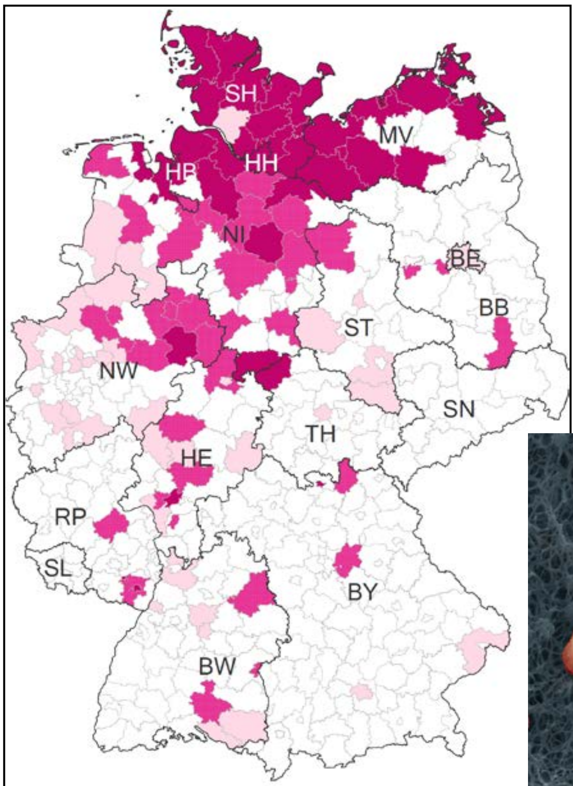
ROBERT KOCH INSTITUT

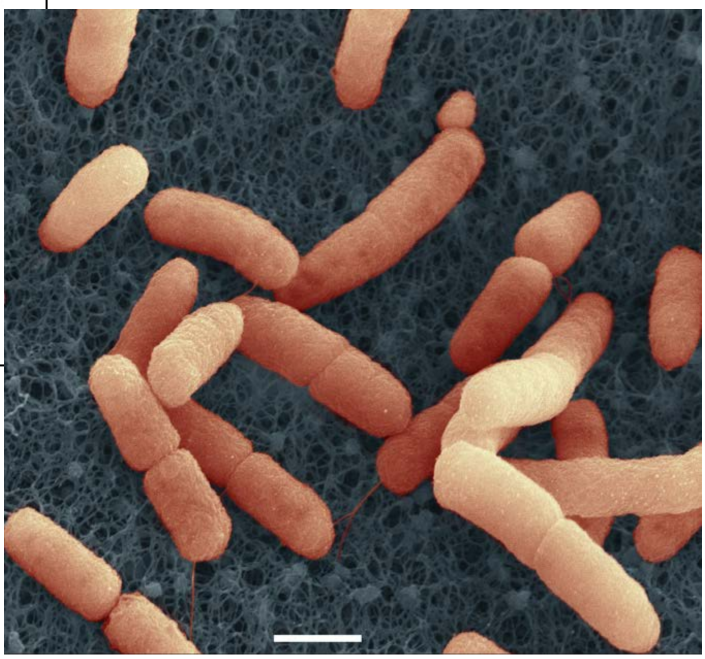
Final presentation and evaluation of epidemiological findings in the

EHEC O104:H4 Outbreak

Germany 2011



Map showing HUS incidence in the outbreak



EHEC bacteria of the outbreak strain O104:H4
Scanning electron microscope. Scale: 1 µm
Source: Holland, Laue (Robert Koch Institute)

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Final presentation and evaluation of the epidemiological findings in the EHEC O104:H4 outbreak, Germany 2011

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Contents

Abstract

From May to July 2011, primarily in northern Germany, there was a large outbreak of illnesses characterized by haemolytic-uremic syndrome (HUS) and bloody diarrhoea associated with infections by enterohemorrhagic *Escherichia coli* (EHEC) of the serotype O104:H4. In this report, results from the surveillance, the epidemiological studies and microbiology of the Robert Koch-Institute (RKI) are presented. The contributions by the RKI to the identification of disease clusters and tracing of food items are found in a report by the EHEC Task Force at the Federal Office for Consumer Protection and Food Safety (BVL).

Epidemiological characterization and course of the outbreak

The outbreak (based on the date of onset of diarrhoea symptoms) began at the beginning of May 2011 and reached its peak on 22 May 2011. Since then, both the number of reported cases of EHEC gastroenteritis associated with the outbreak and the number of new HUS cases decreased. Since mid-June, only sporadic cases of HUS occurred. On 26 July, the Robert Koch-Institute declared that the outbreak had ended. At that point, there had been no new cases clearly associated with the outbreak for 3 weeks, since the last illness on 4 July.

A total of 855 cases of HUS and 2,987 cases of acute gastroenteritis attributed to the outbreak were contracted (as of 16 August 2011). The number of cases affecting women outnumbered men in both HUS (68%) and EHEC (58%). The majority of cases involved adults. This is in stark contrast to the observed cases of EHEC gastroenteritis and HUS reported in the last years, in which small children were predominantly affected. Death was reported for 35 (4.1%) of the patients identified with HUS and 18 (0.6%) of the patients with EHEC gastroenteritis.

Cases of illness were reported from all federal states, but the 5 most northern states were most affected, including Hamburg, Schleswig-Holstein, Bremen, Mecklenburg-Western Pomerania and Lower Saxony, with HUS incidence in these states up to 10 cases per 100,000 persons.

Once the infection vehicle (sprouts) was identified and its distribution was stopped at the beginning of June, there were no further clusters associated with the consumption of this vehicle. In the late stages of the outbreak, cases of secondary transmission by infected persons via close contact within households occured, as well as distinct localized outbreaks that could be attributed to secondary contamination of food products by employees (EHEC shedders) in the food industry. There were also a few recorded laboratory infections.

Intensive surveillance for EHEC O104 was continued after the official end of the outbreak in order to identify a potential transition of the infection to an endemic phase. After July 4, 7 additional infections from EHEC O104 were recorded, as yet exclusively cases of apparent transmission within households or from occupational exposure (data as of August 30, 2011). Overall, the frequency of EHEC and HUS reported after July 4 sharply decreased to a rate interpretable as "background".

Evidence for sprouts as the vehicle of infection

Large-scale EHEC infection outbreaks typically originate from fecal contamination of vegetable or animal foods which are not sufficiently heated prior to consumption or are typically consumed raw. Evidence for sprouts as the responsible vehicle in this outbreak in Germany arose from epidemiological studies by the Robert KochInstitute in collaboration with regional and local public health authorities, as well as with clinics and the investigations of the federal food safety authorities. Epidemiological studies show a statistically

significant association between the consumption of sprouts and risk of disease (e.g. recipe-based restaurant cohort study: relative risk 14.2; 95% CI 2.6 - ∞; all 31 cases in the cohort study explained by the consumption of sprouts). Investigations by the EHEC Task Force at the BVL revealed that 41 of 41 well-documented localities (e.g. restaurants) in each of which several cases were exposed (so-called clusters) can be traced back to sprouts from Company A in Lower Saxony.

In the synopsis of the available results, the RKI, the Federal Institute for Risk Assessment (BfR) and the BVL concordantly concluded that the outbreak in Germany caused by EHEC O104:H4 was attributable to the consumption of contaminated sprouts from Company A. An outbreak involving EHEC O104:H4 reported in France (illness onset between 15 and 20 June) likewise indicated a connection with the consumption of locally produced sprouts. Investigations by national and international food authorities revealed that fenugreek seeds involved in France and in Company A could be traced back to the same supplier.

Conclusions for further epidemiological surveillance and recommendations

This outbreak of EHEC infections is the largest recorded up to now in Germany and, based on the number of cases of HUS, is the largest outbreak of this sort worldwide. Within a relatively short period of time, epidemiological studies and systematic tracing of food products led to the discovery of sprouts as the vehicle of infection. Currently there is no evidence to suggest that the EHEC O104:H4 pathogen has become endemically established in Germany after the end of the outbreak.

The activities of epidemiological surveillance were constant and focussed on surveillance in accordance with the Protection Against Infection Act (IfSG) for EHEC and HUS, as well as emergency room monitoring of cases of bloody diarrhoea in selected hospitals. Furthermore, doctors and the public health services maintained increased vigilance for the occurrence of bloody diarrhoea and HUS including a rapid diagnosis (with differentiation with respect to the outbreak strain) and notification of in- and outpatients. Furthermore, within the context of the notification requirements for EHEC and HUS, all new cases of EHEC and HUS that meet the outbreak case definition continue to be scrutinized by local health authorities based on a questionnaire developed by the RKI (e.g. regarding secondary transmission, laboratory infection) in order to be able to identify the source of infection of these new cases.

The explicit advice to consistently observe personal hygiene and food hygiene measures continues to be vital. Strict adherence to hand hygiene (http://www.bzga.de/?sid=663) and other standard measures of hygiene are of central importance. Stringent adherence to hygienic practices is generally essential in a household, but particularly in the presence of EHEC-infected persons or persons with diarrhoea. This means that the utmost cleanliness is especially imperative in the kitchen and bathroom. Apart from direct consumption of contaminated food, the bacteria can also be transmitted via hand contact or contaminated kitchen utensils. This is of particular importance if potentially contaminated food is not subsequently heated. The risk can be reduced if hands and kitchen utensils are washed thoroughly with water and soap/detergent and dried carefully before preparing food, especially food that will not be subsequently cooked. (The recommendations of the BfR can be found at: www.bfr.bund.de > A - Z Index > EHEC). Objects, clothing or surfaces contaminated with feces or vomit should be immediately washed or cleaned; typical household gloves should be worn if there is contact. Recommendations are available at www.rki.de > Infektionskrankheiten A-Z > EHEC.

1 Descriptive Epidemiology

On 19 May 2011, the RKI was invited by the Health and Consumer Protection Agency in Hamburg to assist the responsible authorities in the investigation of a cluster of three paediatric HUS cases. Upon arrival of the RKI team on 20 May, it quickly became clear that adults were atypically also affected by HUS, and that the number of cases continued to rise rapidly. An outbreak investigation was initiated.

1.1 German Notification Data

The electronic reporting system in Germany has collected standardized data covering HUS and EHEC gastroenteritis cases since 2001. Suspicion of disease, disease and death from HUS are notifiable by the treating physician according to §6 of the IfSG (Protection Against Infection Act), and EHEC-detection is notifiable by laboratories according to §7 IfSG. All information pertaining to the cases is amalgamated by the local public health departments (a case is either an EHEC gastroenteritis case OR an HUS case). In addition to the routine surveillance, on 23 May local and state public health departments were requested in a newsletter to report HUS and HUS suspected cases to the RKI immediately after receiving the notification and to add subsequent case investigation results.

In contrast to the usual surveillance reference definitions of EHEC gastroenteritis (toxin-based laboratory detection, serogroup optional, and illness with symptoms of gastroenteritis) and HUS (purely clinical case definition, EHEC detection by laboratory diagnostics is optional), the following restrictions were set in place in order to define the cases likely attributable to the outbreak: cases with onset of disease (the typical first symptom is diarrhoea) between 1 May and 4 July 2011 were included ("outbreak time period"). Cases with unknown illness dates were counted from reporting week 19 (beginning 9 May) to 28 (ending 17 July). Cases with evidence for EHEC sub types that do not correspond to the characteristics of the outbreak strain were excluded. This applies to EHEC of serogroups other than the outbreak strain O104:H4 (for details see section 3), as well as to EHEC without serogroup information reported to be only stx1-positive. With data as of 1pm on 16 August 2011, all reported HUS cases (including any remaining suspected cases) and all EHEC cases that met the clinical description were analysed.

A total of 855 cases of HUS and 2,987 cases of EHEC gastroenteritis (without development of HUS) were reported, hence a total of 3,842 cases is attributable to the outbreak. For 5% of HUS cases and 9% of EHEC gastroenteritis cases, a date of disease onset is not available.

An additional 19 HUS cases and 719 EHEC cases were recorded, which were not ascribed to the outbreak because of the exclusion criteria described above. In the same time period in the previous 5 years (2006-2010), a median of 13 HUS cases and 218 EHEC gastroenteritis cases were reported. For the 2011 outbreak period, this corresponds to a 67-fold increase in HUS and a 17-fold increase in EHEC. The increase in the number of 2011 EHEC cases not ascribed to the outbreak (n=719), well beyond the total EHEC cases in the previous year (n=218), reflects the strongly increased attention to and higher clinical investigation rates for EHEC.

Among the HUS cases, 68% were female and the median age was 42 years (range from 0 to 91 years). Among EHEC cases, 58% were female and the median age was 46 years (range from 0 to 100 years). Figure 1 shows the incidence of reported HUS cases by age and gender. Hospitalization is likely in all HUS cases (for EHEC reported for 54%). Among HUS patients, 35 (4.1%) died and among the patients with EHEC gastroenteritis, 18 (0.6%) died.

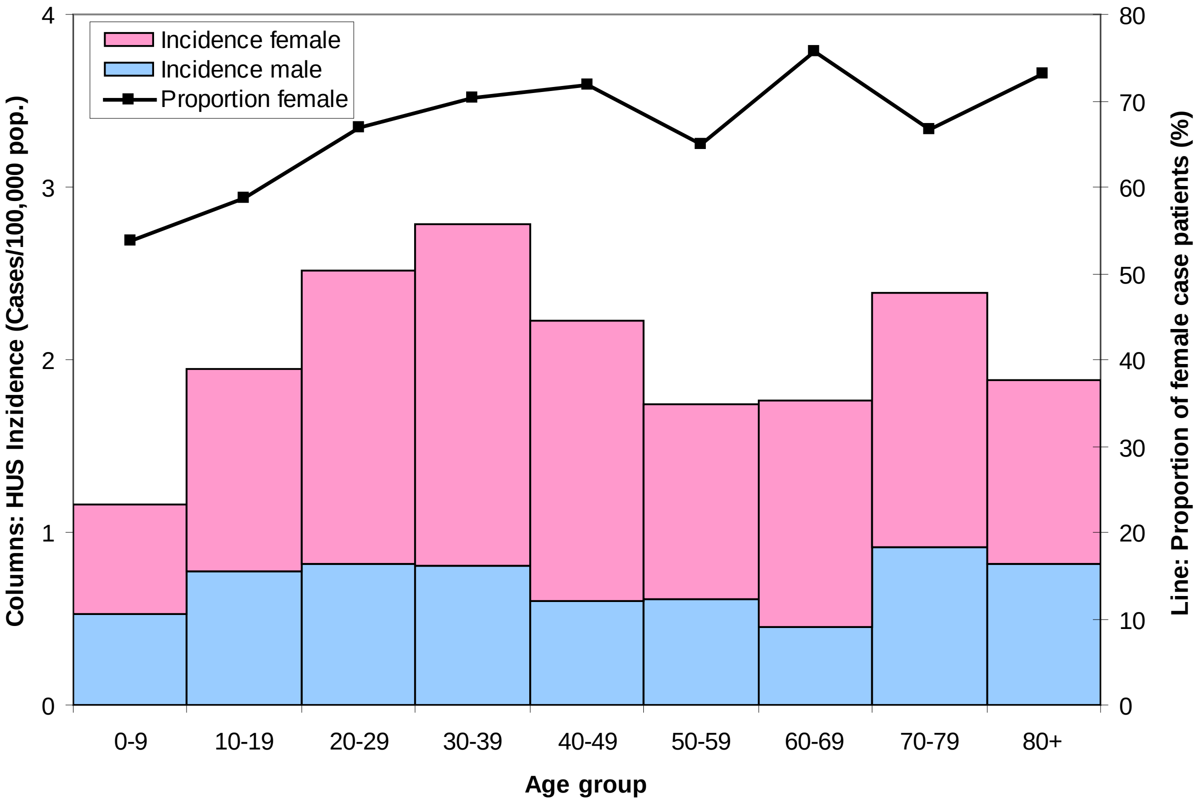

Figure 1: Incidence of HUS by age category and gender (left y axis) and the proportion of cases that were female (right y axis) in each age category (n=855 HUS cases).

Disease onset (based on diarrhoea symptoms) of the first case of HUS in an adult was 8 May, which is also the date of the first case of HUS with detection of EHEC O104. On 1 May, a 45-year-old male from Aachen became ill with EHEC. In that case, other diarrhoea pathogens in addition to EHEC O104 were detected, so it is not clear whether the disease onset with respect to EHEC really is 1 May. In the next EHEC case with detection of O104, onset of illness was 8 May, the same as for HUS. This case involved a 42-year-old female from Lower Saxony.

Among the HUS cases, the proportion of bloody diarrhoea reported was 79%, and among EHEC cases this proportion was 56%. These values are taken as minimum percentages, since the input screen of the current electronic reporting system only offers the options "bloody diarrhoea" and "diarrhoea, not specified".

Data on laboratory detection of EHEC O104 in known cases is currently still forwarded to RKI. Currently there is information confirming the outbreak strain EHEC O104 for 42% of laboratory-confirmed cases of HUS and 21% of EHEC diarrhoea cases. Based on the distinct excess of cases due to the outbreak compared to the previous year as outlined above, it must however be assumed that almost all these HUS cases can be attributed to the outbreak, and a large percentage of the EHEC cases, the size of which cannot be determined precisely because of the lack of microbiological data.

Figure 2 shows the epidemiological curve of HUS and EHEC cases. Both curves sharply rise from 8 May on, peak on the 21 and 22 May, respectively, and then decline quickly at first and more slowly later on. Considering only cases with detection of O104, the peaks are on 20 May (HUS) and 22 May (EHEC gastroenteritis).

As of 27 July, no new cases that could be clearly attributed to the outbreak were reported (criteria: HUS in an adult with O104 or no serogroup confirmed, or EHEC with O104 confirmed). Therefore, whe the outbreak was deemed ended as of 5 July the active phase of the outbreak investigation ended and the post-outbreak surveillance phase began. Under these conditions (as of 30 August 2011), 7 additional cases with disease onset dates between 17 July and 14 August came to the attention of the RKI, in which O104 was detected and/or infection with it was probably: one household secondary HUS case in an adult with detection of O104 in the primary case only, as well as 6 EHEC cases with detection of O104.

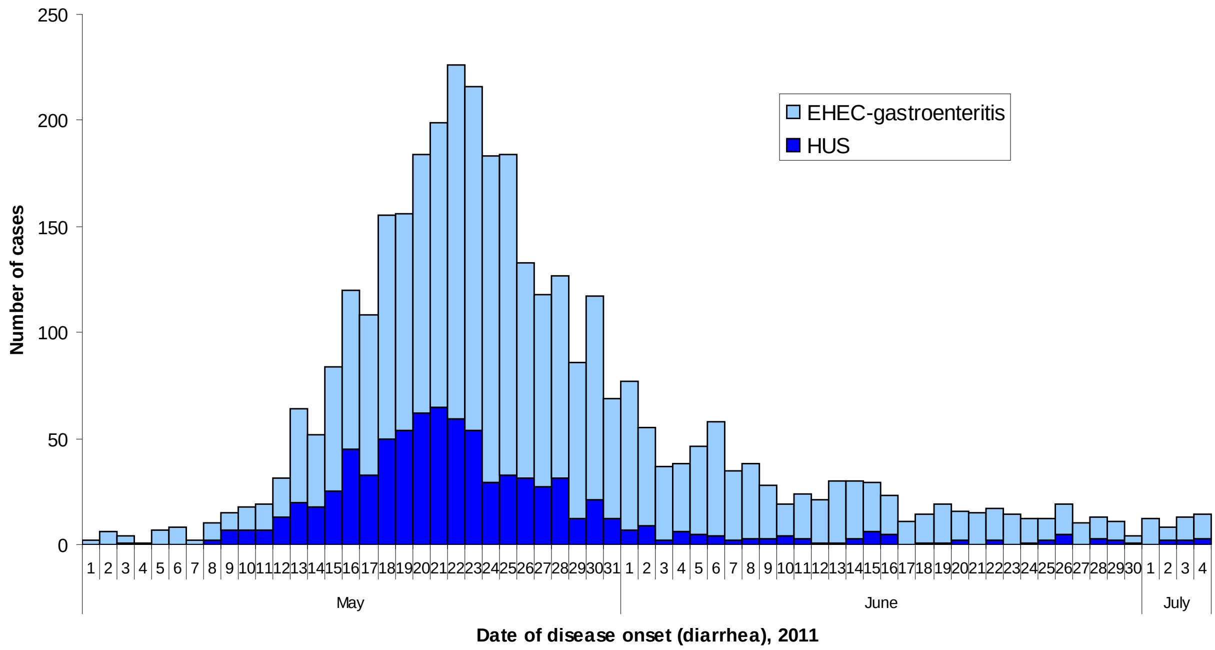

Figure 2: Epidemiological curve for HUS and EHEC outbreak cases (809 HUS and 2,717 EHEC cases with known date of disease onset (diarrhoea) within the outbreak time period.

Within the outbreak time period, cases were reported from all 16 states; however, the five most northern federal states (Hamburg, Schleswig-Holstein, Bremen, Lower Saxony and Mecklenburg-Western Pomerania) were most affected (HUS disease incidences from 1.8 to 10.0 cases per 100,000 persons – all other states had incidence rates <1 cases per 100,000 persons).

Figure 3 illustrates the HUS incidences per county (including independent cities). Case persons with travel history within Germany are counted in the county in which they were probably infected.

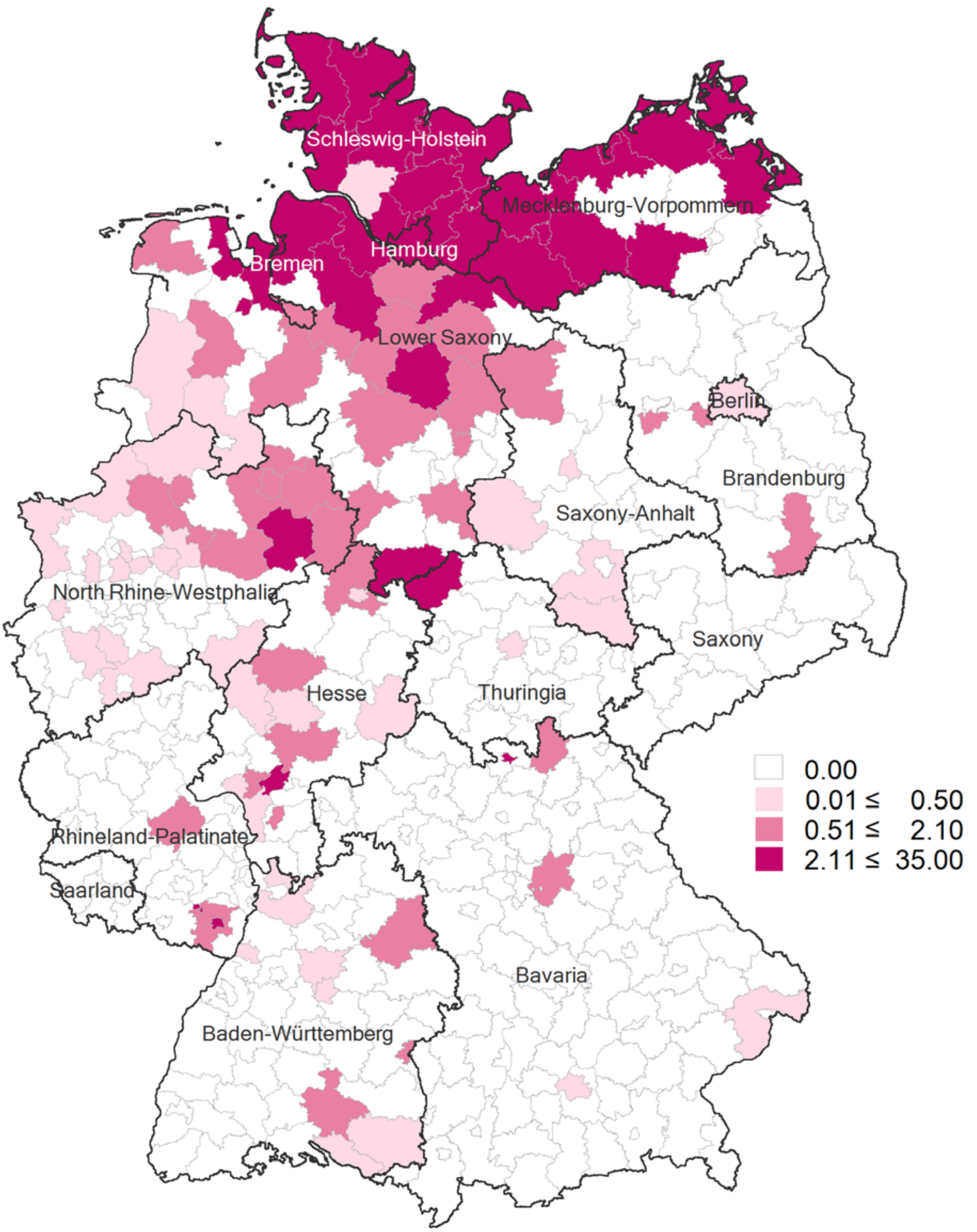

Figure 3: Incidence (cases per 100,000 persons) of HUS during the outbreak, illustrated by county, in which infection likely occurred (residence county, or in cases with travel history, the county of presence at the time of infection).

Compared to the HUS and EHEC data reported from previous years, the following differences can be noted:

- Compared to 696 cases of HUS reported to the RKI from 2001 to 2010, the affected persons in the HUS outbreak cases are much older. Only 2% of outbreak cases are under age 5, compared to 69% of HUS cases in previous years in which HUS was primarily a paediatric problem.

- The proportion of women among the EHEC outbreak cases (58%) is similar to their proportion among the adult EHEC cases from 2001 to 2010 (61%). However, the proportion of women among the HUS outbreak cases (68%) compared to the 63 adult HUS cases from 2001 through 2010 (56%) is higher.

The current outbreak is the largest HUS/EHEC outbreak that has ever been described in Germany, and in terms of the number of reported cases of HUS, it is also by far the world's largest described outbreak of this kind. For a more detailed description of the reported data, see also the various scientific publications on the outbreak in Eurosurveillance[1,2] as well as other publications currently in preparation. A final version of the New England Journal of Medicine[3] article is expected to be published by the end of 2011.

1.2 Surveillance of bloody diarrhoea

Since bloody diarrhoea is frequently the first symptom EHEC patients experience, the development of an EHEC outbreak can be assessed almost real-time by ascertaining patients presenting with these symptoms, e.g. in emergency departments. On 27 May 2011, syndromic surveillance of patients with bloody diarrhoea was established in emergency departments of voluntarily participating hospitals. Because reported numbers of emergency admissions fluctuated daily, on 1 July, daily case reporting was carried on in those emergency departments participating as constantly as possible, in order to enhance interpretation possibilities of the data. The surveillance was continued through 30 September, 2011, since even after the end of the outbreak itself, people could have excreted O104:H4 and isolated EHEC O104:H4 cases could have been reported to the Public Health Service.

The participating emergency departements represented all states, both in **areas more affected** (Bremen, Hamburg, Schleswig-Holstein as well as parts of Lower Saxony (north) and North Rhine-Westphalia (Paderborn)) as well as in **areas less affected** by the outbreak. Data collection included the total daily number of all patients visiting the participating emergency departments and the number of patients with bloody diarrhoea by gender and age group (<20 years, >= 20 years).

The data were sent to the RKI via e-mail or fax on a daily basis. As of 30 June, a total of 193 emergency departments had participated in the syndromic surveillance; of these, 28 were located in areas more affected. As the surveillance continued from 1 July onward, 75 emergency departments were participating; of these, 16 were located in the more affected areas described above. The number of actively participating emergency departments varied from day to day. Therefore, results may subsequently change if further, retrospective data are sent from emergency departments. All the analyses that follow are based only on the data from those emergency departments that took part after the continuation of the surveillance after a July.

From the start of of the bloody diarrhoea surveillance, the proportion of patients with bloody diarrhoea among all patients visiting emergency departments in more affected areas progressively converged with the proportion among patients visiting emergency departments in less affected areas. After the reporting week from 18-24 July, there was no

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[1] Frank C, Faber M, Askar M, et al. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. Euro Surveill 2011;16:pii=19878.
[2] Askar M, Faber M, Frank C, et al. Update on the ongoing outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing Escherichia coli (STEC) serotype O104, Germany, May 2011. Euro Surveill 2011;16; pii=19883.
[3] Frank C, Werber D, Cramer JP, et al. Epidemic Profile of Shiga-Toxin-Producing Escherichia coli O104:H4 Outbreak in Germany - Preliminary Report. N Engl J Med 2011.

difference in the proportion between groups (Table 1). Figure 4 illustrates the proportion of patients with bloody diarrhoea among all emergency department patients by gender and age group in more affected areas. Women were more frequently affected by bloody diarrhoea than men. After 30 May, a decreasing proportion of females among the cases was observed.

Table 1: The proportion of patients with bloody diarrhoea out of all patients visiting emergency departments by area and week. Quotient represents the "Proportion in more affected areas relative to the proportion in less affected areas". EHEC/HUS outbreak, Germany 2011.

Week	more affected area	less affected areas	quotient
30 May – 5 June	5.2 % (315/6,067)	0.9 % (91/10,407)	5.9
6 June – 12 June	3.7 % (218/5,929)	0.8 % (142/17,291)	4.5
13 June – 19 June	1.6 % (70/4,264)	0.6 % (108/17,263)	2.6
20 June – 26 June	1.0 % (29/2,963)	0.5 % (76/16,437)	2.1
27 June – 3 July	0.9 % (31/3,460)	0.4 % (73/16,479)	2.0
4 July – 10 July	0.5 % (22/4,793)	0.3 % (41/16,219)	1.8
11 July – 17 July	0.4 % (20/4,996)	0.2 % (36/15,763)	1.8
18 July – 24 July	0.3 % (15/6,058)	0.3 % (36/13,639)	0.9
25 July – 31 July	0.2 % (9/5,159)	0.2 % (28/14,213)	0.9
1 August – 7 August	0.2 % (9/4,743)	0.1 % (21/14,606)	1.3
8 August – 14 August	0.2 % (9/5,028)	0.2 % (31/14,091)	0.8

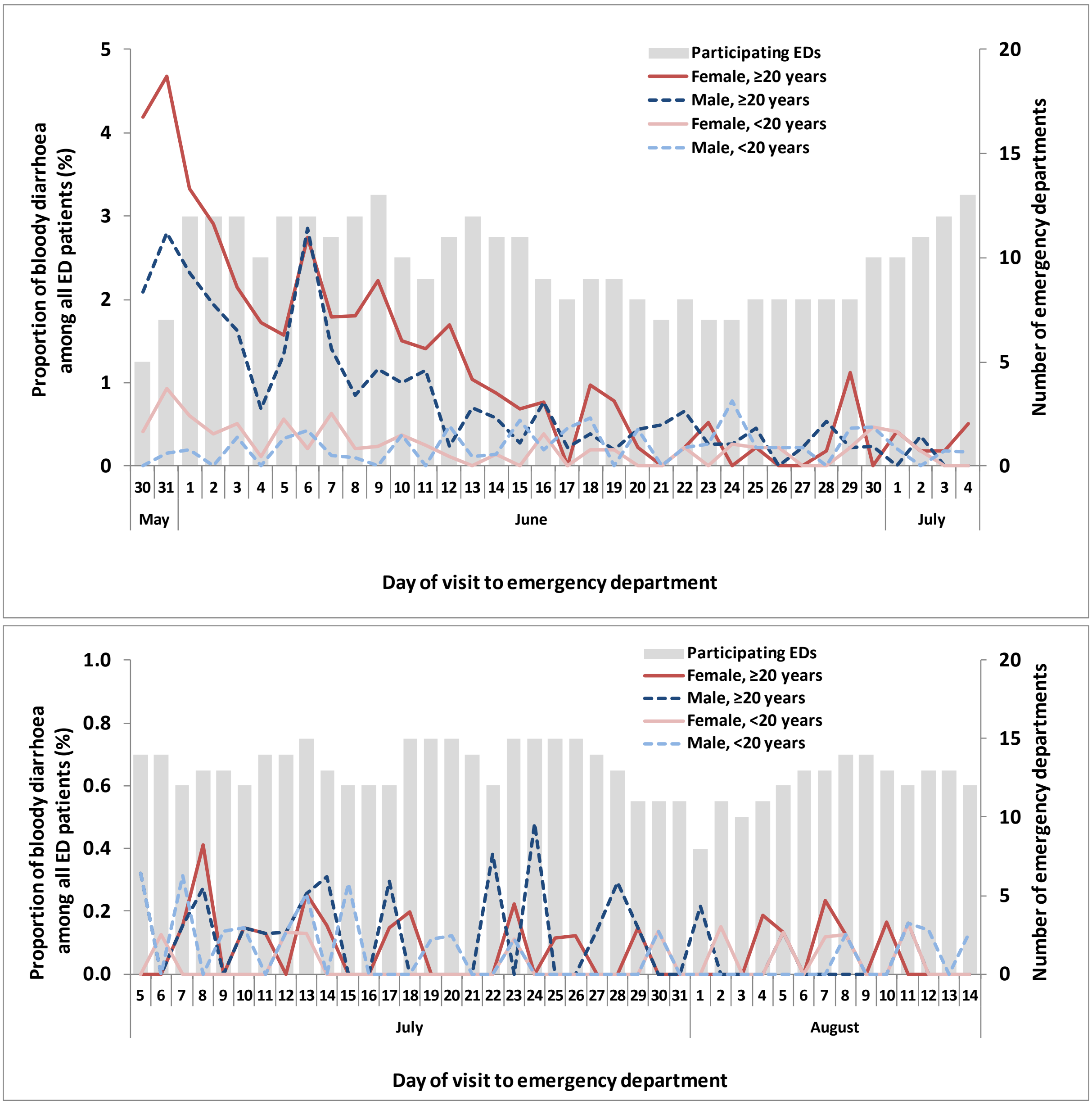

Figure 4: Surveillance of bloody diarrhoea in emergency departments in more affected areas: proportions of patients with bloody diarrhoea among all patients visiting emergency departments, by gender and age group, EHEC/HUS outbreak, Germany 2011 (n=747), for each time period (A): 30 May – 4 July and (B): after 5 July. Forenhanced legibility, the figure have different scales on the y-axis.

1.3 Case reports abroad (as of 18 August, 2011)

France reported a local EHEC O104 outbreak in Bordeaux that was not associated with a stay in Germany[4]. The outbreak comprised 15 cases with onsets of disease between 15 and 20 June, which are probably associated with the consumption of sprouts grown in France. This suggests that contaminated sprouts (and/or their seeds) as a vehicle for infection were not confined to Germany and needed to be taken into consideration as vehicle in other occurring disease clusters domestically and internationally. For details, see Europe-

[4] Gault G, Weill FX, Mariani-Kurkdjian, et al. Outbreak of haemolytic uraemic syndrome and bloody diarrhoea due to Escherichia coli O104:H4, south-west France, June 2011. Euro Surveill. 2011;16(26):pii=19905. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19905

an Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) statement from 29 June 2011[5].

There were two additional cases reported that did not involve stay in Germany or confirmed consumption of sprouts from Sweden (1 case) and Austria (1 case).[6]

In addition to these cases, all EHEC or HUS cases which occurred internationally to date have directly or indirectly been linked to a stay in Germany (see Table 2).

Table 2: Number of cases and deaths in countries in the European Union (Source: ECDC, as of 26 July 2011 11:00 a.m., WHO: as of: 21 July 2011 6:00 p.m.)

States	EHEC (deaths)	HUS (deaths)
EU		
Denmark	16 (0)	10 (0)
France	2 (0)* + 2(0)§	9 (0) §
Greece	1 (0)	0 (0)
Great Britain	3 (0)	4 (0)
Luxembourg	1 (0)	1 (0)
Netherlands	7 (0)	4 (0)
Norway	1 (0)	0 (0)
Austria	4 (0)	1 (0)
Poland	1 (0)	2 (0)
Sweden	35 (0)	18 (1)
Spain	1 (0)	1 (0)
Czech Republic	1 (0)	0 (0)
Total EU	**75 (0)**	**50 (1)**
Non-EU		
Canada	1 (0)	0 (0)
Switzerland	5 (0)	0 (0)
USA	2 (0)	4 (1)
Total Non-EU	**8 (0)**	**4 (1)**
Overall total	**83 (0)**	**54 (2)**

* Cases in connection with stay in Germany

§ Cases in connection with the outbreak in Bordeaux (only 11 of the 15 cases are listed)

1.4 Incubation period

During the investigation of the outbreak, evidence emerged indicating a prolonged incubation period for infections with the outbreak strain (compared to information in the literature and experience from previous outbreak investigations with other EHEC serotypes). Knowledge of the incubation period is of central importance for epidemiological studies (e.g. in order to consider the correct exposure period when interviewing patients), for the evaluation of epidemiological trends and for the establishment of preventative measures. On the basis of known exposure time points in selected cases and their date of disease onset, the incubation period was estimated for the outbreak strain. In this estima-

[5] http://www.efsa.europa.eu/de/press/news/110629a.htm

[6] EFSA/ECDC joint rapid risk assessment: Outbreak of Shiga toxin-producing E. coli (STEC) O104:H4 2011 in the EU 8 July 2011 (updated from 29 June)
http://ecdc.europa.eu/en/publications/Publications/110712_TER_Risk_Assessement_Ecoli.pdf

tion, only cases with known date of onset and a known exposure period of a maximum of 2 days were included (compare publication in NEJM[7]).

Cases for which area of residence and presumed exposure site were exactly the same or located within the outbreak area were excluded. The data analyzed originated from the statutory notification system, from restaurant-clusters and from international cases.

Within these criteria, the incubation period could be analyzed for 91 cases. The resulting probability mass function is illustrated in Figure 5.

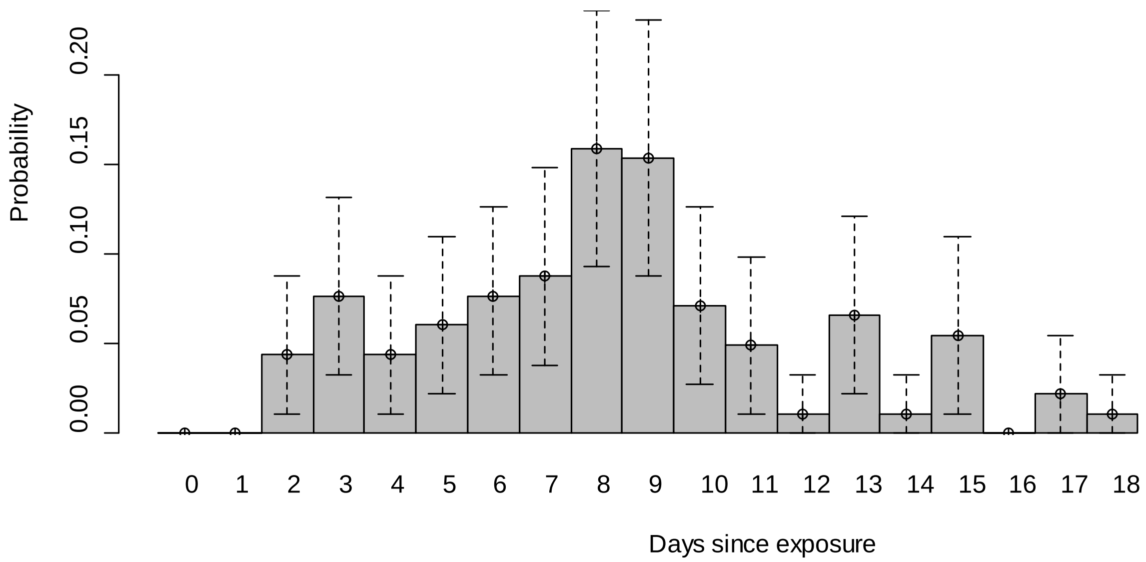

Figure 5: Estimated probability mass function on the incubation period (based on 91 individuals) with corresponding pointwise 95% confidence intervals.

The median incubation period up to the onset of diarrhoea symptoms is 8 days, with the 25th percentile at 6 days and the 75th percentile at 10 days. Compared to the incubation period of EHEC O157 (3 to 4 days), the incubation period for EHEC O104 is significantly longer.

To estimate the duration from the onset of diarrhoea until the onset of HUS, data from the statutory notification system were analysed. The calculation is based on 98 cases: the median between the onset of diarrhoea and the onset of HUS was 5 days, with the 25th percentile at 4 days and the 75th percentile at 7 days. The average duration between the onset of diarrhoea and HUS appears to be shorter for the outbreak strain than for infections with enterohemorrhagic *E. coli* serotype O157 (7 days).

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[7] Frank C, Werber D, Cramer JP, et al. Epidemic Profile of Shiga-Toxin-Producing Escherichia coli O104:H4 Outbreak in Germany - Preliminary Report. N Engl J Med. 2011 Jun 22. [Epub ahead of print]

1.5 Estimate of the exposure period

The incubation period estimated in Section 1.4 can be used to estimate the possible time point of exposure of the cases. For this, HUS cases with known date of onset of diarrhoea were used (as of 23 August 2011: 809 of 854 cases).

The back-projection from the onset of diarrhoea to the time of exposure is performed by the method of Becker et al.[8], which was developed for the back calculation from AIDS incidence to HIV incidence. In Figure 6, the blue bars (i.e. λ_t) indicate the estimates for the expected number of exposures per day. The figure also contains 95% bootstrap confidence intervals for λ_t to account for the uncertainty in the estimate of the incubation period used for the back-projection.

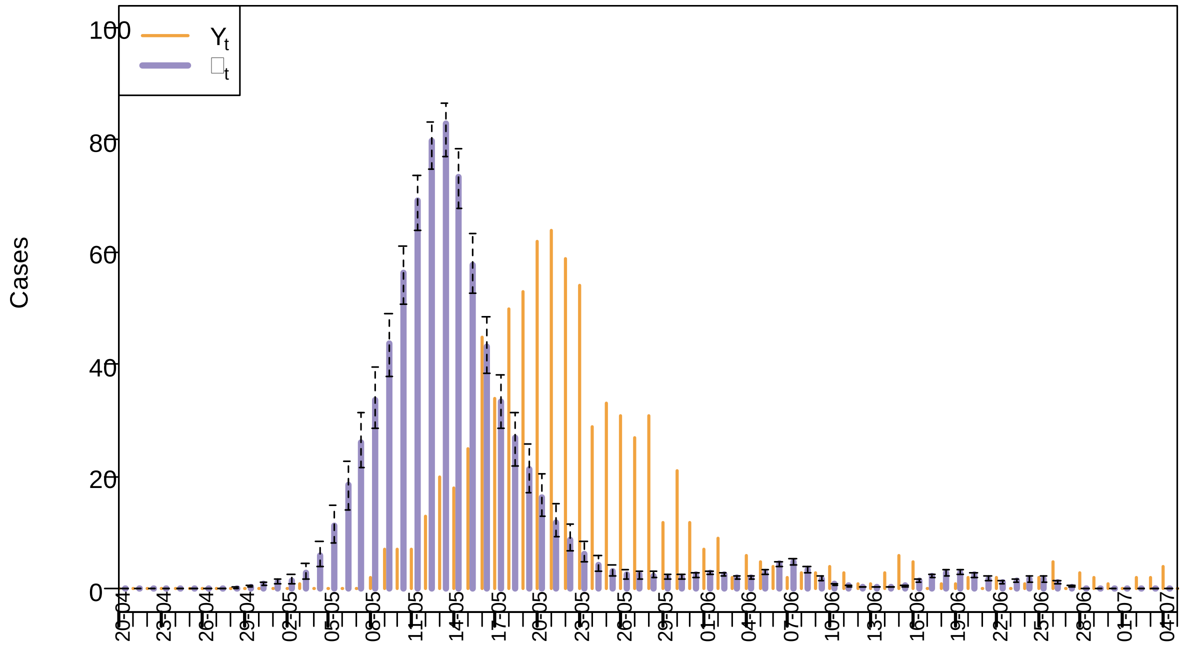

Figure 6: Back-projection from the daily onsets of disease to daily exposures. The thin orange bars Y_t show the actual observed cases by onset of disease, the thick blue bars λ_t show the estimated expected number of exposures per day (including 95% confidence intervals).

The back-projection indicates that 90% of HUS cases, which were reported as part of the outbreak, are likely to have had their exposure before 23 May. Furthermore, the greatest number of new daily infections occurred in the period between 12 May and 14 May.

[8] Becker NG, Watson LF, Carlin JB (1991), A method of non-parametric back-projection and its application to AIDS data, Statistics in Medicine, 10(10):1527–1542.

1.6 Reporting delays

EHEC and HUS cases occur in low numbers throughout the year, without them being attributed to an extraordinary cluster or other unusual event. The RKI was initially informed by email about a small cluster (n = 3) of paediatric HUS cases in Hamburg on 19 May 2011. Before this date, no increase of the reported EHEC and HUS cases compared to the number to be expected was evident. After 20 May 2011, the RKI investigated the outbreak in northern Germany in close collaboration with health and food safety authorities of the federal and state level.

All cases with disease onset after 1 May 2011, but typically reported later, were retrospectively included in the epidemiological analysis. This date was chosen to describe the beginning of the outbreak as completely as possible. Distinction must be made between the date of onset of disease, date of hospitalization, date of diagnosis and date of report to the local health authority and receipt of the notification at the RKI.

According to the specifications of the German Infection Protection Act, a case of disease must be reported by the diagnosing physician and detection of a pathogen by the laboratory to the appropriate local health authority within 24 hours. The local health authority reviews the information and enters it into an electronic database. By the third business day of the following week at the latest, the information is notified electronically to the responsible state health authority and then within another week (at the latest) it is electronically sent to the RKI (according to § 11 IfSG). After the EHEC/HUS outbreak became known, from the week beginning 23 May 2011 onward, the Robert Koch Institute requested daily notification of new HUS cases and updates on previously known cases. Between 24 May and 27 July, more than 50% of the cases were notified to the RKI within two days and 75% were notified within four days after receipt of the report at the local health authority.

In practice, from the onset of disease until the visit with the doctor and/or until hospital admission and then until report to the local health department and electronic notification of these data via the responsible state authorities to the RKI, there is a period between a few days and several weeks. Figure 7 shows the current (as of 26 August 2011, 10:00 a.m.) number of HUS cases reported to the RKI by onset of disease. Displayed in colour is the date (week of receipt of the notification at the RKI) on which the cases were notified to the RKI. It is clear that only one case of HUS with the onset of disease on 3 May 2011 was known to the RKI before 23 May 2011, but this could not be recognized as exceptional due to the undetermined serotype at that time. It was not until the week from 23 to 29 May 2011 that a greater number of HUS cases were notified to the Robert Koch Institute. At that point, investigations conducted by the RKI had already begun.

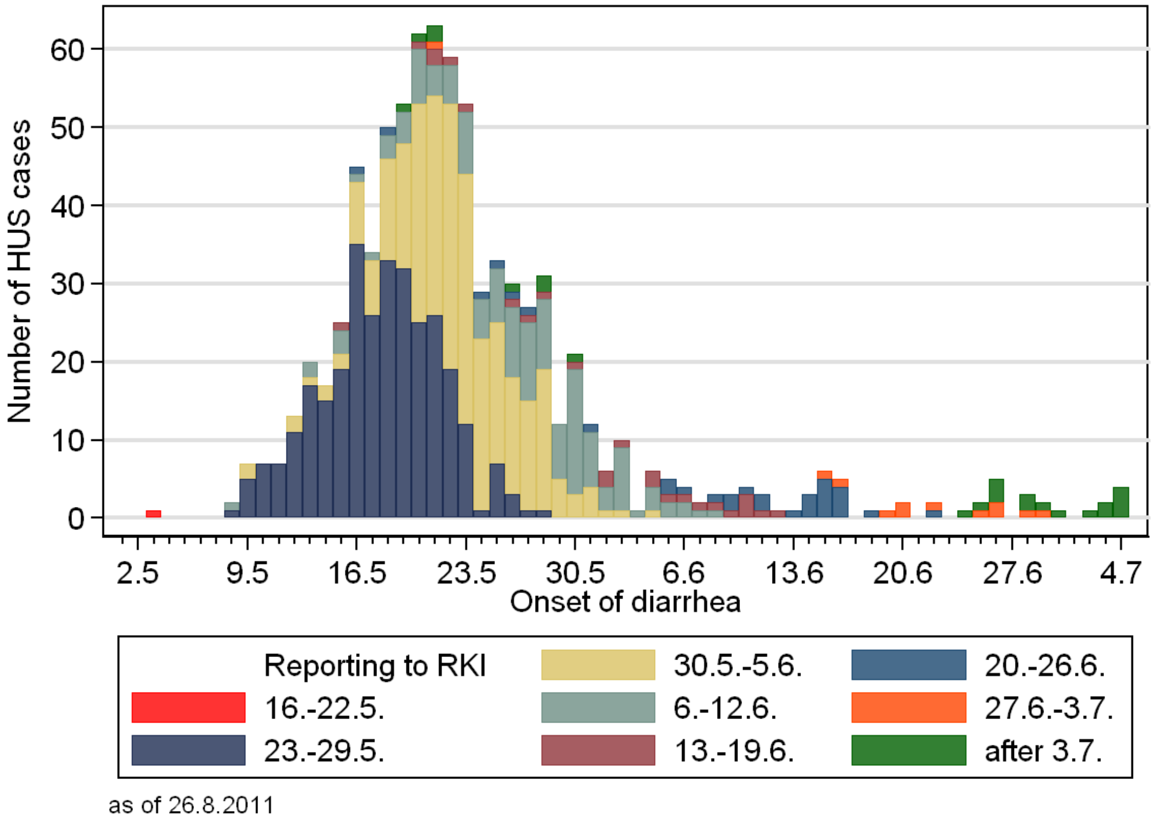

Figure 7: HUS cases by onset of disease and week of receipt of the notification at the RKI.

Likewise in the EHEC reporting category, in which there is a "background" of a few cases per week, only 3 cases were notified in the week from 9 until 15 May 2011, another 5 cases from 16 until 19 May 2011 (in the week from 16 until 22 May 2011, a total of 14 cases) without specified serotype; only in the week from 23 to 27 May 2011 was a larger number of cases sent to the RKI.

Figure 8 illustrates the epidemic curve of HUS cases by onset of disease, beginning of hospitalization, date of diagnosis, date of reporting to the local health department (GA) and receipt of the notification at the RKI. This analysis considered only those date data, which follow the natural temporal sequence from onset of disease, to diagnosis, to reporting and finally notification transmission. The delays between the level of the entire process become apparent. Regarding HUS, at the beginning of the outbreak onset of disease and hospitalization dates lie well before the date of notification, which is also true for the diagnoses dates of the first cases. The receipt of the notification at the RKI is again a few days after the report to the local health authority. The sum of these individual delays leads to the total delay between disease onset and notification received at the RKI[9].

[9] Altmann M, Wadl M, Altmann D, et al. Timeliness of surveillance during outbreak of Shiga toxin–producing Escherichia coli, Germany, 2011. Emerg Infect Dis. 2011 Oct; [Epub ahead of print] http://www.cdc.gov/eid/content/17/10/pdfs/11-1027.pdf

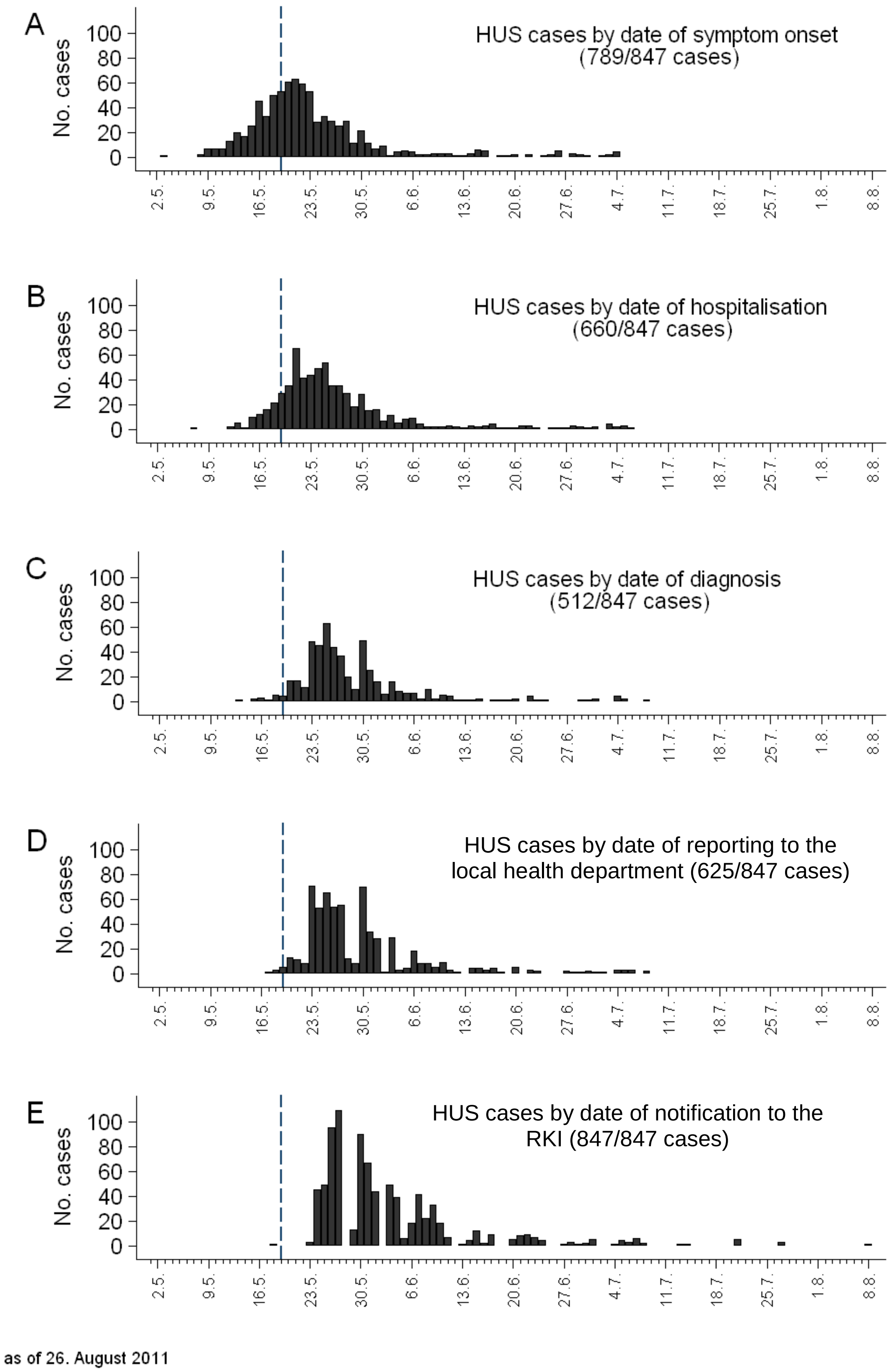

Figure 8: HUS cases, according to outbreak case definition, presented chronologically by: date of disease onset (A), date of hospitalization (B), date of diagnosis (C), date of reporting to health authorities (D), and date of receipt of notification at the RKI (E). The vertical line marks 19 May, the date on which RKI received the report on a cluster of 3 HUS cases in Hamburg.

1.7 *"Now-Casting"*

During the course of the epidemic, it was important to take into account the reporting and notification delay in the interpretation of the number of reported HUS cases by date of disease onset. Therefore, for each day, the number of hospitalized cases, which had not yet been sent to the RKI, was retrospectively estimated (a kind of "now-casting").

In order to estimate the reporting/notification delay distribution, dates of hospitalization and dates of receipt of the corresponding notifiation at the RKI of HUS cases notified to the RKI between 23 May and 1 June were utilised. Based on this distribution, from 2 June on, now-casting was conducted as follows:

Let $y_{t,s}$ be the number of hospitalizations on day t, which were reported to the RKI at the time s≥t, and let y_t be the number of actual hospitalizations on day t, i.e. $y_t = \sum_{s=t}^{\infty} y_{t,s}$. In other words, at time s only $y_{t,s} = F(s-t) \cdot y_t$ hospitalizations were observed at the RKI. Here, F is the estimated distribution function of the notification and reporting delay, using the data from the period 23 May to 1 June. A forecast on day s for the actual number on day t is thus $y_t = \frac{y_{t,s}}{F(s-t)}$.

To take into account the uncertainty in the estimation of the distribution function F in the calculation of the forecast, 95% prediction intervals were also calculated for F and then correspondingly transformed to obtain a 95% prediction interval for y_t.

Figure 9 ilustrates the results of the now-casting on 7 June 2011. The cases, by date of hospital admission, reported to the RKI by this date can be discerned (in green). Also shown are the estimated number of hospitalized cases, which until then had not been reported/notified because of the reporting/notification delay (orange).

Figure 10 depicts the result of the now-casting as of 23 June 2011. It is evident that after 13 June – even when taking into account the delay in notifying and reporting – only few HUS cases were predicted and have occurred.

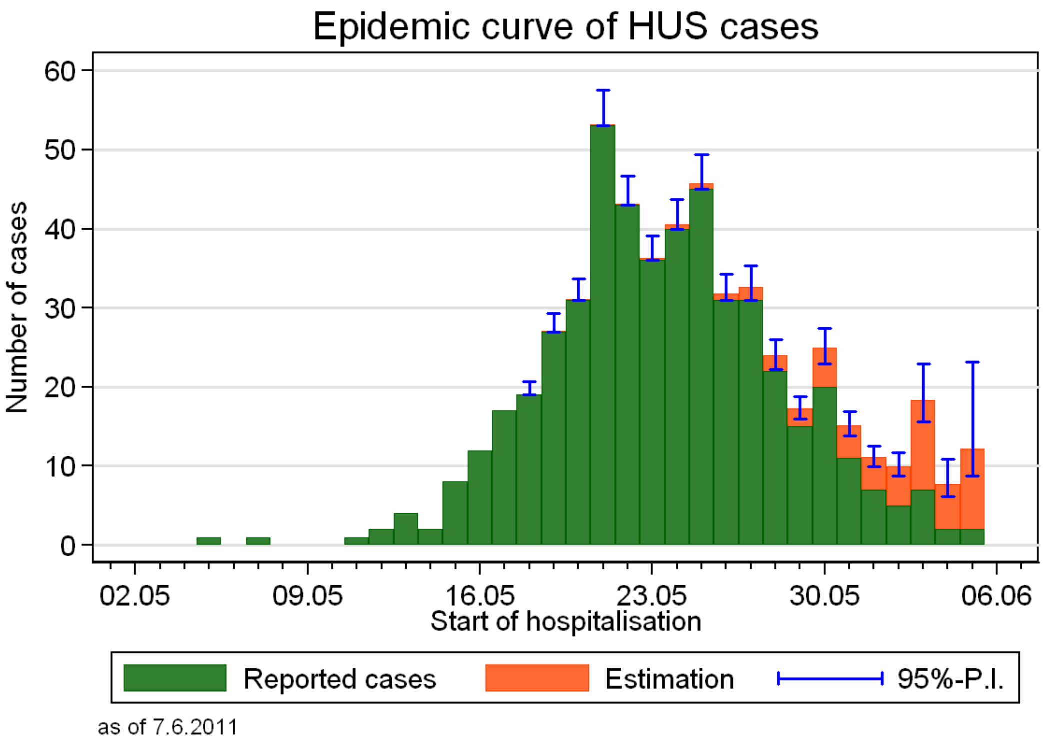

Figure 9: Now-Casting on 7 June 2011. The green bars show the situation of HUS, reflected by the data available to RKI as of 7 June 2011. The now-cast forecasts are illustrated in orange and the 95% prediction intervals (PI) in blue.

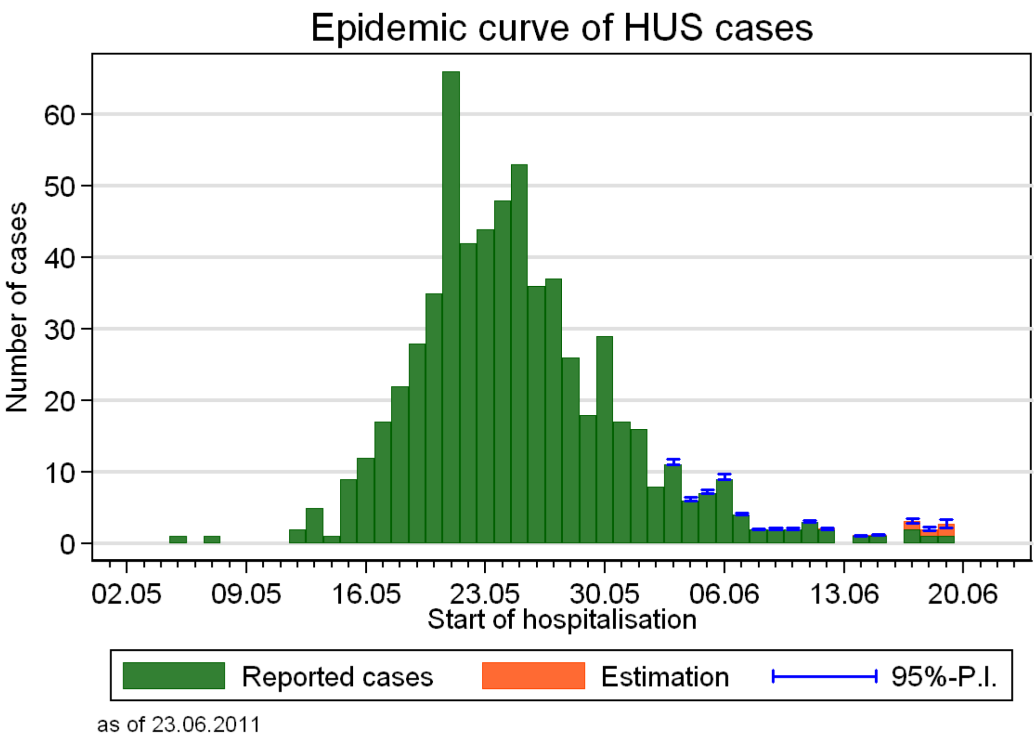

Figure 10: Now-Casting on 23 June 2011. The green bars show the situation of HUS, reflected by the data available to RKI on 23 June 2011. The now-cast forecasts are illustrated in orange and the 95% prediction intervals (PI) in blue.

2 Investigations on the vehicle of infection

Since 20 May 2011, the RKI investigated the outbreak of haemolytic-uremic syndrome (HUS) and bloody diarrhoea associated with infections by enterohemorrhagic *Escherichia coli* in northern Germany in collaboration with health and food safety authorities of the federal and state governments. The cause of the outbreak could be narrowed down by a number of consecutive epidemiological studies. The most important studies and results are presented below.

2.1 Early epidemiological studies

The time course, geographic and demographic distribution of cases and the initial exploratory interviews with patients early on indicated the transmission route in this outbreak to be food-borne. Vehicles such as raw milk, raw meat or sprouts that had been identified as a source of infection in previous EHEC/HUS outbreaks appeared to play no role in current events according to exploratory interviews.

The first case-control study of patients in Hamburg was limited for methodological reasons to those exposures that were able to explain a large proportion of cases. Thel analysis showed a significant association of being a case with consumption of raw tomatoes, cucumbers, and lettuce. On 25 May, these findings were released in a joint news conference with the Federal Institute for Risk Assessment (BfR)[10], communicated within the Early Warning and Response System (EWRS) of the EU and on 26 May together with a preliminary description of the outbreak in a publication in the online-journal Eurosurveillance[11]. Since neither thiss study nor evidence from the food safety sector could narrow down this list of vegetables at this time, the RKI initiated and conducted further studies.

2.2 Analysis of a satellite outbreak in two canteens of a Frankfurt-based company

Between 9 and 17 May 2011, a total of 60 employees at two locations of a Frankfurt-based company contracted bloody diarrhoea, nine of which were laboratory-confirmed (to be EHEC infected); 18 of the 60 employees developed HUS. On 19 May 2011, the health department of the city of Frankfurt am Main was informed of the events by the personnel office of the company and initiated an investigation of the outbreak.

The Health Protection Authority City and the Veterinary Service of Frankfurt am Main, together with the Robert Koch Institute and the operators of the canteens, were able to acquire a list of the purchases made in the canteen in the weeks from 2 to 16 May for case persons (n = 23) and for randomly selected healthy persons (n = 30) with the help of electronic billing documentation. A logistic regression analysis (Table 3) was performed.

The risk of contracting bloody diarrhoea for employees who had bought and consumed a salad in the canteen in the above mentioned period was six times higher compared to employees who had not bought any salad. A total of 20 of the 23 cases (87%) could be explained by the salad purchase. The consumption of other foods from the canteen was not significantly associated with the disease. With this study, a salad bar item available in the canteen could be identified as the most likely vehicle.

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[10] Preliminary results of the EHEC/HUS study (Statement of the BfR and RKI of 25 May 2011), http://www.rki.de/cln_178/nn_467482/DE/Content/InfAZ/E/EHEC/Gemeinsame__Stellungnahme__RKI__BfR.html

[11] Frank C, Faber M, Askar M, et al. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. Euro Surveill 2011;16:pii=19878.

The results of the subsequently conducted nationwide traceback analyses revealed that the operator of the two canteens was connected through one distributor to the producer of sprouts in Lower Saxony (Company A). These were also offered in the two canteens. After becoming aware of this connection, a new, more specific survey among the employees regarding the actual salad bar components to confirm the sprout hypothesis was unfortunately not possible any more [12].

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Table 3: Results of the univariable and multivariable analyses (odds ratios and associated 95% confidence intervals, 95% CI) of risk factors for the development of bloody diarrhoea for guests at two canteens in Frankfurt am Main.

		Univariable	Multivariable
		Odds ratio (95% CI)	Odds ratio (95% CI)
Salad consumption		5.83 (1.42-23.88)	6.57 (1.37-31.39)
Dessert consumption		1.52 (0.48-4.81)	
Fruit consumption		0.53 (0.15-1.81)	
Asparagus consumption		0.75 (0.24-2.41)	
Gender (♀=1)		2.28 (0.73-7.15)	2.63 (0.63-10.96)
Age	<30	2.80 (0.62-12.66)	2.13 (0.41-11.17)
	30-<40	Reference value	Reference value
	40-<50	0.43 (0.09-2.14)	0.53 (0.09-2.98)
	≥50	0.70 (0.09-5.43)	0.31 (0.03-3.07)

2.3 Recipe-based restaurant cohort study

In order to be less dependent on raw vegetable consumption memory of patients and control subjects during the analysis, the RKI pursued the following approach with the help of a "recipe-based restaurant cohort study": [13]

Partly in the context of cluster detection with the support of information from public health services, and partly as a result of active case finding by means of the order books of a restaurant, 10 groups with a total of 176 participants could be identified, who dined in the same restaurant during the period from 12 to 16 May 2011. Individuals who could not be reached and for whom no other person could give details concerning the meal that was served to them, or who could not remember the dish they had eaten, were excluded. A total of 31 (18%) individuals from the groups contracted bloody diarrhoea or EHEC/HUS. This exposure information came from two sources:

[12] Wilking H, Götsch U, Meier H, et al. Use of payment system information for detection of risk factors during an outbreak of Shiga-Toxin–Producing Escherichia coli (STEC) O104. Emerg Infect Dis. 2011 [accepted]

[13] Buchholz U, Bernard H, Werber D, et al. German Outbreak of Escherichia coli =104:H4 Associated with Sprouts. NEJM 2011; [accepted]

- The group participants were asked which meal they had ordered (photographs were used as a reminder), though in principle, the pre-ordered meals were already known for most groups from the booking lists of the restaurant

- The chef of the restaurant was interviewed in detail concerning the quantities of each ingredient went into which meals and how each meal was prepared.

The information from these interviews was evaluated in a cohort approach that retrospectively calculates the relative risk of disease (RR) for restaurant customers in the corresponding period. Here, the analyses showed that customers, who had been served sprouts, had a 14.2 times higher risk of contracting the disease in the univariable analysis (95% CI 2.6 - ∞, p <0.01) according to the case definition, compared to people who had not been served sprouts. All 31 case subjects had been served a meal that contained sprouts. The result of the univariable analysis was confirmed in the multivariable analysis (RR: 14.2, 95% CI 2.4 - ∞, p<0.01). The use of other raw ingredients such as tomatoes, cucumbers or green salad in the served meals yielded no significant p-values (p> 0.15) in the analysis for an increased relative risk of disease.

Table 4: Results of univariable and multivariable analyses of the recipe-based restaurant cohort study. RR = relative risk; 95% CI.

Ingredient	Total	Cases among the exposed	Total number exposed	Cases among the non-exposed	Total number non-exposed	RR	95% CI	p-value
Univariatel								
Sprouts	152	31	115	0	37	14.23	2.55-infinity	<0.01
Tomatoes	152	14	50	17	102	1.68	0.77-3.62	0.18
Cucumbers	152	14	50	17	102	1.68	0.77-3.62	0.18
Chinese cabbage	152	13	45	18	107	1.72	0.77-3.71	0.17
Radicchio	152	13	45	18	107	1.72	0.77-3.71	0.17
Iceberg lettuce	152	13	45	18	107	1.72	0.77-3.71	0.17
Multivariate								
Sprouts						14.17	2.40-infinity	<0.01

In the period in which the groups dined in the restaurant, the restaurant used only one mixture of sprouts, containing fenugreek sprouts, alfalfa sprouts, adzuki bean sprouts and lentil sprouts. The supplier of sprouts for the restaurant received the sprouts from the incriminated Company A in Lower Saxony.

2.4 Findings from additional case-control studies on the consumption of sprouts

A variety of animal and vegetable foods, including sprouts, had already been taken into account during the first intensive survey of patients from Hamburg (20/21 May). In these exploratory interviews, only 3 of 12 patients declared having eaten sprouts. Overall, the patients interviewed stood out because of very conscious and careful eating habits, which made a relevant under-reporting of sprouts unlikely. It is a methodological requirement and standard practice to include only those exposures that are potentially able to epidemiologically explain a large part of the outbreak in further analyses.[14] Otherwise, the risk of false positive correlations increases with the inclusion of an excessive number of exposures. Therefore, the sprouts were not initially pursued as a vehicle for the outbreak. Sprouts were taken into account in subsequent detailed studies conducted by the RKI.

[14] World Health Organization (WHO), 2008: Foodborne Disease Outbreaks: Guidelines for Investigation and Control. http://www.who.int/foodsafety/publications/foodborne_disease/outbreak_guidelines.pdf)

2.4.1 "Raw Vegetables" Case-Control Study

In order to further narrow down the list of raw vegetables suspected as potential vehicles of infection, another case-control study was conducted. The study involved interviewing case subjects in three cities strongly affected by the outbreak (Lübeck, Bremerhaven and Bremen). Case patients were adult HUS patients who were hospitalized during the study period in one of three hospitals in Lübeck, Bremerhaven or Bremen. Controls were individually matched to case patients by age group (18-34 years, 35-44 years, 45 years or older), gender and place of residence, with a target ratio of 1:3. Recruitment of the controls was carried out by contacting residents at their homes. A starting point was set approximately 50m from the address of the case subject.

On the basis of previous exploratory interviews with other HUS patients, the cases and controls in this study were interviewed regarding the consumption of primarily vegetable foods, such as fruits and raw vegetables, during the 2 weeks before the onset of diarrhoea (cases) or prior to the interview (controls). In addition, sprouts were included in the list of potential risk foods, although only 25% of HUS patients had reported their consumption in exploratory interviews. Conditional logistic regression was used for statistical analyses. Multivariate models were determined using a manual forward/backward strategy based on p-values.

A total of 26 cases (9 men, 17 women) and 81 controls were included in the study. The median age of cases was 47.5 years (interquartile range 29-75 years). With respect to sprout consumption, 6 (25%) of the 24 cases indicated they had eaten sprouts in the assumed infection period, compared to 7 (9%) of the 80 controls with information on this item. Table 5 lists all exposures with p <0.1.

Table 5: Fruit and vegetable exposures associated with adult HUS (p-value <0.1) in univariate analysis of the "raw vegetable" case-control study.

Exposure	Exposed cases	Exposed controls	Matched Odds Ratio (95% CI)	p
	No./Total No. (%)	No./Total No. (%)		
Sprouts	6/24 (25)	7/80 (9)	4.35 (1.05-18.0)	0.043
Cucumbers	22/25 (88)	52/79 (66)	3.53 (0.96-12.9)	0.057
Apples	22/24 (92)	57/81 (70)	3.91 (0.86-17.7)	0.077
Peppers	16/24 (67)	35/80 (44)	2.66 (0.90-7.9)	0.077
Strawberries	19/26 (73)	43/81 (53)	2.33 (0.90-6.0)	0.082

*Exposures with p>0.1: raw onions, lettuce, asparagus, carrots, tomatoes, basil.

In multivariate analysis, sprouts and cucumbers were first examined together: both variables remained significant, with an Odds Ratio (OR) of 5.8 for sprouts (95% CI 1.2-28.6; p=0.032) and an OR of 6.0 (95% CI 1.1-31.3) for cucumber. None of the other above-mentioned exposures that were then tried in the model had a significant associations with disease.

In the study, information on the source of supply for some of the exposures was also collected. Consumption of raw vegetables (cucumbers, carrots, tomatoes or lettuce) outside the home was positively associated with HUS (OR=9.4, 95% CI, 2.7-32.8). This could indicate that people were infected, for example, by eating salads outside the home (e.g. in canteens, restaurants). The above-mentioned foods are commonly consumed together (in the form of a salad), wherein a contamination may have affected these and/or another food product which was not remembered.

As part of a subsequent survey of cases and controls who at first had denied eating sprouts or could not remember having done so (20 cases, 73 controls), 8 (40%) cases and 37 (51%) controls were re-interviewed. Overall, 3 of the 8 (37.5%) interviewed cases changed their original statement and now reported to have *definitely* eaten sprouts in the surveyed period. None of the 37 re-interviewed controls changed their original statement. If the results of the re-interviewed cases are extrapolated to all cases that denied having eaten sprouts or were uncertain they had done so, the proportion of remembered sprout consumption out of all cases can be estimated as 52%.

2.5 Investigations of disease clusters

2.5.1 Cooperation with the EHEC Task Force

The EHEC Task Force convened by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) consisted of experts from the state food safety authorities in five states (Lower Saxony, Schleswig-Holstein, Mecklenburg-Western Pomerania, Hamburg and Bavaria), the BVL, BfR, EFSA and RKI. The EHEC Task Force was coordinated by the BVL and the Bavarian Health and Food Safety Agency (LGL). So-called disease clusters were analyzed with respect to supply channels and supply chains for certain food items.

A cluster was defined as occurrence of at least one case of disease (EHEC or HUS) at an exposure site, when there were strong indications that the infection could only have occurred at this place. This was the case, for example, when members of a tour group, in which there were several cases of disease, had eaten together only at one particular restaurant. Locations where individual cases had consumed food were considered for follow-up, if only a single exposure site was likely, e.g. Danish tourists travelling through northern Germany had only eaten at one specific motorway service station. As a first step, the identification of clusters was essentially conducted by means of information on cases obtained by the RKI. The information came from physicians, e.g., in hospitals, local and state health departments, health authorities from other countries, e.g., Sweden, Denmark and USA, from data reported to the RKI via the surveillance system and also from patients themselves, e.g., through patient questionnaires in epidemiological studies conducted by the RKI.

Overall, the Task Force was able to identify 41 outbreak clusters according to the above definition in six affected states (Lower Saxony, Schleswig-Holstein, Mecklenburg-Western Pomerania, Hamburg, Hessen, and North Rhine-Westphalia). These 41 clusters involved more than 300 cases. All 41 clusters could be linked to sprouts from sprout-producing Company A in Lower Saxony.

Two individuals in Lower Saxony infected with the outbreak strain had exclusively consumed sprouts that they themselves had grown from a sprout seed mix (“self-sprouters”). This suggested that contaminated sprout seeds must underlie the outbreak. Fenugreek and lentil seeds presented as the common denominator among all 41 outbreak clusters and the “self-sprouters”. The results of the investigation into the HUS/EHEC O104:H4 outbreak in France in June 2011, in which the disease was similarly linked to consumption of privately-grown sprouts (see Section 1.3), allowed the identification of the vehicle of infection to be narrowed to fenugreek seeds from Egypt. The detailed results of this investigation are presented and explained in a separate report on the work of the EHEC Task Force at BVL[15] and the European Task Force coordinated by the EFSA[16].

2.5.2 Cohort studies of selected clusters

Disease clusters were identified using reporting data, international data sharing, anecdotal evidence and standardized interviews of HUS patients in clinics17. Before sprouts were known to be the likely infection vehicle, cohort studies were carried out by the RKI using three early identified clusters. In this epidemiological approach, individuals from travel groups were interviewed in detail regarding their food consumption with the help of menu item lists, when a common exposure site was probable. The aim of the cohort studies was to identify the food item(s) the consumption of which was associated with increased risk of bloody diarrhoea/HUS. Groups of persons with a manageable number of members (diseased and healthy individuals) and with available contact information (phone numbers or e-mail addresses), who had a limited period of exposure (e.g. a particular weekend) at a common potential exposure site (e.g. a restaurant, hotel) and who were willing to participate in a survey by the Robert Koch Institute were suitable for cohort studies. A statistically significant association between consumption of particular foods (primarily lettuce, cucumbers and tomatoes) and the disease was partly found in the initial analyses of the cohort studies, but there was no single meal or food item that could explain all the disease cases. After there had been indications from other studies suggesting sprouts as infection vehicle, follow-up inquiries were conducted in the restaurants. Retrospectively, all cases could be explained through the consumption of sprouts (for example, in salads or as garnish for certain dishes). The fact that consumption of sprouts was commonly not remembered is consistent with the follow-up interviews of case persons in the “raw vegetable case-control study”. Through analysis of the supply channels, the sprouts could be traced back to the sprout-producing Company A in Lower Saxony and, moreover, to seed batches imported from Egypt for use in sprout production (see Section 2.5.1)

[15] Die Task Force EHEC: Adolphs J, Appel B, Bernard H, et al.: Ergebnisbericht der Task-Force EHEC zur Aufklärung des EHEC O104:H4 Krankheitsausbruchs in Deutschland. Journal für Verbraucherschutz und Lebensmittelsicherheit (http://www.springerlink.com/content/1661-5751), DOI 10.1007/s00003-011-0710-7, 2011

[16] European Food Safety Authority (2011): Tracing seeds, in particular fenugreek (Trigonella foenum-graecum) seeds, in relation to the Shiga toxin-producing E. coli (STEC) O104:H4 2011 Outbreaks in Germany and France, http://www.efsa.europa.eu/de/supporting/doc/176e.pdf

[17] Deleré Y, Eckmanns T, Krause G, et al.: Screening-/Kurzfragebogen zur explorativen Befragung von Patienten mit blutigem Durchfall während des EHEC/HUS-Ausbruchs in Deutschland. DN-Mitteilungen (Mitteilungsblatt der Deutschen Gesellschaft für Nephrologie). Heft 3, 2011.

3 Bacteriology of the outbreak strain

3.1 Detection and characteristics of the pathogen

A cluster of patients with the clinically very characteristic disease pattern for HUS in Northern Germany reported to the RKI on 19 May 2011 quickly directed attention to the Shigatoxin-producing *E. coli* (EHEC) as the pathogen.

On 23 May 2011, the information was received at the National Reference Centre (NRC) for Salmonella and other Bacterial Enteric Pathogens that samples received there on 19 and 20 May 2011 might be connected to the outbreak. The result of the detection of the EHEC virulence markers stx_1 (negative), stx_2 (positive) and *eae* (negative) routinely tested by means of PCR was available on 23 May 2011 for 2 isolates of the outbreak strain (11-01997, 11-02027). On 24 May 2011, the consulting laboratory for HUS was informed about the determination of the O-antigen O104. Further characterization of the pathogen as Shigatoxin 2 (variant *vtx* 2a)-producing *E. coli* of serovar O104:H4 took place on 25 May 2011, coinciding with the results from the consulting laboratory for HUS at the University of Münster (Prof. Karch). On 25 May 2011, preliminary information on the strain (*E. coli* O104, stx_2+, *eae*-, *hly*- with resistance to 3rd-generation cephalosporines (ESBL)) was placed on the ECDC outbreak information platform EPIS. On 26 May 2011, the NRC demonstrated by means of macrorestriction analysis (PFGE) on five selected isolates (including isolates from Bremerhaven and Frankfurt) that it is very likely that the respective patients belong to one epidemiological event.

Further investigations (see also the ongoing updates of the EHEC-data sheet on the website of the Robert Koch Institute[18] and at EPIS) showed numerous specifics of the pathogen, which are described in detail in the appendix, together with concrete information on microbiological diagnostics.

For international communication and strain comparisons, the PFGE pattern was submitted to the EPIS platform and a reference strain was sent to the Clinical Reference Laboratory for *E. coli* (Dr. Caprioli) in Rome, the WHO RL for *E. coli* (Dr. Scheutz) in Copenhagen, the National Reference Laboratory for *E. coli* at the BfR in Berlin (Dr. Beutin) and the consulting laboratory for HUS (Prof. Karch) in Münster.

Although Shigatoxin-producing *E. coli* of the serotype O157:H7 or O157:H- are most frequently responsible for causing HUS globally, other EHEC serotypes (non-O157) with the potential to cause HUS have also been identified. The most comprehensive collection of EHEC strains of different serovars, known as the HUSEC collection, is located at the consulting laboratory for HUS (Prof. Karch, University of Münster). Among others, the O104:H4 HUSEC041 strain isolated at the NRC/RKI in 2001 was also integrated into this collection.

The current outbreak involves the rare EHEC serotype O104:H4, which up to now had not been described in animals and only rarely in humans [Germany 2001/HUSEC041, France 2004, Korea 2006, Georgia 2009 and Finland 2010, additional isolates of the O-antigen type O104 exhibit a different H-antigen (O104:H2; O104:H7; O104:H16; O104:H21)].

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[18] For characteristics of the pathogen as well as information and assistance of the RKI in the diagnosis of the outbreak strain, visit www.rki.de; http://www.rki.de/cln_117/nn_467482/DE/Content/InfAZ/E/EHEC/EHEC__Diagnostik.html

Particularly noteworthy is that the outbreak strain in addition to the Shigatoxin 2a

- possesses **virulence characteristics of enteroaggregative *E. coli*** (the typical EAEC virulence plasmid with the adhesion fimbriae type AAF/I described here for the first time in EHEC, any other to date known EAEC or STEC/EAEC O104:H4 had AAF/III fimbriae) and
- has **special resistance characteristics** (see Appendix: Characteristics of the pathogen as well as information and assistance of the RKI in the diagnosis of the outbreak strain currently circulating, www.rki.de).

Meanwhile, there are sequence data from the outbreak strain, showing a strong homology to enteroaggregative *E. coli* (55989) [19].

Since the species *E. coli* is also part of the normal flora of the human intestine, the detection of pathogenic *E. coli* variants requires specific sub-taxonomic diagnostic methods. Here, in particular, the detection of certain virulence markers, including an isolation and availability of a pure culture, plays an important role. This is especially important in the framework of less characteristic disease patterns, such as (bloody) diarrhea, but also for the detection in asymptomatic shedders.

In particular, the search is facilitated by the *E. coli* antibiotic resistance phenotype (ESBL), unusual for intestinal *E. coli,* because it allows for the use of corresponding selective media for a targeted search. The NRC detected this resistance and immediately used it for the systematic search for the pathogen (using the ESBL- selective medium combined with a multiplex PCR screening for stx_1, stx_2 and *eae* or using the ESBL-selective medium combined with a multiplex PCR for the stx_2, *rfbO104* and *fliCH4* genes (protocol according to Prof. Karch from 06 June 2011)).

Especially in the late phase of the outbreak of the Shigatoxin-producing *E. coli* O104:H4, the differentiation from sporadically circulating strains without the ability to produce ESBL becomes increasingly important to correctly identify infected individuals and shedders with strains different from the outbreak strain. The labs need to follow a search strategy that does not only focus on the use of selective media.

Such a non-selective search strategy (without ESBL selective medium) is routine practice at the NRC. This was continued parallel to the epidemic strain special diagnostics. These investigations are critical for assessing the "background action" and hence for the final assessment of the progression of the outbreak.

3.2 Laboratory tests at the NRC

Between 20 May 2011 and 5 August 2011, a total of 3224 samples from suspected cases were sent to the NRC. 1023 strains were allocated to the outbreak by means of serovar determination, PCR of virulence factors ESBL production and the resistance profile. In 590 samples, no evidence for an EHEC/EPEC infection was found. Submissions from several northern German laboratories are included in the total number, which have made collections of their samples available to the NRC. These 450 samples are processed separately.

Apart from the outbreak strain O104:H4, 87 EPEC and 702 EHEC cases were also identified as additional *E. coli* pathogens. A total of 311 of these EHEC isolates and 53 of the EPEC isolates could be allocated to 42 different serotypes with very different virulence

[19] Mellmann A, Harmsen D, Cummings CA, et al. 2011: Prospective Genomic Characterization of the German Enterohemorrhagic Escherichia coli O104:H4 Outbreak by Rapid Next Generation Sequencing Technology. PLoS ONE 6(7): e22751. doi:10.1371/journal.pone.0022751

patterns. Strain isolation for further molecular subtyping was not successful for 209 samples.

From the perspective of the NRC, the collected data still provides no evidence for another concurrent event.

4 Focus of other epidemiological studies

4.1 Studies of population consumption patterns

Between 24 May and 24 June, repeated surveys with participants of an online-panel were carried out in order to gather information on population consumption patterns. In particular, this forum addressed the following issues:

- How much have consumption patterns changed in the regions more affected by the outbreak following the consumption recommendations and the media coverage?
- How much have patterns changed with respect to personal hygiene (e.g. more frequent hand washing) or in meal preparations?

The interviews were conducted as an online survey by the Society for Consumer Research (GfK), with preparation of the questions by RKI. Invitation to participate in the survey, programming of the questions, and the delivery of information was conducted by the GfK in order to achieve a representative random sample (by age group, gender and socio-economic status). The random sample comprised individuals from all federal states; however, the analysis on which this report is based considered only the data from Northern Germany. Registered and "selected" participants were invited by email to take part in the online survey. For each round of questions, the online survey was open for a period of a week, as in a survey wave. Overall, there were 4 waves: wave 1 from 27 May to 3 June, wave 2 from 3 June to 10 June, wave 3 from 10 June to 17 June, and wave 4 from 17 June to 24 June. For each survey wave, 2100 individuals were surveyed, of whom 1831 individuals took part in all four weeks and were subsequently analysed. In order to reflect the opinions of all levels of the population and to account for variable response rates (e.g. between different age groups), the sample was weighted by age, gender, socio-economic status and state.

Based on the statements of 1831 wave 3 study participants that were included in the analysis, more than two-thirds (76%) of the population in northern Germany completely or partially quit consuming raw vegetables following the 25 May 2011 public recommendation (in the middle of the 21st calendar week; not to consume raw tomatoes, cucumbers or lettuce in northern Germany).

The analysis by gender indicated only a marginal difference (men 73%, women 78%). Almost half the population (43%) of northern Germany completely stopped eating "raw vegetables".

In a differentiated treatment of consumption of tomatoes, cucumbers and lettuce, it was apparent from wave 3 that cucumbers (66%) and lettuce (64%) in particular were completely relinquished, whereas the percentage of the population completely giving up tomatoes was somewhat lower (57%). There was only negligible difference between men and women.

On 10 June 2011 (at the end of the 23rd calendar week) a new public advisory ecommended consumers avoiding eating sprouts. At the same time, previous advisories recommending consumers avoid eating raw cucumbers, tomatoes and lettuce were repealed. In wave 4 approximately 20% of individuals who had previously quit eating raw cucumbers, tomatoes and lettuce stated that they were once again consuming as much of these raw vegetables as they had prior to the warning (tomatoes 22%; cucumbers 18%; lettuce 16%). Almost a quarter (tomatoes 25%; cucumbers 23%, lettuce 18%) of these individuals ate more of these vegetables again, although not in the same quantities as before the consumption warning. In this case too, there was no difference between genders.

Once sprouts came under suspicion of being the EHEC O104 outbreak vehicle, 84% of the population quit eating raw sprouts according to the statements in wave 3, and 80% gave them up according to wave 4.

Since the increased reporting of EHEC cases, approximately 2/3 (63%) of the population paid more attention to personal hygiene, for example in the form of more frequent hand washing. In this, there was a more obvious gender difference (men 59%; women 68%). For people involved in meal preparations, 62% (men 57%; women 67%) of the population of northern Germany paid more attention to cleanliness.

Figures 11 and 12 illustrate the proportion of the consumption of each of raw tomatoes, cucumbers, lettuce and carrots by the population during the calendar weeks 19t through 23. For all food products shown, there was a clear decrease in consumption particularly within the 21st and 22nd calendar weeks, although the trend for carrots is less pronounced. Consumption of sprouts is included after the 21st calendar week. Overall, only a very small percentage of those surveyed declared eating sprouts. Figure 12 shows the development of cucumber consumption for different age groups and genders. The trend in cucumber consumption is similar for men and women, but starts at the lowest level for men in all age groups (with the exception of over 59 year olds). The decrease between weeks 19 and 20 is conspicuous and could be related to a subjective influence of memory against the background of the consumption warning during the week in which the question was asked. In the 23rd calendar week, the proportion of the population eating these foods clearly increased, but did not reach the same magnitude as in the 19th week. This corresponds to the statements given above, where only around 20% of the population returned to their previous consumption behaviour after the consumption warning was repealed. Overall, these data make clear how the population actually reacted to the consumption warning, and also that consumption of other raw vegetables, such as carrots, decreased somewhat even though no warning was issued against them.

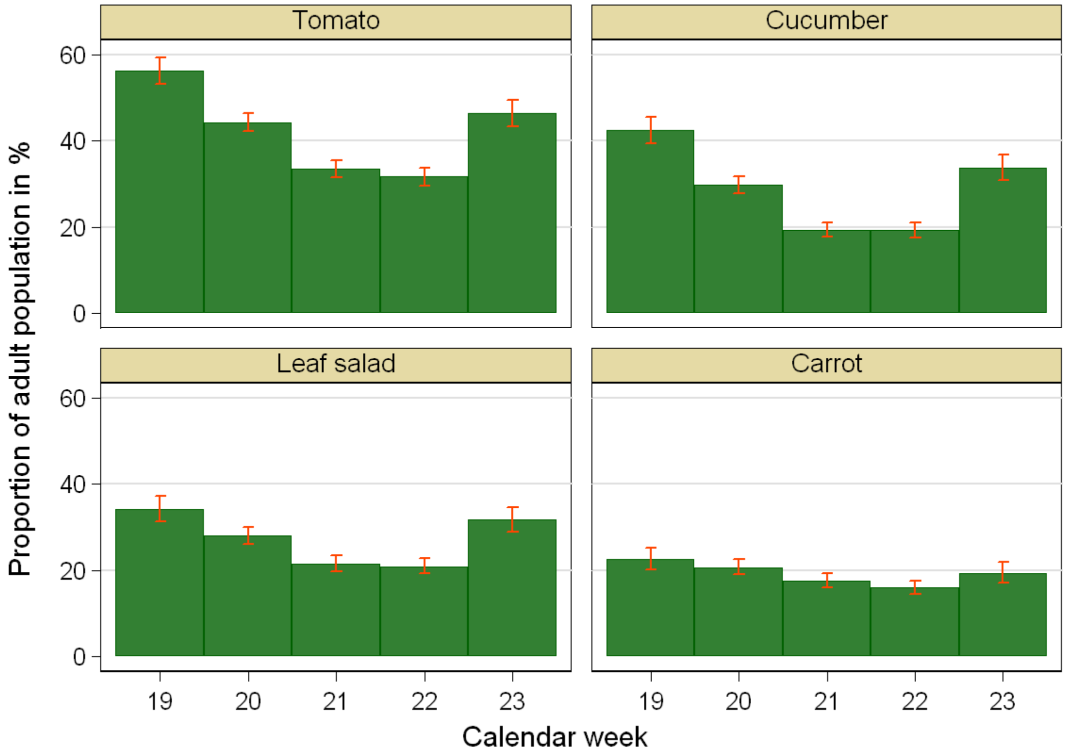

Figure 11: Estimated proportion of the adult population in northern Germany who said they had consumed raw tomatoes, cucumbers and lettuce during the 19th through 23th calendar week.

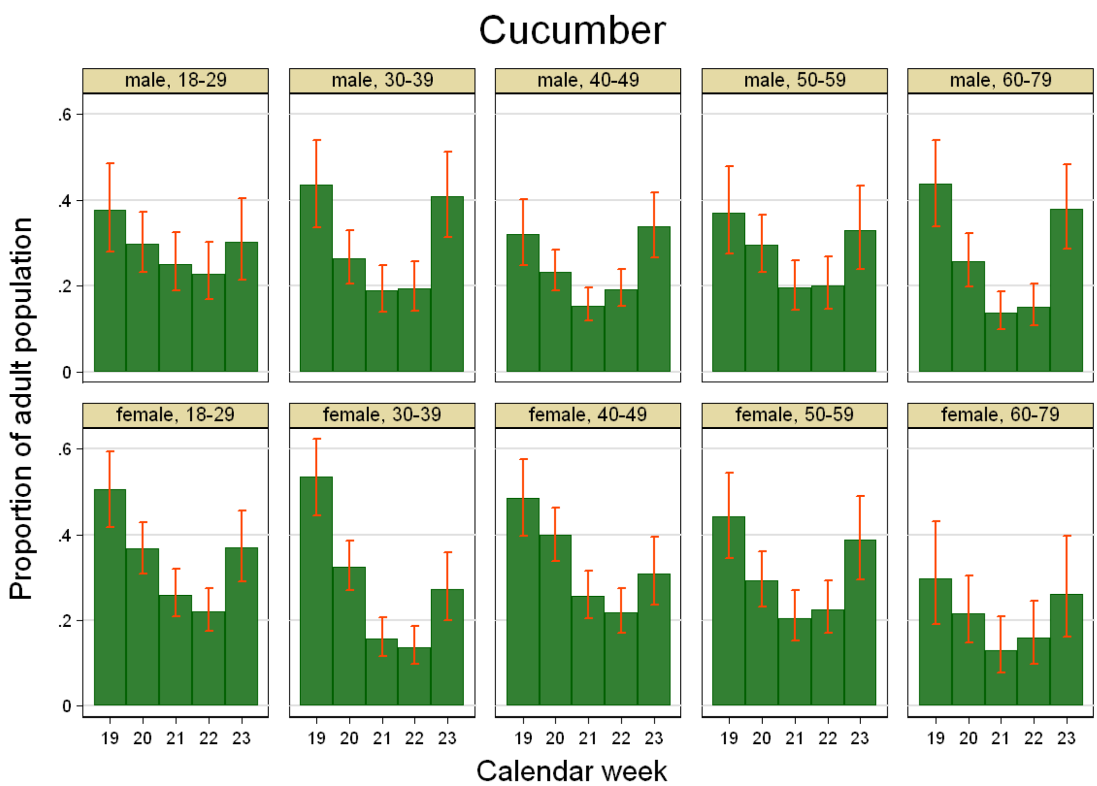

Figure 12: Estimated proportion of the northern Germany adult population, displayed by age group and gender, who said they had consumed raw cucumbers during the 19th through 23th calendar week.

4.2 "Late cases"

Sprout production in Company A in Lower Saxony was suspended in early June and the public warning regarding the consumption of sprouts was issued 10 June. If contaminated sprouts were no longer consumed after 10 June, only isolated cases of new infections with EHEC O104 that are associated with the consumption of sprouts from Company A would be expected after 24 June, taking into account the incubation period (median of 8 days, for 90% of patients less than 15 days). It was more probable that later, new cases occurred as a result of secondary transmissions.

The RKI and the public health services in states and counties paid special attention to the investigation of the new cases after the halt of putting into circulation of contaminated sprouts and the public consumption warning ("late cases"). The following questions were to be investigated:

- What proportion of these new cases was caused by the outbreak strain EHEC O104:H4?
- It was to be estimated what proportion of the late cases caused by the outbreak strain could still be explained by (a) the consumption of contaminated sprouts, (b) secondary transmission or (c) neither of these modes of transmission.

To examine the second question, a standardized questionnaire was developed, to be completed by the local health authorities for every new EHEC/HUS patient (with onset of disease on 10 June or later).

A total of 787 EHEC cases and 71 HUS cases with disease onset on 10 June or later were reported (as of 24 August 2011). Of these, 510 (65%) EHEC cases and 62 (87%) HUS cases conformed to the published outbreak case definition (see appendix). Of these, 358 EHEC cases and 48 HUS cases had onset of disease between 10 June and 4 July. The most recent onset of an EHEC case that met the outbreak case definition occurred on 12 August, and the most recent HUS case fell ill on 7 August.

For 238 (47%) of the 510 EHEC cases with onset of disease after 10 June and 35 (56%) of the late 62 HUS cases, information was available that the pathogen strain was Stx1 negative and Stx2 positive or corresponding to the serotype O104. The following analysis takes into account these very specific cases.

The age range for the 238 **EHEC cases** was between 0 and 97 years (mean 40 years, median 37 years) and 126 (53%) were female. Information on the probable mode of transmission was available for 79 (33%) of the 238 EHEC cases:

- 4 cases (5%) from different cities or counties could be explained by probable laboratory infection.
- 1 case (1%) with disease onset on 22 June affirmed consumption of sprouts within the presumed exposure time period.
- 23 cases (29%) were related to diarrhoea in individuals in close contact (secondary infection via person-to-person transmission).
- 1 case (1%) with disease onset on 20 June reported both consumption of home-grown sprouts and diarrhoea in individuals in close contact.

- 37 cases (47%) with none of the aforementioned mode of transmission lived either permanently or stayed temporarily during the incubation period in one of the more affect areas (Bremen, Hamburg, Schleswig-Holstein, parts of Lower Saxony (north) or North Rhine-Westphalia (Paderborn)).
- 13 cases (16%) denied both having consumed sprouts and being in close contact with individuals with diarrhoea and did not report a stay in one of the more affected areas. O104 was detected in 4 of these 13 cases.

The 35 **HUS cases** were in individuals between 0 and 88 years of age (mean 25 years, median 18 years); 17 (49%) of the 35 HUS cases were female. Information on the mode of transmission was available for 26 (74%) of the 35 HUS cases:

- 1 case (4%) with disease onset on 11 June affirmed consumption of sprouts.
- 13 cases (50%) were related to diarrhoea in individuals in close contact (secondary infection).
- 1 case (4%) with disease onset on 16 June reported both consumption of sprouts and diarrhoea in individuals in close contact.
- 9 cases (35%) with none of the aforementioned mode of transmission lived either permanently or stayed temporarily during the incubation period in one of the more affect areas.
- 2 cases (8%) denied both having consumed sprouts and being in close contact with individuals with diarrhoea and did not report a stay in one of the more affected areas. O104 was not detected in either of these 2 cases.

Only a few isolated late outbreak cases affirmed consumption of sprouts within the exposure period. Despite the heightened public sensitivity resulting from the consumption warning and media coverage regarding sprouts, it cannot be excluded that some other late cases consumed sprouts but could not recall it. However, overall it must be assumed that sprouts no longer played a role in the late cases after the suspension of sprout production at Company A and following the consumption warnings.

In contrast, approximately one-third of the late EHEC cases and one half on the late HUS cases can be explained by secondary transmission. Of note among these are 9 cases that can be related to consumption of meals that were prepared by a catering company in which employees were infected with EHEC O104. The other cases of secondary transmission primarily involved infection within families and common households. These cases suggest that increased vigilance with respect to hygiene measures should still be maintained in close environment with EHEC patients in order to prevent secondary infection.

Four other cases were explained by laboratory infection. It is not clear whether these infections are attributable to specific problems in the operations within these laboratories.

Based on the information from the survey, a substantial proportion of the late case can be associated with none of the aforementioned modes of transmission. It is striking that approximately three-quarters of these unexplained cases stayed in more affected areas within the incubation period. Infection, more likely secondary infection, could have taken place there. Furthermore, it cannot be excluded that the survey conducted was potentially not sensitive enough to resolve the probable mode of transmission for every particular case.

4.3 Domestic environment and shedders

In collaboration with local health authorities in northern Germany, the RKI conducted a household-based case-control study including 44 case households (112 individuals) with at least one EHEC or HUS case, which was reported in the context of the outbreak, and 36 control households (89 individuals) near the residences of the cases, with no reported EHEC or HUS case . Individuals were tested for shedding of the outbreak strain EHEC O104.

In 14 case households (32 individuals), the outbreak strain EHEC O104 was isolated from the stool of 17 case patients at the time of the investigation.

In cases with the outbreak strain EHEC O104, the period of time between the onset of disease and the provisioning of the stool sample was 10 to 44 days. The time period between onset of disease and the provisioning of the stool sample did not differe between cases with or without evidence of EHEC O104.

In 3 of 14 households, the outbreak strain was detected in 2 cases each. All cases showed clinical symptoms. In 2 of these households, household transmission seems unlikely, because of the short time between the onsets of the cases' disease. In the third household, household transmission is likely, since there were 34 days between onset of disease for the initial and the secondary case. At the time of investigation, the initial case was still an EHEC shedder. In the 36 control households, there was no evidence of the outbreak strain in any of the participants at the time of the investigation.

A prospective study was carried out in collaboration with the same local health authority listed above as well as an additional one. Preliminary results support the results of the household-based case-control study. A total of 17 households with at least one EHEC or HUS case that shed the outbreak strain beyond the cessation of symtpoms were investigated in the prospective study. In addition to the 14 case households that were identified in the context of the study described above, 3 of 15 households studied from the other county were included.

In the still ongoing prospective study, no case of household transmission has yet been detected. Currently (as of the results of the present investigation on 30 August 2011), the longest documented period of shedding of an adult case included in the prospective study is 13 weeks.

5 External communication by the RKI during the outbreak

Rapid flow of information is important in an outbreak. Important players within the community of experts, primarily the Public Health Service (Öffentliche Gesundheitsdienst (ÖGD)) and the professional societies should be informed about the event in a timely manner. Daily updates sent by the RKI via email to a distribution list enabled a rapid and comprehensive circulation of information on the epidemiological situation and new developments. When necessary, special information reports or teleconferences (TCs) took place. The general public was kept informed through press releases, press conferences and interviews by the media with the RKI as well as on the RKI website. In addition, there was a series of scientific articles published in the RKI journal Epidemiological Bulletin (submissions on 30 May, 6 June, 14 June, 20 June, 27 June, 4 July and 11 July), and in multiplicator journals (Berliner Ärzte) and renowned international peer-reviewed journals (New England Journal of Medicine, Lancet Infectious Diseases, Emerging Infectious Diseases, PLoS One, Eurosurveillance; for an overview see www.rki.de > Infektionskrankheiten A-Z > EHEC and the Appendix)

5.1 Forwarding of information within the Public Health Service (ÖGD) and to national and international public authorities

Figure 13 illustrates some of the information activities at the RKI. Following the preliminary information regarding a local outbreak on 19 May and the invitation of the RKI to support the investigation of the outbreak by the Hamburg Authority for Health and Consumer Protection, on 20 May the RKI outbreak team was sent to Hamburg.

On 22 May, the European Health Authorities and the WHO were first informed about the situation via the Early Warning and Response System (EWRS) of the EU. Over the course of the outbreak, 44 state reports were written by the RKI, initially on a daily basis and later weekly, which summarized all the current state of knowledge regarding epidemiology and detail information. These reports were made available to representatives of the state health authorities, as well as the participating federal authorities, professional organizations and experts, among others.

Once the end of the outbreak had been declared on 26 July 2011 during a press release by the RKI, Germany had sent a total of 103 dispatches via the EWRS, including 20 bilateral messages between Member States in the "Selective Exchange" function, and had provided 60 documents. Among the latter were RKI updates translated into English (33), tables with detailed case numbers (17) as well as additional documents, for example recommendations, press releases, and also case definitions and questionnaires (10). A list of the individual documents (by date and contents) is provided in the Appendix.

Reports and information were also shared with the WHO.

Figure 13 shows a selection of teleconferences the RKI was involved that took place within the period of the outbreak. At the beginning of the outbreak, up to 4 TCs took place daily, and overall there were more than 80 TCs. Between 3 and 17 June, representatives of the RKI participated in a total of 10 sessions of the EHEC Task Force at the Federal Office for Consumer Protection and Food Safety (BVL).

Date	May																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Preliminary information																			→												
RKI-operating situation centre																															
RKI-report																															
reports																															
EWRS report (/day)																						1	1	2	4	4	4		1	1	1
additional WHO/IHR reports																															
Teleconferences = TC																															
TC with ESL																															
TC with BfR/BVL																															
TC with EU/ECDC																															
TC with WHO																															
TC with various experts																															

Date	June																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Preliminary information																															
RKI-report																															
Reports																															
EWRS report (/day)	4	4	3	1	1	2	2	4	2	1	2	3	2	2	2	3	1			2	2	2	2	2			2	2	2	3	
additional WHO/IHR report																															
Telconferences = TC																															
TC with BL																															
TC with BfR/BVL																															
TC with EU/ECDC																															
TC with WHO																															
TC with various experts																															
Members of the RKI Task Force EHEC																															

Date	July																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
RKI-operating situation centre																															
RKI-report																															
Reports																															
EWRS report (/day)	3			1	1	1	2	2			2	1	1	2	1			1	1	2	1	1			1	1	1				
additional WHO/IHR reports																															
Teleconferences = TC																															
TC with BL																															
TC with BfR/BVL																															
TC with EU/ECDC																															
TC with WHO																															
TC with various experts																															
Outbreak end is explained																											←				

Figure 13: Chronology of the exchange of information among RKI, the Public Health Service and other national and international public authorities. FS = Federal States; IHR = International Health Regulations.

5.2 Media- and public relations

Visits to the RKI homepage in the past decade have steadily increased – from 2.6 million pageviews in 2000 to the highest level so far (as a result of the "swine flu") of 166 million in 2009 and 35.8 million in November 2009 alone. In 2010, there were 64.6 million pageviews. At the beginning of the currentyear 2011, there were between 4-6 million visits per month, and in May this number rose to 16.5 million, in June to 17.9 million and in July there were 10.4 million pageviews (Figure 14).

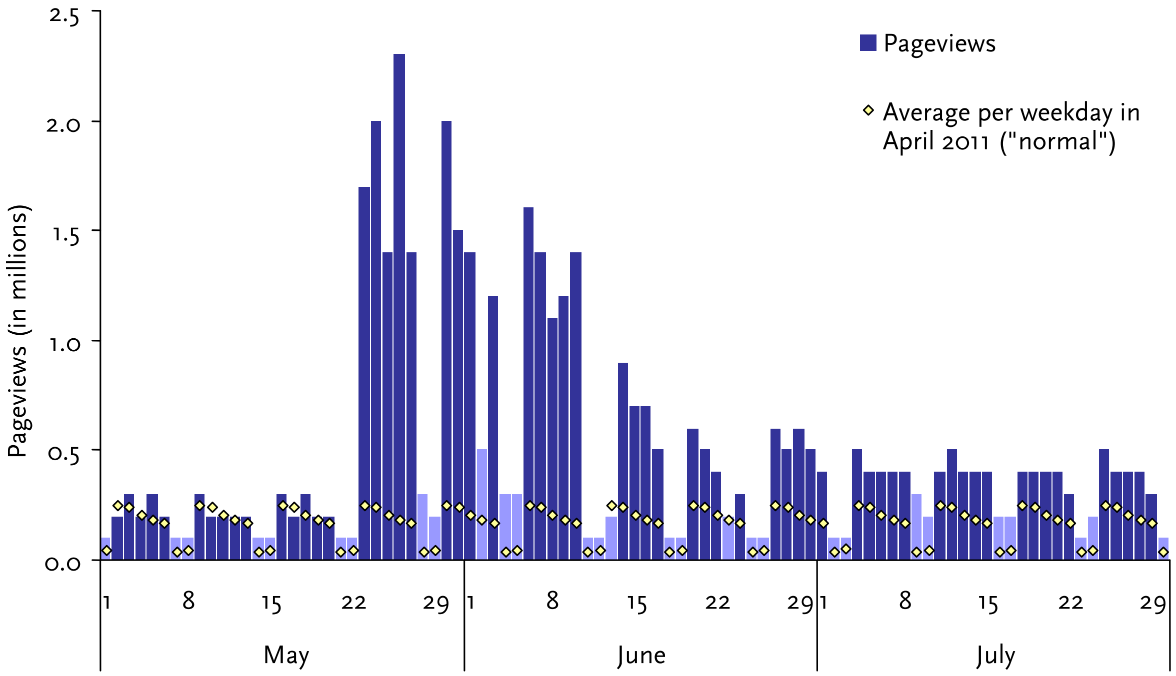

Figure 14: The number of RKI homepage (www.rki.de) pageviews between 1 May and 30 July 2011 by date, and the average number of pageviews in April 2011 by weekday. Light blue bars indicate weekends and holidays.

Working documents (outbreak case definitions, sample reporting forms, diagnosis procedures, hygiene information and surveys), a situation assessment updated daily, including case numbers (23 May to 26 July), special texts (e.g. study explanations, assessment reports, a hotline overview) and links to RKI publications in scientific journals and other external sites (treatment information from the professional society, ECDC, etc.) were made public on the RKI website. The website information could be found on the homepage for the entire outbreak period. An overview of the documents available on the RKI webpages is provided in the Appendix.

On Sunday 22 May, the first evidence regarding possible causes was made public in a dpa interview. Immediately after the risk factors were identified, they were elucidated in a press conference on 25 May. All press releases and media conferences were joint efforts of the RKI and the BfR responsible for consumption recommendations, and when required, the BVL was involved. Joint press conferences or press releases took place on 25 May, 3 June, 10 June, 5 July, 21 July and 26 July 2011.

By the time of the 26 July 2011 press release in which the RKI declared the end of the outbreak, 1825 media inquiries had been received either by email or telephone. On average, 27 media inquiries were answered per day during this time period. A separate evaluation of the media inquiries up to 10 June 2011 (when sprouts were identified as the source of infection) yielded a total of 1321 media inquiries and an average of 63 per day.

Informing and advising the public are not responsibilities of the RKI. However, the Institute's self-image does include informing the general public about relevant health risks or suggesting sources of information. This includes the daily updated situation assessments and an overview of the hotlines and public/consumer information compiled by the RKI. Both were available on the website. Approximately 4600 emails and 160 letters were received by the RKI and were answered with the assistance of volunteers, among others.

6 Appendix

6.1 Schedule of documents provided via Early Warning and Response System during the outbreak

Date	Document
26 May	HUS Outbreak case definition, 26.5.
27 May	Case survey_24.5.
27 May.	Survey controls_24.5.
29 May.	RKI Proposal_report form_HUS
30 May	RKI Update report, English
31 May	RKI Update report, English
1 June	HUS Outbreak case definition, 1.6.
1 June	RKI Update report, English
2 June	Overview of the HUS survey
2 June	Exploration survey, 1.6.
3 June	General press release RKI BfR, 3.6., English
3 June	RKI Update report, English
4 June	RKI Update report, English
5 June	RKI Update report, English
6 June	DGI position paper_Antibiotics, English
6 June	RKI Update report, English
6 June	RKI Update report, English
7 June	RKI Update report, English
8 June	RKI Update report, English
8 June	RKI Update report, English
9 June	RKI Update report, English
10 June	General press release RKI BfR-BVL, 10.6., English
11 June	RKI Update report, English
12 June	RKI Update report, English
13 June	RKI Update report, English
14 June	RKI Update report, English
15 June	RKI Update report, English
16 June	RKI Update report, English
17 June	RKI Update report, English
20 June	RKI Update report, English
21 June	Weekly RKI Update report, English
22 June	RKI Update report, English
23 June	RKI Update report, English
24 June	RKI Update report, English
27 June	RKI Update report, English
28 June	RKI Update report, English
28 June	Weekly RKI Update report, English
29 June	RKI Update report, English
30 June	RKI Update report, English
1 July	RKI Update report, English
4 July	RKI Update report, English
5 July	Weekly RKI Update report, English
5 July	Itemized table of cases, English
6 July	Itemized table of cases, English
6 July	RKI Update report, English
7 July – 27 July	each weekday: itemized table of cases, English

6.2 Schedule of documents provided during the outbreak on the RKI website (new or updated, as of 25 August 2011)

Date	Document	Format
23 May	Teaser (updated almost every workday until mid July)	Text
23 May	Information on EHEC/HUS outbreak events (updated every workday until mid July)	Text
23 May	EHEC/HUS pages of the Robert Koch Institute	Link
24 May	EHEC/HUS pages of the BfR, including consumer advice for protecting against EHEC	Link
24 May	BZgA: Information on EHEC for the general public	Link
25 May	Reporting form for haemolytic uraemic syndrome (HUS) suspected illness, illness, death	Pdf
25 May	EHEC wave: National research platform for zoonoses provides background information	Link
26 May	BfR-RKI statement "Preliminary results of the EHEC/HUS study" (25 May 2011)	Text
26 May	"Information in English" (Teaser for the English pages)	Link
26 May	EHEC diagnosis: Current information and suggestions by the RKI	
26 May	Hygiene measures for inpatients with haemolytic uraemic syndrome (HUS)	Pdf
26 May	Eurosurveillance: Large and ongoing outbreak of haemolytic uraemic syndrome, Germany	Text/Link
May 2011	Consulting laboratory for haemolytic uraemic syndrome: At the Institute for Hygiene at the University of Münster	Link
May 2011	National Reference centre for Salmonella and other bacterial enteritis pathogens	Link
May 2011	National veterinary medicine reference laboratory for E. coli	Link
27 May	Hamburg press release: EHEC pathogen detection in cucumbers (Hamburger Institute for Hygiene and Environment, 26 May 2011)	Link
27 May	Case definition for HUS case in the outbreak	Pdf
27 May	Treatment recommendations from the German Nephrology Society	Link
30 May	BfR statement No. 015/2011 of 26 May 2011	Link
30 May	Appeal for participation in the surveillance of bloody diarrhoea in emergency departments	Text
30 May	Survey questionnaire "Surveillance of bloody diarrhoea"	Pdf
30 May	Participant form "Surveillance of bloody diarrhoea"	Pdf
1 June	BfR statement No. 016/2011 of 31 May 2011	Link
2 June	Eurosurveillance: Update on the ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011 (02 June 2011)	Text/Link
3 June.	Press release BfR / RKI: New epidemiological data corroborate previous consumption recommendations of the BfR	Text
3 June	Recommendation of the German Association for Infectiology on EHEC and antibiotic treatment	Link
4 June	RKI statement (and median reports and RKI activities)	Text
6 June	Epidemiological Bulletin: On the trend in numbers of diseased in the current EHEC/HUS outbreak in Germany	Pdf
8 June	On the epidemiological EHEC/HUS studies by the RKI	Text
8 June	Epidemiological studies and surveys	Pdf
10 June	Press release BfR, BVL, RKI: New evidence in the EHEC outbreak (repeal of the consumption warning for raw cucumbers, tomatoes and lettuce, consumption warning for raw sprouts)	Text
June 2011	RKI advice booklet for physicians (updated)	Text
14 June	Epidemiological Bulletin: On the trend in numbers of diseased in the current EHEC/HUS outbreak in Germany - Update	Pdf
20 June	Epidemiological Bulletin: On the trend in numbers of diseased in the current EHEC/HUS outbreak in Germany - Update II	Pdf
16 June	Information on EHEC on the internet and hotlines	Pdf
22 June	FAQ at the Federal Environment Agency on EHEC and water	Link
27 June	Epidemiological Bulletin: Intensified surveillance during a large EHEC-/HUS outbreak in Germany	Pdf

June 2011	Network for bacterial enteritics Enter-net at ECDC	Link
June 2011	Information from the Friedrich Loeffler Institute on EHEC and STEC	Link
June 2011	ECDC pages on EHEC	Link
June 2011	EFSA pages on EHEC	Link
June 2011	WHO Regional Office of Europe on EHEC	Link
23 June	New England Journal of Medicine: Epidemic Profile of Shiga-Toxin–Producing Escherichia coli O104:H4 Outbreak in Germany - Preliminary Report, 22.6.2011 (10.1056/NEJMoa1106483)	Pdf/Link
23 June	The Lancet Infectious Diseases: Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study, Early Online Publication, 23.6.2011	Pdf/Link
23 June	Eurosurveillance: Enhanced surveillance during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing Escherichia coli in Germany, May to June 2011 (16.6.2011)	Text/Link
27 June	Epidemiological Bulletin: Intensified surveillance during the outbreak in Germany in May/June 2011, Epid. Bull. 25/11	Pdf
1 July	Assessment report on the EHEC/HUS outbreak (30 June 2011)	Pdf
1 July	Outbreak case definition for EHEC- and HUS cases in the context of the outbreak in spring 2011 in Germany	Pdf
5 July	Electron microscope image of EHEC O104:H4	Photo, Text
5 July	Press release BfR, BVL, RKI: EHEC O104:H4 The outbreak in Germany explained: the triggers sprouts grown from fenugreek seeds imported from Egypt	
13 July	Cases of HUS/EHEC disease by federal state	Text
21 July	Press release BfR, BVL and RKI: BfR, BVL and RKI consumption recommendations narrowed down to raw sprouts and seedlings	
26 July	Press release RKI: EHEC/HUS O104:H4 – The outbreak is considered to be over	

English pages

26 May	Teaser (updated regularly until mid July)
26 May	Preliminary results of the STEC/HUS Case Control Study
27 May	Case definition for HUS-cases associated with the outbreak in the spring 2011 in Germany (PDF, 78 KB)
27 May	Characterization of EHEC O104:H4 (PDF, 99 KB)
27 May	Eurosurveillance: Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011
23 June	New England Journal of Medicine: Epidemic Profile of Shiga-Toxin–Producing Escherichia coli O104:H4 Outbreak in Germany - Preliminary Report, June 22, 2011 (10.1056/NEJMoa1106483)
23 June	The Lancet Infectious Diseases: Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study, Early Online Publication, 23 June 2011
23 June	Eurosurveillance: Enhanced surveillance during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing Escherichia coli in Germany, May to June 2011 (16 June 2011)
4 July	Technical Report - EHEC/HUS O104:H4 Outbreak
7 June	German Association for Infectiology (dgi): EHEC infection and antibiotic therapy
8 July	Electron microscopy: EHEC bacteria, O104:H4 outbreak strain
21 July	Press release: BfR, BVL and RKI issue specified consumption recommendations for uncooked sprouts and shoots (germ buds)
26 July	Press release RKI: EHEC/HUS O104:H4 – The outbreak is considered to be over

Supplement

Case definition for HUS-cases associated with the outbreak in the spring 2011 in Germany - last version (as of 1 July 2011) is not attached but available in German at:
http://www.rki.de/DE/Content/InfAZ/E/EHEC/EHEC_O104/Falldefinition_EHEC_O104.html

Characteristics of the pathogen and information and assistance by the RKI in diagnosis of the currently circulating outbreak strain (attached) – a German version is available online:
http://www.rki.de/DE/Content/InfAZ/E/EHEC/EHEC_O104/EHEC_O104_Diagnostik.html

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