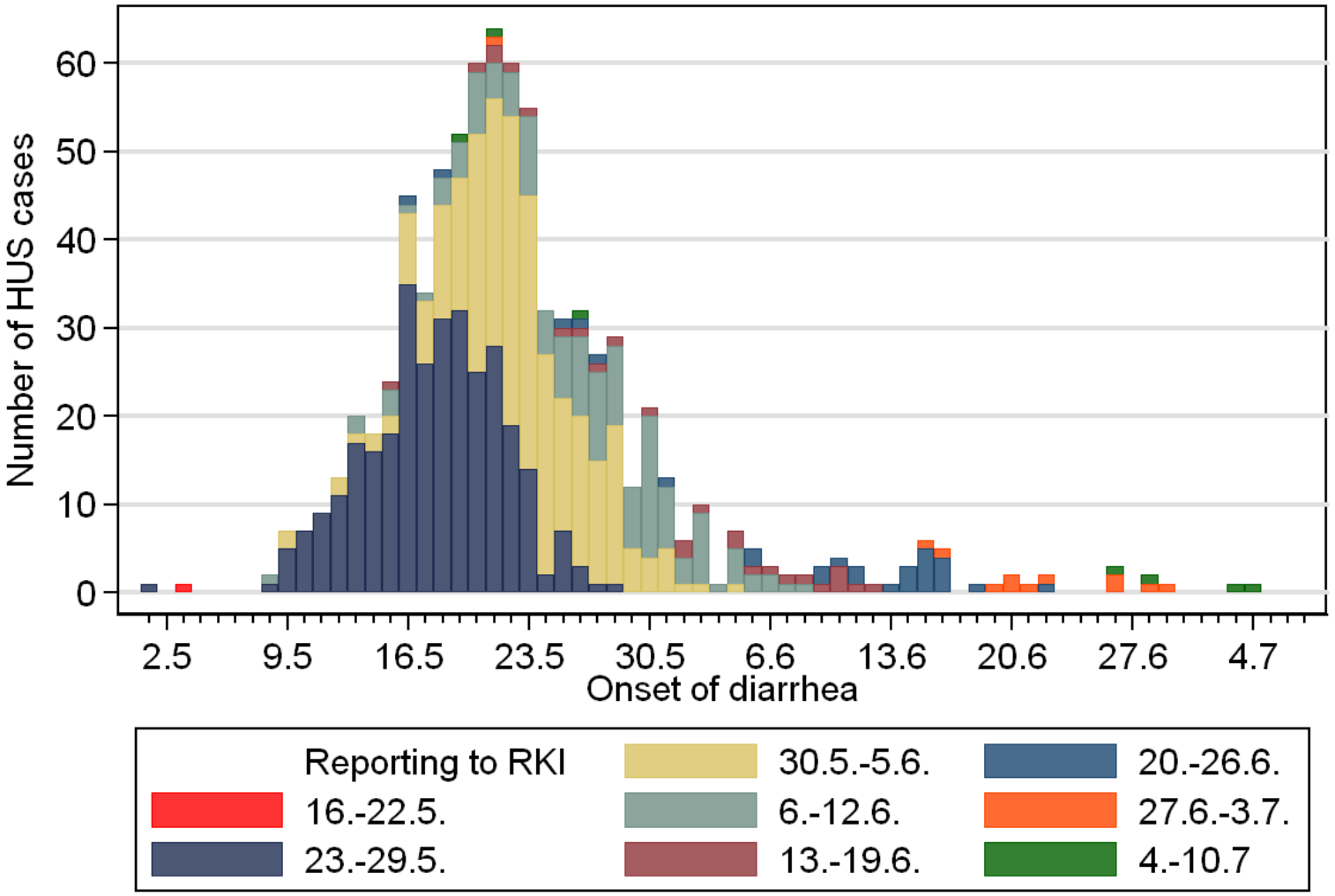


Epidemiological curve according to date of illness onset, diagnosis, hospitalisation and notification to local health authority and RKI (smoothed for public holidays and weekends)



Source: Robert Koch Institute, 20.6.2011

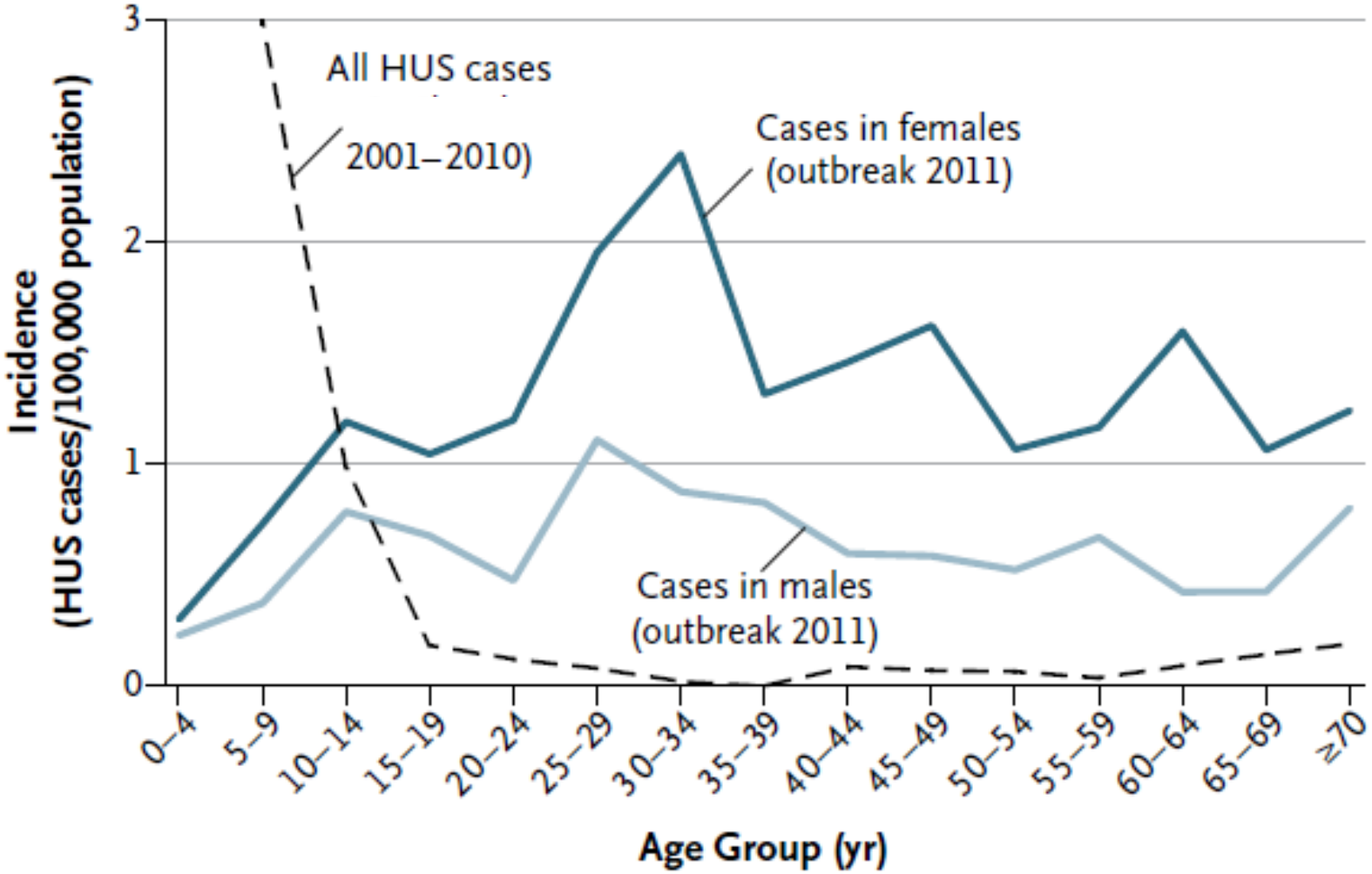
Epidemic curve for HUS cases - by date received at RKI (as of July 7, 2011)



as of 7.7.2011

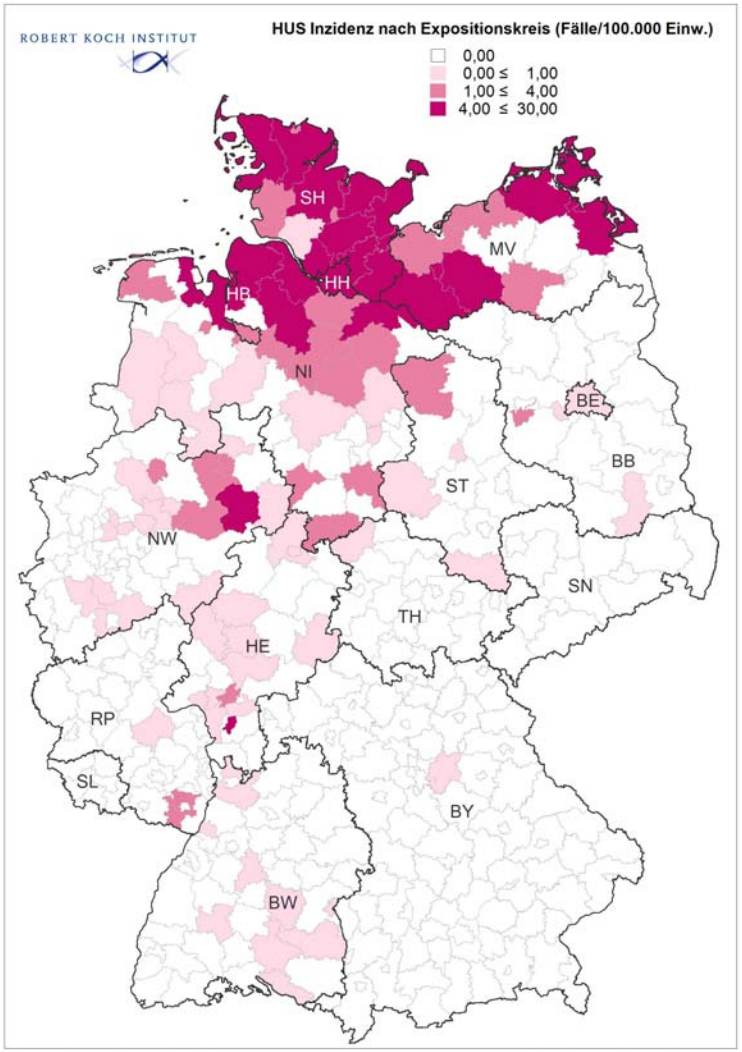
Incidence of HUS – by age and gender

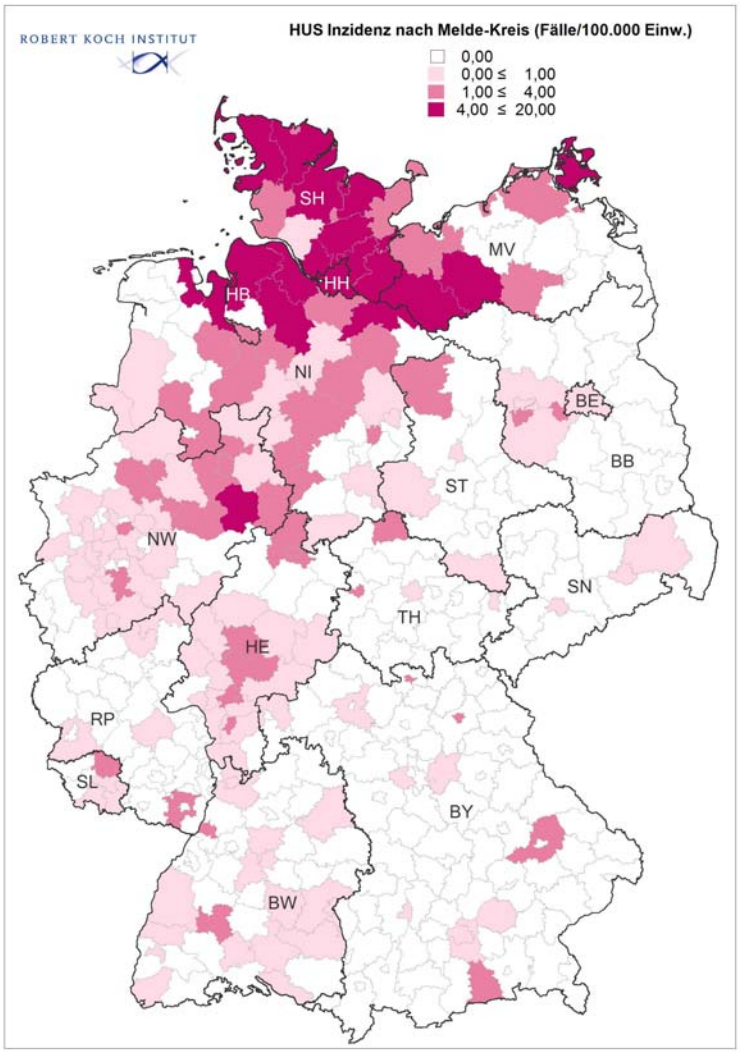
(as of June 17, 2011)



Quelle: Frank C, Werber D, Cramer JP et al. NEJM 2011

Incidence of HUS (cases/100,000 population) according to county of exposure and county of residence (June 17,2011)





Source: Robert Koch Institute, 20.6.2011

Personnel involved

Epidemiology (> 85 staff members)

- Situation room
  - 24(14)/7; 5-12 staff/shift; 1-2 shifts/day
- Field-teams
  - >13 teams; 1-4 days duration; 2-15 persons/team
- Data entry
  - 5-12h/day; 4-14 persons/day
- Surveillance
  - > 10 persons
- Trace back, cluster analysis and liaison with Food Safety Authorities
  - > 7 persons
- Studies and analyses
  - 6-10 persons
- Liaison with international and federal authorities
  - 2 persons
- Contact laboratory-epidemiology
  - 1-2 persons
- Logistics
  - 2-4 persons

Laboratory and Hygiene

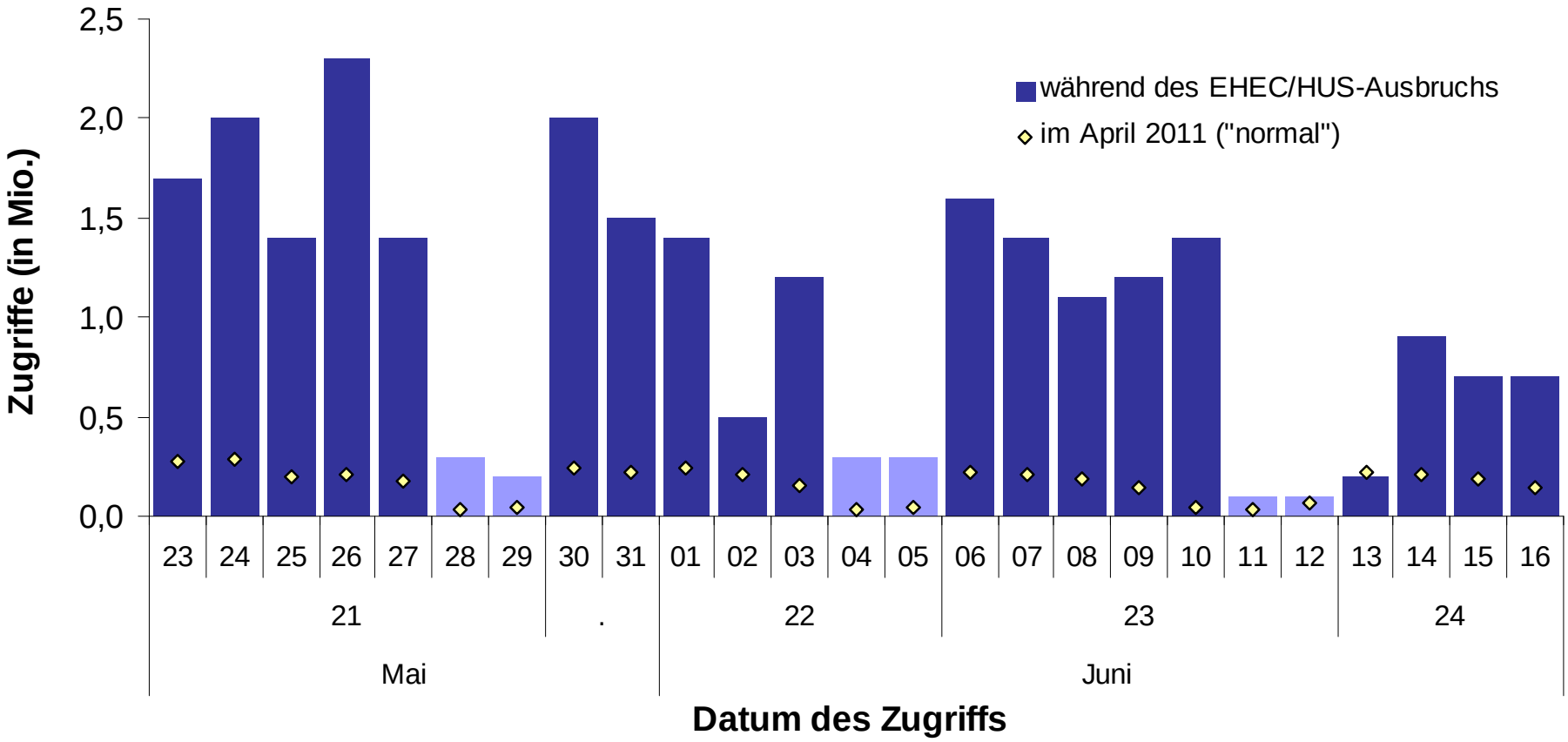
- Primary and reference laboratories typing:
  - > 10 persons

Communication

- Press and internet
  - 7 persons

Source: Robert Koch Institute, 20.6.2011

Frequency of RKI website access
May 23 - June 16, 2011



Source: Robert Koch Institute, 20.6.2011

Japan, 1996: EHEC O157 outbreak caused by contaminated radish sprouts served at school meals

- >12 000 patients with diarrhoea
- 121 HUS (all children under 15 y. o.)
- Deaths: 3 or 11 depending on the information source
- Duration: May 15 - August 15, 1996
- Detected on July 13, 1996
- Time from outbreak detection to identification of the source:
  - probably > 4 weeks

USA, 2008: Salmonella Saintpaul outbreak caused by contaminated chili pepper

- 1500 patients with diarrhoea
- Deaths: 2
- Duration: April 16 – August 28, 2008
- Detected on May 22, 2008 (4 weeks after first case)
- Time from outbreak detection to identification of the source:
  - ~ 7 weeks

"Stealth food" in Salsa

- Early June: warning against tomato consumption;
  July 9 and July 30: warning against chili pepper consumption

Germany, 2011: EHEC O104:H4 from contaminated sprouts

- > 4000 EHEC confirmed cases, > 800 HUS
- Deaths: 17 EHEC + 32 HUS
- Duration: End of 1st week of May 2011 - ongoing
- Detected on May 18, 2011: local health authority notified on afternoon
- Time from outbreak detection to identification of the source :
  - ~ 3 weeks
- May 25, 2011: warning against consumption of tomatoes, cucumbers and lettuce; June10: warning against sprout consumption

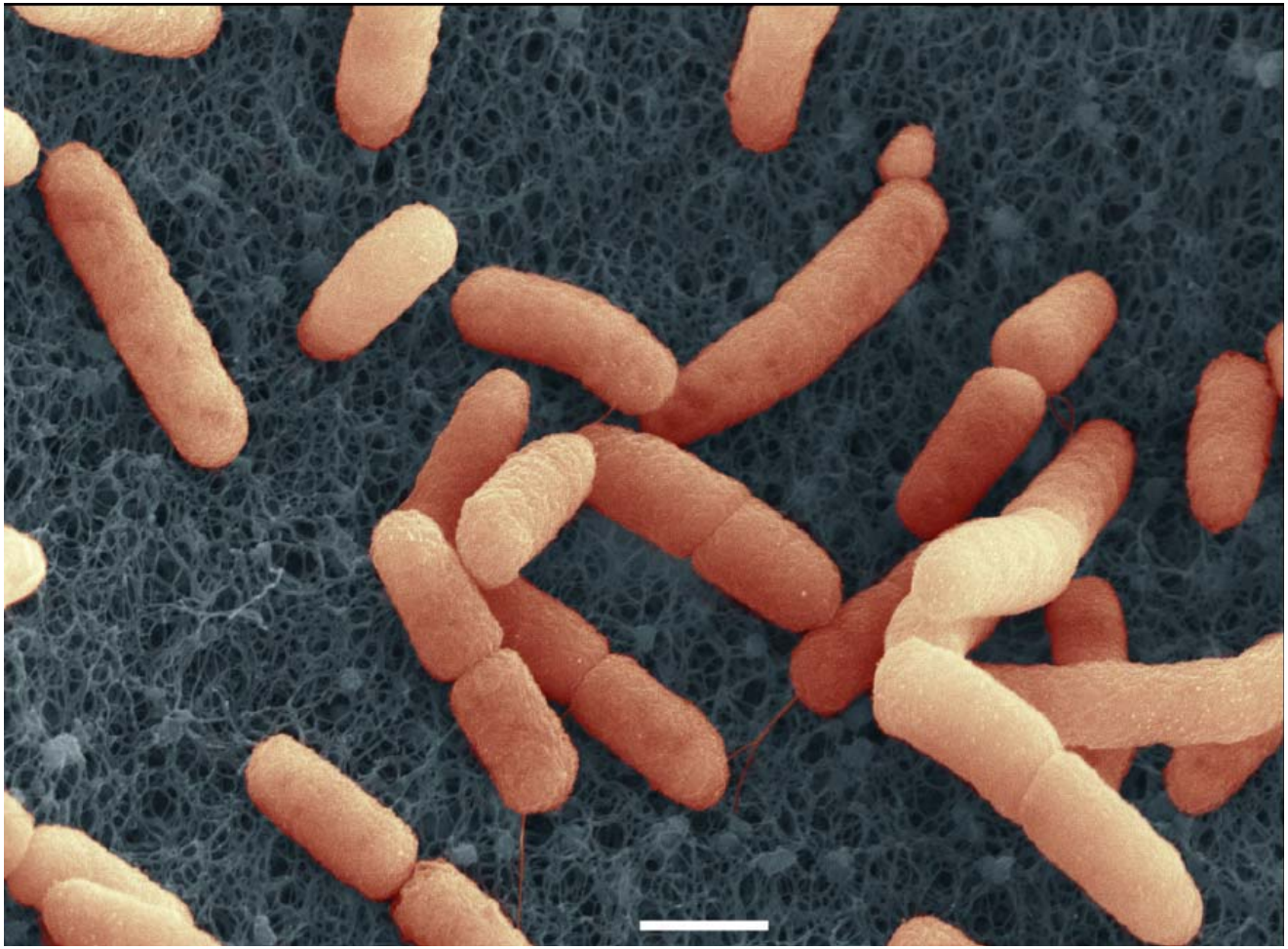
Comparable outbreaks

	Japan, 1996: EHEC O157 (radish sprouts)	USA, 2008: *S. Saintpaul* (chili peppers)	Germany, 2011: EHEC O104:H4 (sprouts)
Deaths	3-11	2	>50
Time to detection	> 7 weeks	~ 4 weeks	~ 2 weeks
Time from detection to identification of source	> 4 weeks	~ 7 weeks	~ 3 weeks
Total duration	~ 12 weeks	~ 16 weeks	➢6 weeks
No	> 12000	1500	> 4000

Epidemiology: Summary and conclusions

- **EHEC/HUS outbreak in Germany**
  - Unusually large; 4,181 EHEC and 859 HUS cases (June 17, 2011)
  - Significant number of severe cases and fatalities (49 deaths)
  - Unusual age and gender distribution
- **Surveillance**
  - accelerated reporting and use of additional surveillance-instruments
- **Studies**
  - Successful implementation of classical and novel epidemiological study types
- **Interdisciplinary work**

Microbiological characterisation of EHEC O104:H4



Microbiological characterisation of EHEC O104:H4

Determination of serotype

0-Antigen O104

(available May 24, 2011)

Severe outbreak in Germany May 2011

EHEC O104:H4

- Rare EHEC serotype
- Not described in animals previously
- Only rarely in humans (total: 7)

Germany	2001	HUSEC041 (Karch)
Korea	2006	
Georgia	2009	
Finland	2010	

Microbiological characterisation of EHEC O104:H4

Virulence markers

- **Shigatoxin 1:** **-** **(negative)**
- **Shigatoxin 2 (vtx2a) :** **+** **(positive)**
- **Intimin (eae) :** **-** **(negative)**

- Enterohemolysin (hly): - (negative)

Tests by PCR available: May 23, 2011, tests of two outbreaks isolates

Variant vtx2a of Shigatoxin 2: May 25, 2011, (Karch and Natl. Ref. Center RKI)

Microbiological characterisation of EHEC O104:H4

Virulence characteristics of enteroaggregative E. coli (2)

- aatA-PCR: + (positive) (ABC-transporter protein gene)
- aggR-PCR: + (positive) (master regulator gene of Vir-plasmid genes)
- aap-PCR: + (positive) (secreted protein dispersin gene)
- aggA-PCR: + (positive) (AAF/I-fimbral subunit-gene) #
- aggC-PCR: + (positive) (AAF/I-fimbral operon-gene) #

MLST Sequence Type:

ST678 (adk 6, fumC6, gyrB 5, icd 136, mdh 9, purA 7, recA 7)

MLST : Prof. Dr. H. Karch; Münster

Microbiological characterisation of EHEC O104:H4

(H. Karch, Münster)

- **Virulence characteristics of enteroaggregative E. coli (1)**
  - Typical EAEC virulence plasmid with adhesion fimbriae type AAF/I
  - First time described in EHEC
  - Any other known EAEC or STEC/EAEC O104:H4 had AAF/III fimbriae
  - Sequence data:
    Strong homology to an Enteroaggregative E. coli (55989)

→ **Virulence combination two different pathogens !**

Microbiological characterisation of EHEC O104:H4

- **ESBL resistance phenotype**
  - Unusual for intestinal E. coli
  - Allows use of corresponding selective media for targeted search
  - ESBL-plate with multiplex PCR screening for

genes	stx1	or alternatively	stx2
	stx2		rfbO0104
	eae		fliCH4

Resistance to 3rd-generation cephalosporins (ESBL)

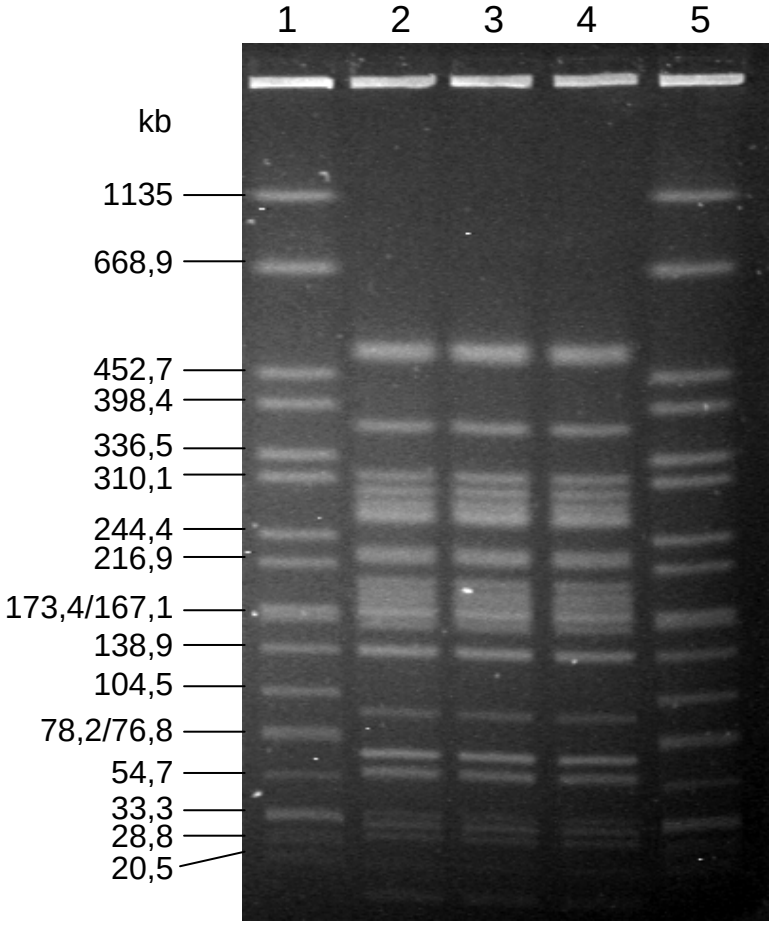
Ampicillin	R
Amoxicillin/Clavulanic acid	R
Piperacillin/Sulbactam	R
Piperacillin/Tazobactam	R*
Cefuroxim	R
Cefuroxim-Axetil	R
Cefoxitin	R
Cefotaxim	R
Cetfazidim	R
Cefpodoxim	R
Imipenem	S
Meropenem	S
Amikacin	S

Gentamicin	S
Kanamycin	S
Tobramycin	S
Streptomycin	R
Nalidixinsäure	R
Ciprofloxacin	S
Norfloxacin	S
Tetracyclin	R
Nitrofurantoin	S
Trimethoprim/Sulfamethoxazol	R
Chloramphenicol	S
Fosfomycin	S

* defined as resistent (AES VITEK)

ESBL: CTX-M-15 + (positive)
plasmid encoded
Other ß-Lactamases: TEM-1 + (positive)

Macrorestriction pattern (*XbaI*) from human E. coli O104:H4 isolates from the current outbreak



PFGE; Prager et al. (2011) IJMM 301:181

Lanes 1 and 5 MW-Standard Salmonella Braenderup H9812

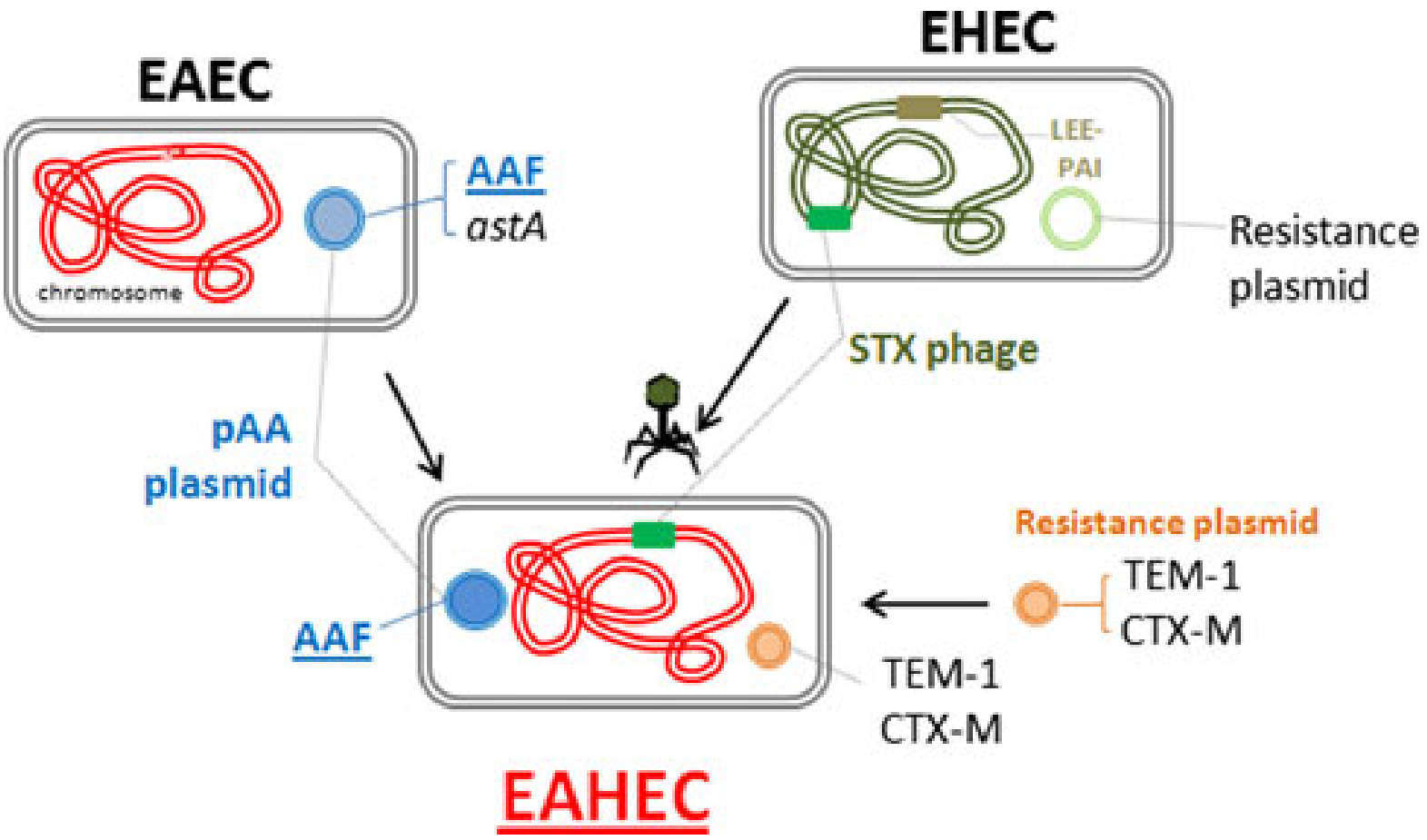
Lane 2: RKI-11-02027 **(HUS)**

Lane 3: RKI-11-02034 **(diarrhea)**

Lane 4: RKI-11-02060 **(bloody diarrhea)**

The macrorestriction-PFGE-pattern (XbaI) of the current EHEC O104:H4 is different compared to HUSEC041

Proposed scheme of the origin of the new *E.coli* pathotype



(Brzuszkiewicz, E et al, Arch. Microbiol., Doi 10.1007, June 2011)

Bacteriological screening of sprouts and seeds and of production site

ALL samples negative!!

Exception: in rinse water of one single opened package that had contained sprouts previously, recovered from trash, in household with EHEC cases

Several secondary infections

- to household partners
- in hospital
- via food distribution

→ Raised awareness !

First suggestions based on current experience

- Cross-sectoral coordination and communication
- Within Public Health Sector
  1. Crisis management: cross-sectoral
  2. Risk communication: cross-sectoral
  3. Involvement of physicians regarding reporting
  4. Personnel capacity in public health sector
  5. Task force on infectious disease epidemiology
  6. Appropriate information exchange (including professional societies etc.)
  7. Modern information technologies
  8. Detailed pathogen typing (diagnostics in primary laboratories)
  9. Accelerated data transfer (within framework of existing law)
  10. Mortality surveillance
  11. Ascertainment of treatment capacity through federal states
  12. Need for research

Robert Koch Institute units involved

- Department Infectious Disease Epidemiology

  Prof. Gerard Krause and many colleagues
  (e.g. Dr. Frank, Dr. Werber, Prof. Stark, Dr. Buchholz)

- Department Infectious Diseases

  Prof. Martin Mielke, Dr. Angelika Fruth

- RKI-Expert Laboratory for HUS / EHEC

  Prof. Helge Karch, Münster