ROBERT KOCH INSTITUT

Technical Report

EHEC/HUS O104:H4 Outbreak
Germany, May/June 2011

As of: 30 June 2011

Presentation and Evaluation of Previous Epidemiological Findings regarding the EHEC/HUS O104:H4 Outbreak, May/June 2011

As of: 30 June 2011

Table of Contents

Introduction

Since early May 2011, there has been an increased incidence of hemolytic-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 illustrated. The contributions of the RKI to the identification of disease clusters and traceback of food items are found in a report by the Task Force EHEC at the Federal Office for Consumer Protection and Food Safety (BVL). This report is meant to be preliminary since the domestic and international epidemiological studies are still ongoing. Results from individual statistical analyses may differ in detail from previously or still to be published reports. Likewise, the national and international epidemiological situation can change and require an adaptation of the assessment.

Summarized Assessment

Time Course of the Outbreak (Epidemiological Trend)

The outbreak (based on the date of disease onset) began, as far as it is up to now determinable, on 8 May 2011 and reached its peak on 22 May 2011. Currently, it cannot yet be definitively determined whether the outbreak, which was predominantly associated with the consumption of sprouts from company A in the state of Lower Saxony, has ended. However, various epidemiological indicators suggest that the outbreak is drawing to a close. The peak in relation to the onset of diarrhoeal symptoms was on 22 May 2011. Since then, both the number of reported infections of enterohaemorrhagic *Escherichia coli* (EHEC) and the number of new cases of hemolytic-uremic syndrome (HUS) have been decreasing. Since 13 June 2011, only sporadic HUS diseases have occurred. The latest date for the onset of diarrhoea for HUS and EHEC cases is 23 June, and for a case with the detection of serogroup O104 it has been 12 June for the past 6 days. Due to the notification and reporting delay, however, reporting of further cases as well as detection of EHEC O104 in patients are to be expected for the following days (relative to the onset of disease).

Due to the increased attention and increased diagnostics,a persistently increased level of sporadic (not associated with the outbreak) cases than before the outbreak is particularly expected to occur in the reporting category EHEC. Therefore, the number of cases reported daily will for some time probably not drop to the level of the expected "background" rate of the previous years. However, among HUS-cases with recent dates of onset, the proportion of cases characteristic for background cases has been increasing. Thus, the cases occurring since 1 June are younger, less often female than the typical cases of the outbreak and a smaller proportion of them (63%) has a clear connection to northern Germany (previously 86%). Until now, no disease clusters have become known in Germany which occurred after mid-June and in which sprouts were the vehicle of infection.

The public warning by the Federal Institute for Risk Assessment (BfR), BVL and RKI regarding the consumption of sprouts was issued on 10 June. If contaminated sprouts were no longer consumed after that date, only few isolated cases of new infections 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). In the future, more cases of new infections could occur due to secondary transmission or previously unrecognized sources. There is currently no epidemiological evidence for other foods as vehicles of infection.

Evidence for Sprouts as Vehicle of Infection

EHEC infections typically originate from fecal contamination of vegetable or animal foods which are not sufficiently heated before consumption or habitually consumed raw. Evidence for sprouts as the responsible vehicle in this outbreak in Germany comes from epidemiological studies of the Robert Koch Institute and the investigations of the Food Safety Authorities. Epidemiological studies of the Robert Koch Institute (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) show a statistically significant link between the consumption of sprouts and risk of disease. Investigations by the Task Force at the BVL revealed, that 41 of 41 well-documented and studied clusters in six states can be explained by the consumption of sprouts of company A in the state of Lower Saxony.
In the synopsis of the results available so far, the RKI in accordance with the Federal Institute for Risk Assessment (BfR) and the BVL reaches the conclusion, that the outbreak of disease in Germany caused by EHEC O104:H4 can be attributed to the consumption of contaminated sprouts from company A. A recent outbreak involving EHEC O104:H4 reported from France (onset of disease between 15 and 20 June) shows a probable connection to the consumption of locally grown sprouts. This suggests that sprouts could also have acted as vehicle of infection independent from company A in Lower Saxony and are possibly to be considered as vehicle in other disease clusters domestically and internationally.

Conclusions for Further Epidemiological Surveillance and Recommendations

The activities of epidemiological surveillance continue unabatedly and focus on surveillance (Infection Protection Act, "Infektionsschutzgesetz, IfSG") of EHEC and HUS as well as surveillance of cases of bloody diarrhoea in ermergency departments of selected hospitals. Furthermore, increased vigilance applies for the occurrence of bloody diarrhoea and HUS including a rapid diagnosis (differentiation with respect to the outbreak strain) and notification of in- and out-patients. In the context of notification requirements for EHEC and HUS, all new EHEC and HUS cases, that meet the case definition, are to be interviewed by local health authorities based on a questionnaire of RKI (i.e. with respect to sprout consumption, possible secondary transmission) in order to be able to identify the source of infection of these new cases. These activities should be continued for at least 2-3 months, given the long incubation period and the unclear situation with regard to any still existing but not yet active sources of infection.

The explicit advice to consistently pay attention to personal hygiene and food hygiene measures is still vital. The strict adherence to hand hygiene (http://www.bzga.de/?sid=663) and other standard measures of hygiene is of central importance. Strict adherence to hygiene measures is generally essential in a household, but particularly in the presence of EHEC-infected persons or persons with diarrhoea. This means that it is especially urgent to maintain extreme cleanliness in the kitchen and bathroom. Apart from the direct consumption of contaminated food, the bacteria can also be transmitted via hands or contaminated kitchenware. This is particularly important if potentially contaminated food is not heated afterwards. The risk can be reduced if hands and kitchen accessories are washed thoroughly with water and soap/detergent and dried carefully before preparing food, especially food that is not cooked afterwards. (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; usual household gloves should be worn when in contact with them. Recommendations at http://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber__EHEC.html

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 also affected by HUS, which is unusual, and that the number of cases continued to rise rapidly. An outbreak investigation was initiated.

1.1 Current Epidemiological Situation

1.1.1 German Notification Data

The electronic reporting system in Germany collects standardized data covering HUS and EHEC gastroenteritis cases since 2001. Suspicion of disease, disease and death from HUS are notifiable according to § 6 IfSG (Infection Protection Act) by the attending physician, EHEC-detection according to § 7 IfSG by laboratories, whereas all information concerning the cases is collected at the local public health departments (one case is either an EHEC gastroenteritis case OR an HUS case). In addition to routine surveillance, local and state public health departments were requsted in a newsletter from 23 May to report HUS and HUS suspected cases to the RKI immediately after receiving the notification and to add subsequent case based findings as available.

In contrast to the usual surveillance reference definitions of EHEC gastroenteritis (toxin-based laboratory detection, serogroup optional, and disease with symptoms of gastroenteritis) and HUS (purely clinical case definition, optional EHEC detection by laboratory diagnostics) the following limitations were set: Cases with onset of disease (with the typical first symptom diarrhoea) from 1 May 2011 are included (- an end date has not yet been defined). Cases with the detection of EHEC of serogroups other than the outbreak strain (O104 - for details see Chapter 3), and EHEC without serogroup information that is reported as isolated stx1-positive are excluded (because it does not match the characteristics of the outbreak strain). In contrast to the number of EHEC cases published in the epidemiological report with the same data set, asymptomatic infections are not analysed.

Until 28 June 2011, 838 cases of HUS and 2,764 cases of EHEC gastroenteritis (without development of HUS), thus a total of 3,602 cases attributable to the outbreak, were reported to the RKI. Among the HUS cases, 68% are female (EHEC: 59%) and the median age is 43 years (range from 0 to 91 years; EHEC: 47 years, range from 0 to 99 years). Hospitalization is likely in all HUS cases (for EHEC for 59% reported). Among HUS patients, 30 (3.6%) died, among the patients with EHEC gastroenteritis, 17 (0.6%) died.

Figure 1 shows the epidemiological curve of HUS and EHEC cases. Both curves sharply rise from 8 May on, peak around 22 May and then decline. The latest date of disease onset was contracted is 23 June. Whether or not cases with disease onset prior to 8 May, belong to the outbreak, cannot yet be assessed conclusively.

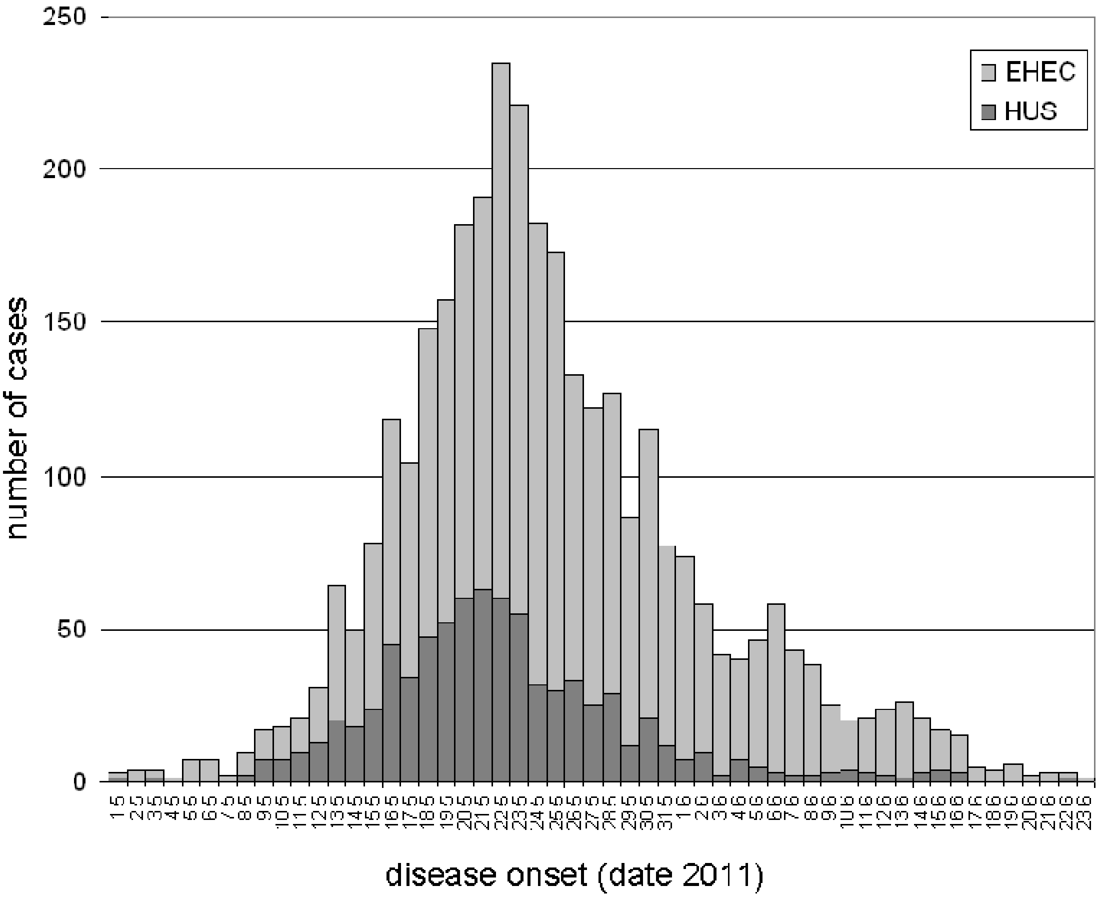

Figure 1: Epidemiological curve of HUS and EHEC outbreak cases (as of 28 June 2011, 773 HUS and 2,507 EHEC cases with diarrhoea as known onset of disease)

Cases were reported from all 16 states, however, the five most northern federal states Hamburg, Schleswig-Holstein, Bremen, Lower Saxony and Mecklenburg-Western Pomerania are affected most (HUS disease incidences from 1.8 to 10.5 cases per 100,000 inhabitants). Figure 2 shows the HUS incidences (cases/100,000 residents) per district, whereas persons suffering from the disease with travel history within Germany are counted for the district in which they were probably infected.

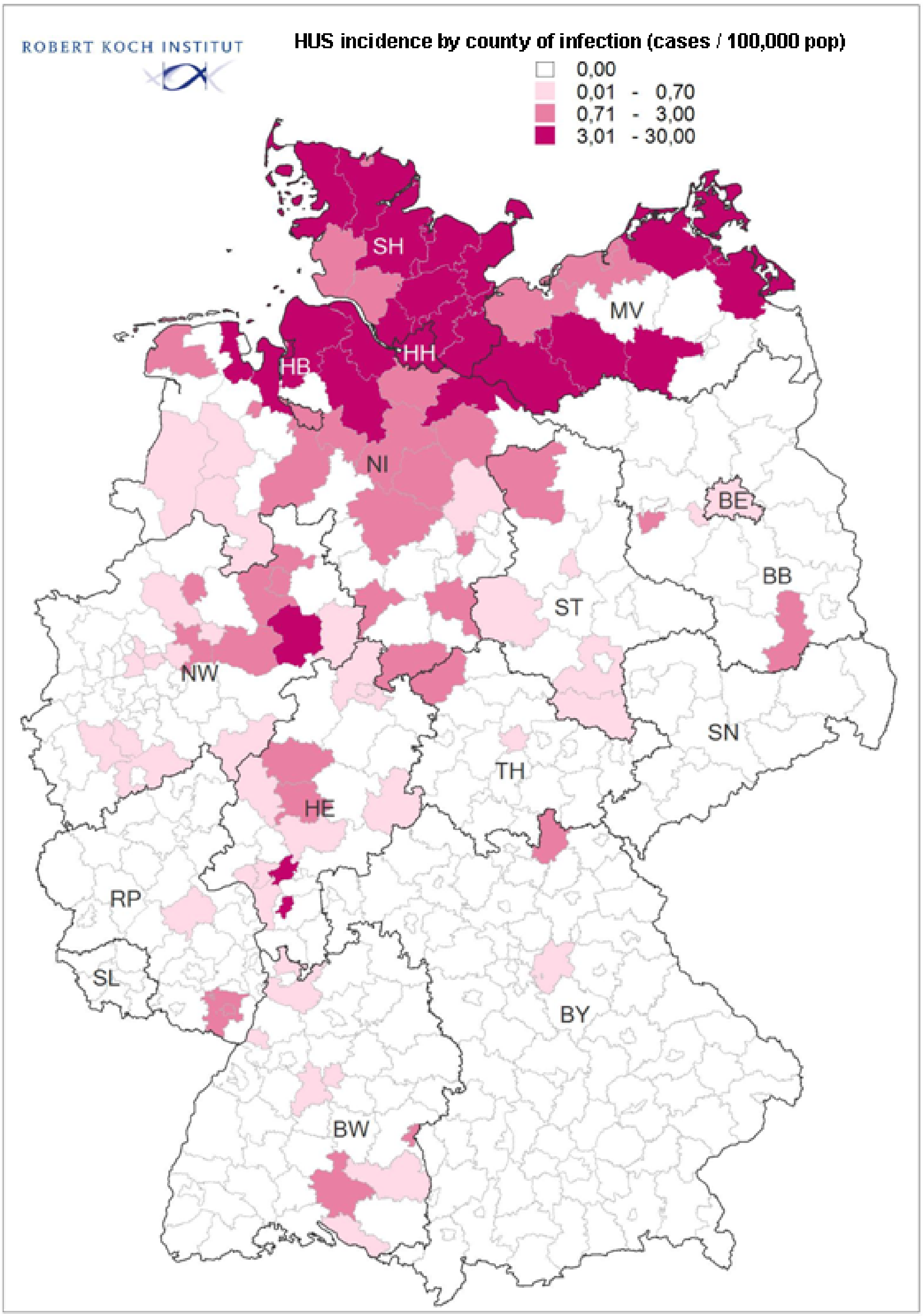

Figure 2: Incidence of HUS during the outbreak according to district, in which the infection has probably taken place (home district or in cases with travel history the area of residence at the time of infection)

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

- Compared to 696 cases of HUS, which were reported to the RKI from 2001 to 2010, the HUS outbreak cases are much older. Only 1% of outbreak cases are under age 5, compared to 50% of HUS cases in previous years in which HUS was primarily a paediatric problem.
- The proportion of women among the EHEC outbreak cases (59%) 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 increased.

So far, microbiological information which could help clearly distinguish sporadic (non-O104) cases from the outbreak cases towards the end of the outbreak is only available for a small number of reported cases. However, the 61 HUS cases with later onset of disease (from 1 June) differ in several ways from the profile of the earlier outbreak cases:
The late cases are younger than the previous cases (median age 37.5 years compared to 43 years), women represent a smaller proportion (50% of the late vs. 69% of the early cases), and a smaller proportion of them (63%) has a clear connection to northern Germany (HH, SH, HB, MV, NI) compared to the early cases (86%). Because the always to be anticipated "background" of HUS cases is characterized by a very young age, an almost balanced sex ratio and residences throughout the country, the observed shifts are compatible with a waning outbreak, which is also illustrated by the decreasing numbers of newly reported cases.
As of 28 June, the latest known date for the disease onset of an HUS or EHEC case with the detection of serogroup O104 is (since 23 June and thus unchanged for 6 days) 12 June (Figure 3).

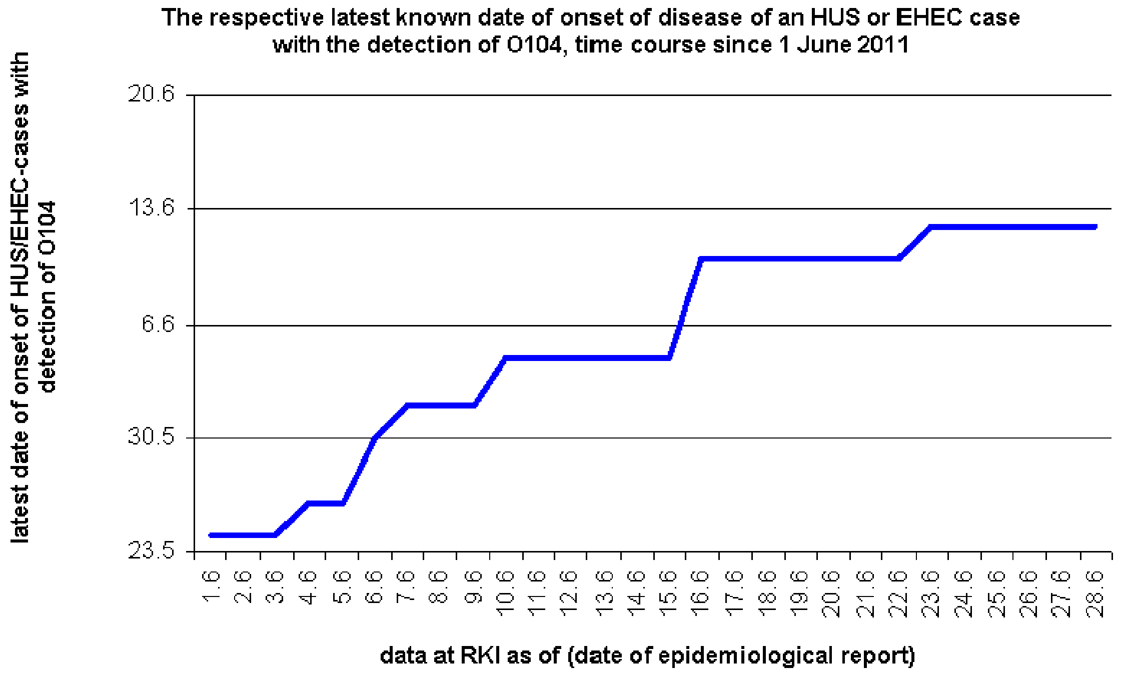

Figure 3: The respective latest known date for the onset of disease of an HUS or EHEC case with the detection of O104, progressing since 1 June 2011

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 the worlds largest described outbreak of this kind. For a more detailed description of the reported data, see also the various scientific publications on the outbreak [1, 2, 3]

1.1.2 Surveillance of Bloody Diarrhoea

Since bloody diarrhoea is a common first symptom of EHEC patients, the trend of an EHEC outbreak can, in a timely manner, be assessed in emergency departments. On 27 May 2011, syndromic surveillance of patients with bloody diarrhoea was established in emergency departments of voluntarily participating hospitals.
The participating emergency departments were located in all states, both in areas more affected by the EHEC/HUS outbreak (Bremen, Hamburg,

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.

Schleswig-Holstein as well as parts of Lower Saxony (north) and North Rhine-Westphalia (Paderborn)) and in less affected areas. 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 are sent to the RKI via e-mail or fax on a daily basis. As of 26 June, a total of 181 emergency departments participated in the syndromic surveillance; 27 of which were in areas more affected. 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. Between 28 May and 26 June, the proportion of patients with bloody diarrhoea among all patients visiting emergency departments in more affected areas was 3.3% (882/26,475); this proportion was 0.7% (759/112,512) in the less affected areas. Figure 4 shows the proportion of patients with bloody diarrhoea among all patients and the number of emergency departments participating in the more affected areas. Women were more frequently affected by bloody diarrhoea than men, whereas after 30 May, decreasing proportions of female cases were observed. In the last week (20-26 June), the proportion of patients with bloody diarrhoea among patients visiting emergency departments in more affected areas was 0.8% on average.

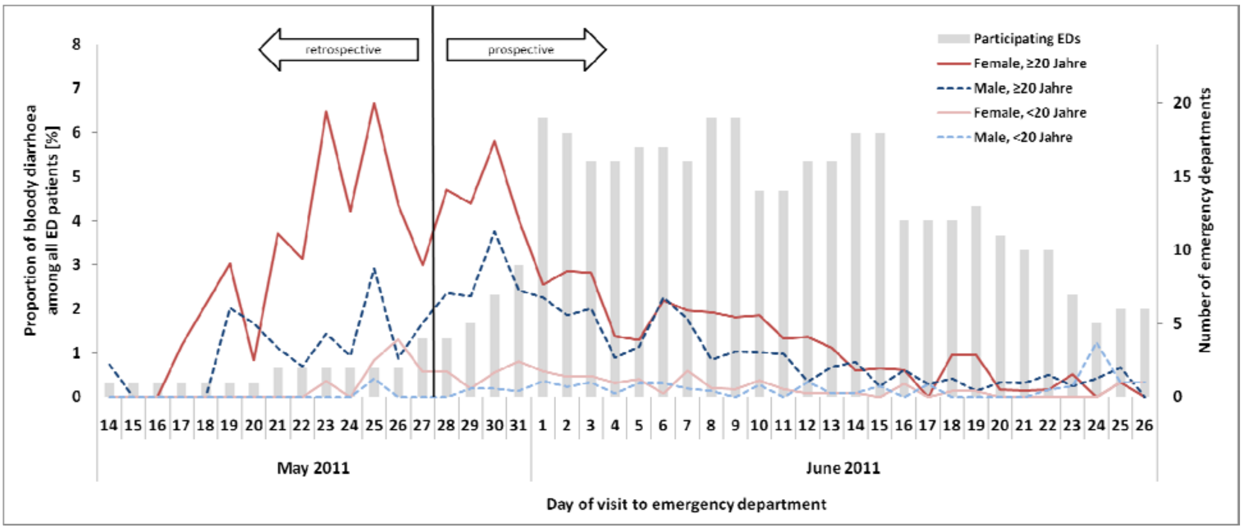

Figure 4: Proportions of patients with bloody diarrhoea among all patients visiting emergency departments, by age and sex as well as number of participating emergency departments in areas more affected by the EHEC/HUS outbreak, EHEC/HUS outbreak, Germany, May-June 2011 (n=1,021)

1.1.3 Case Reports Abroad (as of 28 June 2011)

The French Public Health Institute INVS reported a local EHEC O104 outbreak via EWRS on 24 June 2011. This outbreak includes 15 cases (onsets of disease between 15 and 20 June) which are probably associated with the consumption of sprouts grown in France. This suggests

This suggests that contaminated sprouts as a vehicle for infection have not been confined to Germany and would possibly have to be taken into consideration as vehicle in other occurring disease clusters domestically and internationally. For details see EFSA/ECDC statement from 29 June 2011 http://www.efsa.europa.eu/de/press/news/110629a.htm

On 28 June, the Swedish authorities reported a patient with a detected EHEC O104:H4 infection in southern Sweden, who had not resided in Germany, no contact with an infected case linked to Germany and had not been able to recall eating sprouts. Investigations are ongoing.[4]
In addition to these cases, all EHEC or HUS cases which occurred internationally have directly or indirectly been linked to a stay in Germany. (See Table 1)

Table 1: Number of cases and deaths in countries within and outside the European Union (Source: ECDC, as of 27 June 2011 11:00 a.m., WHO: as of: 24 June 2011 3:00 p.m.)

States	**EHEC (Deaths)**	**HUS (Deaths)**
EU		
Denmark	14 (0)	9 (0)
France	2 (0) + 3(0)*§	5 (0)*
Greece	1 (0)	0 (0)
Great Britain	3 (0)	3 (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	33 (0)	18 (1)
Spain	1 (0)	1 (0)
Czech Republic	1 (0)	0 (0)
Total EU	**72 (0)**	**39 (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)**
Total Sum	**80 (0)**	**43 (2)**

* In connection with the outbreak in Bordeaux (only 8 of the 15 cases are given)
§ suspected cases

4 http://ecdc.europa.eu/en/publications/Publications/2011June29_RA_JOINT_EFSA_STEC_France.pdf

1.2 Incubation Period

During the investigation of the outbreak, evidence for a prolonged incubation period for infections with the outbreak strain was found (compared to information in the literature and experience from previous outbreak investigations). Knowledge of the incubation period is of crucial importance for epidemiological studies (e.g. in order to consider the correct exposure period when interviewing patients). On the basis of the known exposure period in selected cases and the date on which the disease was contracted, the incubation period was estimated for the outbreak strain. In this estimate, only cases with known date of onset and a known exposure period of a maximum of 2 days were included. Cases, where area of residence and presumed exposure site were exactly the same or located in the outbreak area, were excluded. The analyzed data originates from the statutory notification system, from restaurant-clusters and international cases. The incubation period was estimated for 73 cases, the resulting probability function is illustrated in Figure 5. The median incubation period is 8 days. A total of 38 (52%) of the 73 cases became infected on the 7th-10th day after exposure. Compared to the incubation period of EHEC O157 (3 to 4 days) the incubation period for EHEC O104 seems to be extended significantly.

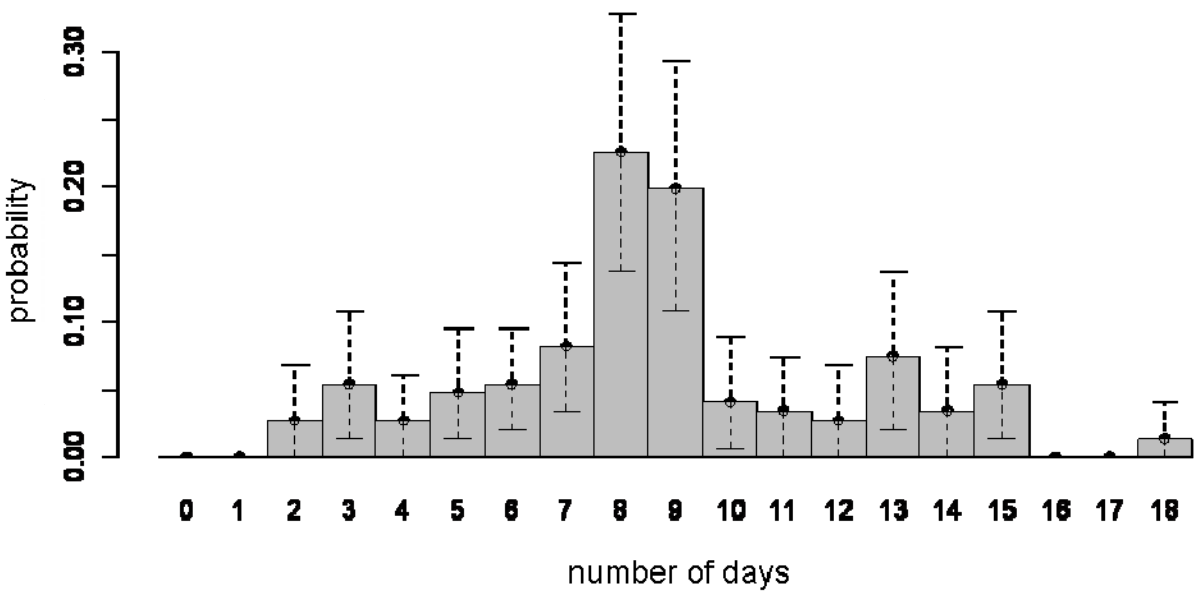

Figure 5: Estimated probability function of the incubation period (based on 73 individuals) with corresponding point-by-point 95% confidence intervals.

To estimate the duration from the onset of diarrhoea until the onset of HUS, data were evaluated which originate from the statutory notification system. The calculation is based on 98 cases: The median between the onset of diarrhoea and the onset of HUS is 5 days. A total of 52 (53%) of the 98 cases developed HUS 4-6 days after the onset of diarrhoea. The 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).

1.3 Estimation of the Exposure Period

The incubation period estimated in Section 1.2 can be used to determine the possible exposure period of the cases. For this, HUS cases with known date of onset of diarrhoe are used (as of 27 June 2011: 773 of 838 cases). The back projection from the onset of diarrhoea to the time of exposure is done by a method by Becker et al.[5] which was developed for the back calculation from the AIDS incidence to the HIV incidence. In Figure 6, the blue bar (i.e. λ_t) indicates the calculated estimate for the expected number of exposures per day. The figure contains extra 95% bootstrap confidence intervals for λ_t to account for the uncertainty of the incubation period estimation in the back calculation.

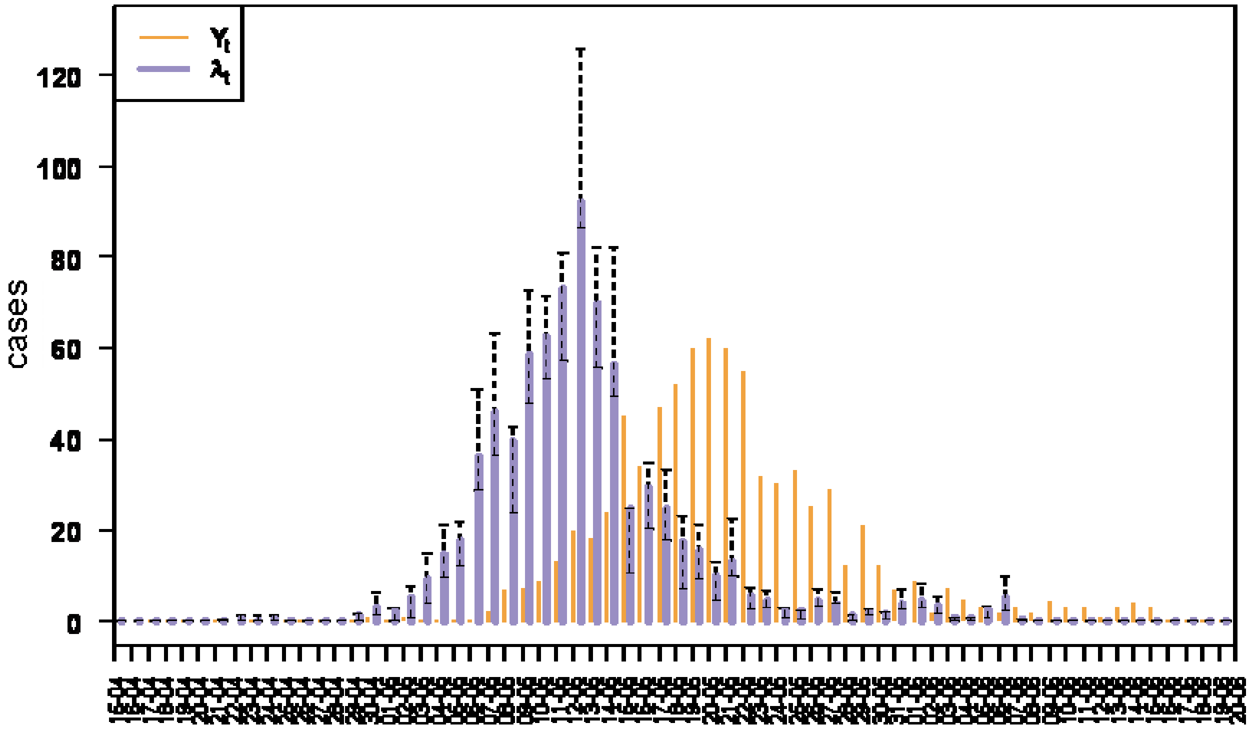

Figure 6: Back projection from the daily onsets of disease to the exposure period. The thin orange curve Y_t shows the actual observed onsets of disease, the thick blue curve λ_t shows the estimated number of exposures per day (including 95% confidence intervals).

The back calculation shows that the time of infection for up to 90% of HUS cases probably falls within the period between 5 May and 24 May.

5 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.

1.4 Reporting Delays

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

All cases with disease onset since 1 May 2011, which were usually notified later, were retrospectively included into the epidemiological analysis. This date was chosen to consider the beginning of the outbreak as completely as possible. One has to differentiate between the date of onset of disease, date of hospitalization, date of diagnosis and date of notification to the local health authority and receipt of the notification at the RKI (report).

According to the specifications of the German Infection Protection Act,a case of disease and detection of a pathogen must be notified to the local health authority by the diagnosing physician and the respective laboratory within 24 hours. The local health authority reviews the information and enters it into an electronic database. On the third business day of the following week at the latest, the information is reported 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, the Robert Koch Institute requested the responsible authorities from the week of 23 May 2011 for daily reporting of HUS case and updated data. Between 24 May and 22 June, more than 50% of the cases were reported to the RKI within four days after receipt of the notification at the local health authority.

In practice, from the onset of disease to the doctor's visit or hospital admission and then until notification to the local health department and electronic transmission of this data via the responsible state authorities to the RKI, a period between a few days and several weeks passes by. Figure 7 shows the current (as of 26 June 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 received at the RKI. It is clearly visible that only one case of HUS with the onset of disease on 3 May 2011 was known to the RKI before 20 May 2011, but this could not be recognized as exceptional due to the undetermined serotype at that time. Only in the week from 23 to 29 May 2011, a large number of HUS cases were reported to the Robert Koch Institute. At this point, investigations conducted by the RKI had already begun.

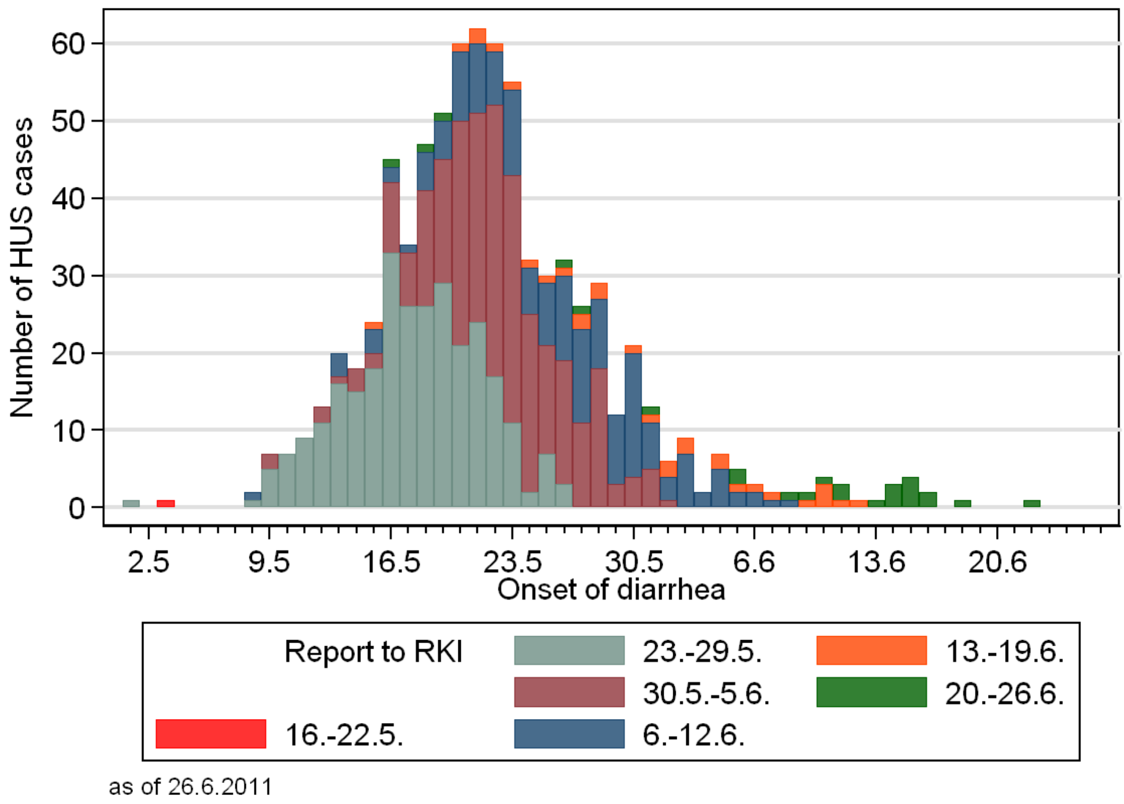

Figure 7: HUS cases by onset of disease and week of receipt of the notification at the RKI (transmission)

In the reporting category "EHEC," in which there is a "background" of a few cases per week, only 3 cases were transmitted in the week from 09 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 specifying the serotype; only in the week from 23 to 27 May 2011 a larger number of cases was 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 notification to the local health department (GA) and receipt of the notification at the RKI (report). The delay between each level during the notification and reporting process becomes visible. Onset of disease and hospitalization are well before the date of notification at the beginning of the outbreak of HUS, the diagnosis of the first cases is also a few days earlier, the receipt of the notification at the RKI is again a few days after the date of notification. The sum of these individual delays leads to the total delay between disease and reporting to the RKI.

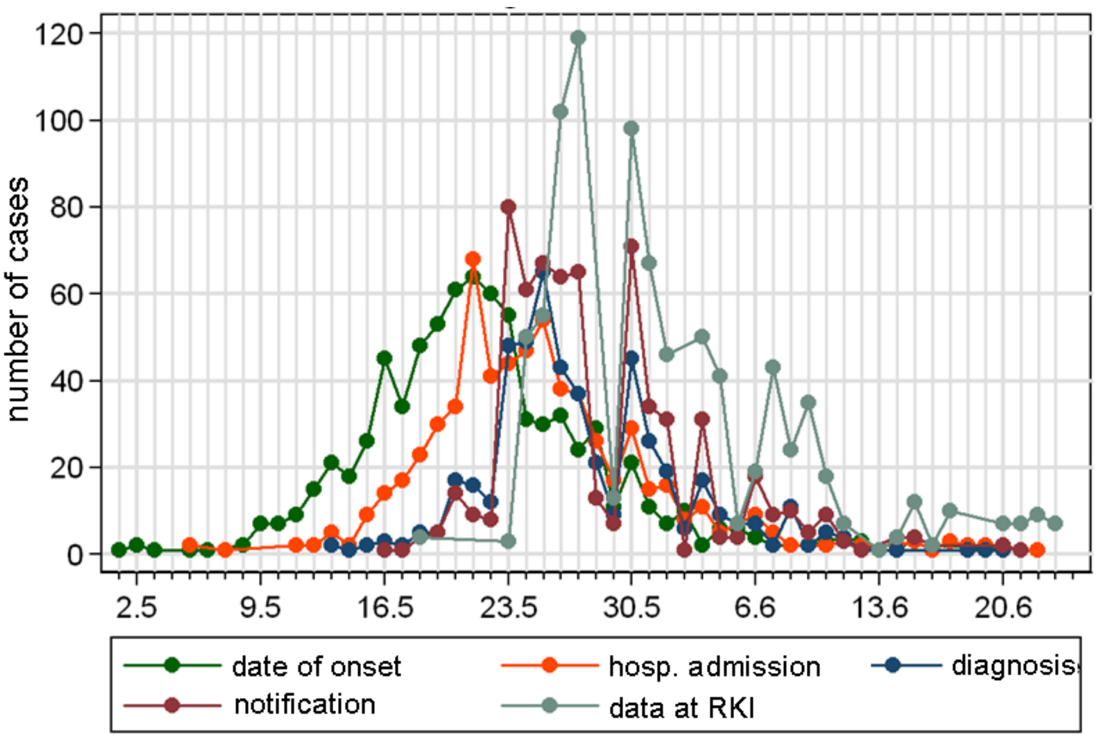

Figure 8: HUS cases presented chronologically: Onset of disease, date of hospitalization, of diagnosis, of notification to health authorities, and of receipt of notification at the RKI (reporting)

1.5 "Now-Casting"

In the course of the epidemic, it was important to take into account the notification and reporting delay in the interpretation of the epidemic curve of notified and reported HUS cases. 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 necessary reporting delay distribution, it was assumed that the distribution of time between hospitalization and receipt of the corresponding report at the RKI had not changed in the period between 23 May and 1 June (period A). From June 2, this distribution was then used for the forecast.

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 actual number of hospitalizations on day t,

i.e. $y_t = \sum_{s=t}^{\infty} y_{t,s}$ In other words, at the time s only $y_{t,s} = F(s-t) \cdot y_t$

hospitalizations are observed at the RKI. Here, F is the estimated distribution function
of the notification and reporting delay using the data from period A. A forecast on

day s for the actual number on day t is therefore $y_t = \frac{y_{t,s}}{F(s-t)}$.

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 illustrates the result of the now-casting on 7 June 2011. The cases reported

to the RKI by date of hospital admission (in green) and an estimated number of hospitalized cases, which until then had not been reported because of the notification delay (orange) are shown.

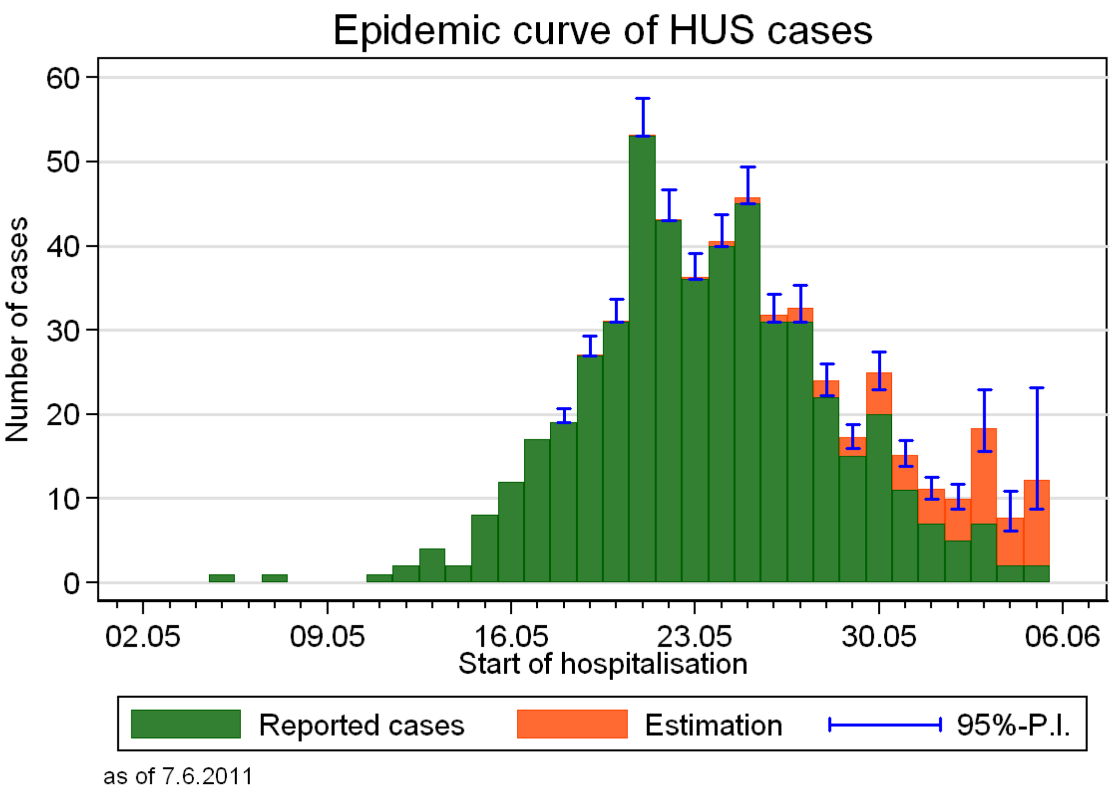

Figure 9: Now-Casting on 7 June 2011. The green bars show the situation of HUS, as the data of 7 June 2011 reflect. The now-cast prognoses are illustrated in orange and the prediction intervals (PI) in blue.

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

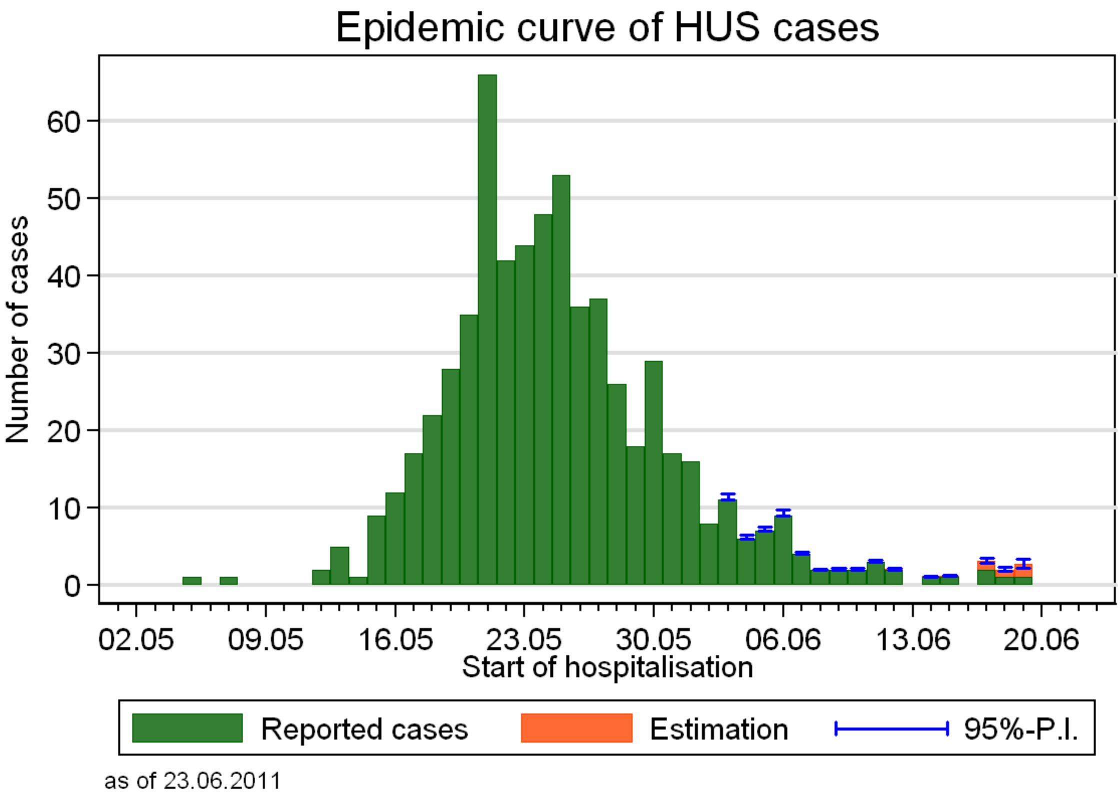

Figure 10: Now-Casting on 23 June 2011. The green bars show the situation of HUS, as the data of 23 June 2011 reflect. The now-cast prognoses are illustrated in orange and the prediction intervals (PI) in blue.

2 Investigations on Exposure

Since 20 May 2011, the RKI has been investigating the outbreak of hemolytic-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. It was possible to progressively isolate the cause of the outbreak through a large 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 and initial exploratory interviews with patients early on indicated the source of infection to be a food product contaminated with EHEC. Vehicles such as raw milk or raw meat that had been identified as a source of infection in previous EHEC/HUS outbreaks appeared to play no role in current events according to the survey results. The first case-control studies were limited for methodological reasons to those exposures that were able to explain a large proportion of cases.

These epidemiological analyses showed that affected patients had significantly more often consumed raw tomatoes, cucumbers and lettuce than healthy study participants. Since neither these studies nor evidence from the food safety sector could narrow down this list of vegetables, the RKI initiated or carried out 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 of two locations of a Frankfurt-based company contracted bloody diarrhoea, nine of which were confirmed by laboratory diagnostics; 18 of which developed an 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 the investigation of the outbreak.
The health department of Frankfurt, together with the Robert Koch Institute and the operators of the canteens, was able to acquire a list of the purchases made in the canteen in the weeks from 02 to 16 May for case persons (n = 23) and for randomly selected healthy persons (n = 35) with the help of electronic billing documentation and perform a logistic regression analysis (Table 2). The risk of contracting bloody diarrhoea to 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 a 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 component of the salads sold in the canteen (salad bar) could be identified as a very likely vehicle.

The results of the subsequent nationwide conducted traceback analyses revealed that the operator of the two canteens was connected through a vendor 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 with the employees regarding the actual salad components to confirm the sprout hypothesis was unfortunately not possible.

Table 2: Results of the univariate and multivariate analysis of risk factors for the development of bloody diarrhoea in two canteens in Frankfurt am Main

		Univariate Odds ratio (95% CI)	Multivariate 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 the recollection of the raw vegetable consumption of the surveyed patients and control subjects during the analysis, the RKI pursued the following approach with the help of a "recipe-based restaurant cohort study": Partly in the context of cluster detection with the support of public health services, partly as a result of active case finding by means of order books of the 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. Persons 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. There were 168 persons included in the analysis. A total of 31 (18%) persons from the groups contracted bloody diarrhoea or EHEC/HUS. This exposure information came from two sources:

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

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

This information 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 univariate 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 univariate analysis was confirmed in the multivariate 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 showed no significant p values (p> 0.15) in the analysis for an increased relative risk of disease.

Table 3: Results of univariate and multivariate data analyses of the restaurant recipe-based cohort study. RR, relative risk; 95% CI, 95% confidence interval (CI).

Ingredient	Total	Cases among the exposed	Total number exposed	Cases among the non-exposed	Total number of non-exposed	RR	95% CI	P-value
Univariate								
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 group dined in the restaurant, only one mixture of sprouts was used, containing fenugreek sprouts, alfalfa sprouts, adzuki bean sprouts and lentil sprouts. The supplier of the restaurant supplying the sprouts received his goods from the incriminated company A in Lower Saxony.

2.4 Findings from 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 stated to have eaten sprouts. The patients interviewed stuck out because of very conscious and careful eating habits, which made a relevant under-coverage 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.[6] Otherwise, the risk of false positive correlations increases with the inclusion of an excessive number of exposures. Therefore, the sprouts were not pursued initially. Sprouts were taken into account in subsequent detailed surveys of the RKI.

6 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

From 29 May to 04 June 2011, another case-control study was conducted, which aimed to more accurately differentiate between the relationship of the consumption of various vegetable foods and the disease. The study was conducted in three cities strongly affected by the outbreak (Lübeck, Bremerhaven and Bremen). Case subjects were adult HUS patients who were hospitalized during the study period in one of three hospitals in Lübeck, Bremerhaven or Bremen. Controls were individually associated with a target ratio of 1:3 by age group (18-34 years, 35-44 years, 45 years or older), gender and residence. The 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 intensive surveys of other HUS patients, the cases and controls in this study were interviewed regarding the consumption of vegetable foods, such as fruits and raw vegetables in the 2 weeks before the onset of the disease or prior to the interview. Moreover, the consumption of sprouts was also surveyed, although only 25% of HUS patients surveyed in the exploratory interviews indicated that they had consumed sprouts. The 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 who presented this information. Table 4 lists all exposures with p <0.1.

Table 4: Fruit and vegetable exposures associated with the incidence of HUS (p-value <0.1) in the univariate analysis of the raw vegetable case-control study.

Exposure	Exposed Cases No./Total No. (%)	Exposed Controls No./Total No. (%)	Matched Odds Ratio (95% CI)	p
Sprouts	6/24 (25)	7/80 (9)	4.35 (1.05-18.0)	0.043
Cucumber	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
Pepper	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 in-depth multivariate analysis for fruit and vegetable variables, sprouts and cucumbers were first examined together: both variables remained significant, with an 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. No other of the above mentioned exposures is significantly associated with disease in a multivariate model (with sprouts and cucumbers).

In the study, the source of supply for some of the exposures was also collected. It was noticeable that the 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 may indicate that people were infected i.e. eating salads outside the home (e.g. in canteens, restaurants). Oftentimes, the above mentioned foods are consumed together (in the form of a salad), while a contamination may have affected these and/or another food product which was not remembered.

As part of a re-survey of cases and controls who denied to have eaten sprouts at first or could not remember to have done so, 8 (33%) cases and 37 (46%) controls were interviewed again. Overall, 3 of the 8 (37.5%) interviewed cases changed their original statement and indicated to have eaten sprouts in the surveyed period. None of the 37 controls surveyed again 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 in all cases can be estimated to be 52%.

2.5 Investigations of Disease Clusters

2.5.1 Cooperation with the Task Force EHEC

In close cooperation with the RKI, BfR, BVL, state food safety authorities and the states most affected by the outbreak (Lower Saxony, Schleswig-Holstein, Mecklenburg-Western Pomerania and Hamburg), disease clusters were analyzed in the Task Force EHEC (coordinated by the BVL and Bavaria) in terms of supply channels and chains of certain foods. The occurrence of at least one case of disease (EHEC or HUS) at an exposure site was defined as cluster, when there were strong indications that the infection could only have come from this place. This was the case for example when members of a tour group, in which there were several cases of disease, had just eaten at a restaurant together. Consumption locations of individual cases were then considered to be important enough to trace, when only a single exposure site served as possible explanation, e.g. Danish tourists traveling through northern Germany had only eaten at one specific motorway service station. In a first step, the identification of clusters was essentially conducted by means of information on cases of disease sent to the RKI. The information came from doctors, e.g. in hospitals, local and state health departments, health authorities from other countries, e.g. Sweden, Denmark and USA, from free-text data reported to the RKI via the surveillance system and also by patients themselves, e.g. through patient surveys in the context of epidemiological studies of the RKI.

Overall, the Task Force was able to identify 41 outbreak clusters according to the above mentioned definition in six affected states (NI, SH, MV, HH, HE, NW). These 41 clusters involve more than 300 cases of disease. The results of food traceability of these 41 clusters in relation to the producer A are shown and explained in a separate report on the work of the Task Force EHEC.

2.5.2 Cohort Studies of Selected Clusters

Before sprouts were known to be the likely food vehicle, cohort studies were carried out by the RKI using three early identified clusters. In this epidemiological approach, cases and healthy individuals of travel groups were interviewed in detail regarding their food consumption with the help of menus, when a common exposure site was probable. The aim of the cohort studies was to identify the food vehicle related to the diseases. Groups of persons with a manageable number of members (cases and healthy individuals) with contact information present (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 relationship between disease and particular foods could not be found in the analysis of the cohort studies. After there had been indications from other studies suggesting sprouts as a vehicle, follow-up research was conducted in the restaurants. Retrospectively, all cases of disease could be explained through the consumption of sprouts which, for example, had been used as garnish for certain dishes. By means of an analysis of the supply channels, the sprouts could be traced back to the sprout producing company A in Lower Saxony (see Section 2.5.1).

3 Bacteriology of the Outbreak Strain

3.1 Detection and Characteristics of the Pathogen

The cluster of patients with the clinically very characteristic disease pattern of the hemolytic-uremic syndrome in north-west Germany, which was reported to the RKI on 19 May 2011, quickly directed the attention to the Shigatoxin-producing E. coli (EHEC) as pathogen.
On 23 May 2011, the information, that samples received at the National Reference Centre (NRZ) for salmonella and other bacterial enteritics on 19 and 20 May 2011 might be connected to the outbreak, was available to the NRZ. The result of the detection of the EHEC virulence markers stx1 (negative), stx2 (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 Shigatoxin2 (variant vtx2a) producing E. coli of serovar O104:H4 was done 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, stx2+, eae-, hly- with resistance to 3rd-generation cephalosporines (ESBL)) was placed on the outbreak information platform EPIS. On 26 May 2011, the NRZ 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 and at EPIS) showed numerous specifics of the pathogen, which are described in detail in the appendix, together with concrete information on clinical-microbiological diagnostics (characteristics of the pathogen as well as information and assistance of the RKI in the diagnosis of the outbreak strain, www.rki.de).

For international communication and strain comparisons, the PFGE pattern was submitted to the EPIS platform and a reference strain was sent to the CRL for E. coli (Dr. Caprioli) in Rome, the WHO RL for E. coli (Dr. Scheutz) in Copenhagen, the NRL 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 hemolytic-uremic syndrome worldwide, other E. coli serotypes have also been identified as EHEC. 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 NRZ/RKI in 2001 was also integrated into this collection.

The current outbreak involves the rare EHEC serotype O104:H4, which has not been described in animals and only rarely in humans (Germany 2001/HUSEC041, Korea 2006, Georgia 2009 and Finland 2010).

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 here for the first time described in EHEC, any other 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 an E. enteroaggregative E. coli (55989).

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), play an important role. This is especially important in the framework of less characteristic disease patterns, such as (bloody) diarrhoea, but also for the detection in asymptomatic excretion.

In any particular case, the search is facilitated by the resistance phenotype (ESBL) unusual for intestinal E. colis in a way that it allows for the use of corresponding selective media for a targeted search. The NRZ detected this resistance and immediately used it for the systematic search for the pathogen (using the ESBL-plate combined with a multiplex PCR screening of stx1, stx2 and eae or using the ESBL-plate combined with a multiplex PCR for the stx2, 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 persons infected 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 NRZ. This was continued parallel to the epidemic strain special diagnostics. These investigations are critical for assessing the "background action" and hence for assessing the progression of the outbreak.

3.2 State of Laboratory Tests at the NRZ

Between 20 May 2011 and 24 June 2011, a total of 2324 samples from suspected EHEC/HUS cases were sent to the NRZ. 202 of them were fully typed by means of serovar determination, PCR of virulence factors and the resistance profile and were allocated to the current outbreak. Another 650 were allocated to the outbreak by means of PCR and ESBL production. In 354 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 NRZ. These 450 samples are processed separately.

Apart from the outbreak strain O104:H4, 24 EPEC and 370 EHEC cases were also identified. A total of 72 of these EHEC isolates and 7 of the EPEC isolates could be allocated to 17 different serotypes with very different virulence patterns. The typing for another 204 will be available at the beginning of next week. Strain isolation for fine typing was not successful for 94 samples.

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

4 Focus of Current and Planned Epidemiological Studies

4.1 "Late Cases"

Since 22 May, the number of newly diagnosed cases of EHEC caused diarrhoea and HUS has been declining. Since the sprout production in company A in Niedersachsen was suspended in early June and the public warning regarding the consumption of sprouts followed, it cannot be excluded that people have still eaten contaminated sprouts in early June.

If contaminated sprouts were no longer consumed after 10 June, only few isolated cases of new infections would be expected that are associated with the consumption of sprouts from company A, taking into account the incubation period (median of 8 days, for 90% of patients less than 15 days) after 24 June. In the future, new additional cases can occur due to secondary transmissions.
The RKI and the public health services in states and counties paid special attention to the investigation of the new cases of disease after the halt in production and the public consumption warning ("late cases”). The following questions should be investigated:

- What proportion of these new cases is caused by the outbreak strain EHEC O104:H4? The case definition for reporting cases to the RKI also includes cases with stx2-detection (or with positive ELISA detection, which does not differentiate between stx1/stx2) that are not (yet) serotyped and are perhaps not caused by the outbreak strain.

- It should be estimated what proportion of the late cases, which are really caused by the outbreak strain, can still be explained by the consumption of contaminated sprouts, how many of them are due to secondary transmission and to what proportion neither of these explanations fit. The potential recall bias with regard to sprout consumption needs to be taken into account.

However, if a significant proportion of late cases definitely caused by the outbreak strain O104 cannot be explained by sprout consumption or secondary transmission, further epidemiological studies are needed to uncover other existing sources of infection.

To examine the second question, a standardized questionnaire was developed, which should be completed by the local health authorities for every new EHEC/HUS patient (with onset of disease on 10 June). In the first questionnaires sent to the Robert Koch Institute, information regarding the sprout consumption (three times yes, 5 times no) was available for 8 patients (including 3 of them with HUS) and information regarding preceded diarrhoea in the close environment was available for 10 patients (4 of them with HUS) (three times yes, seven times no). Two patients who denied having consumed sprouts mentioned contact to a person suffering from diarrhoea.

4.2 Domestic Environment and Secretor

In collaboration with local health authorities, the RKI is conducting a household-based study regarding the prevalence of EHEC O104 carriers in 50 households with a reported EHEC case in the context of the outbreak and 50 control households located near their residences. In further studies, households with an EHEC O104 carrier are included in a prospective cohort study to identify possible household transmissions and risk factors for household transmissions as well as to investigate the shedding period of the EHEC O104 carriers.

5 Appendix

Characteristics of the pathogen as well as information and assistance of the RKI in the diagnosis of the outbreak strain (Updated: 24 June 2011)
http://www.rki.de/DE/Content/InfAZ/E/EHEC/EHEC__Diagnostik.html

Publications:

Frank C, Faber MS, Askar M, Bernard H, Fruth A, Gilsdorf A, Höhle M, Karch H, Krause G, Prager R, Spode A, Stark K, Werber D, on behalf of the HUS investigation team. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. Euro Surveill. 2011;16(21):pii=19878. Available online:
http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19878

Askar M, Faber MS, Frank C, Bernard H, Gilsdorf A, Fruth A, Prager R, Höhle M, Suess T, Wadl M, Krause G, Stark K, Werber D. 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(22):pii=19883. Available online:
http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19883

Wadl M, Rieck T, Nachtnebel M, Greutélaers B, an der Heiden M, Altmann D, Hellenbrand W, Faber M, Frank C, Schweickert B, Krause G, Benzler J, Eckmanns T, on behalf of the HUS surveillance and laboratory team. 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 . Euro Surveill. 2011;16(24):pii=19893. Available online:
http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19893

Frank C, Werber D, Cramer JP, Askar M, Faber M, Heiden MA, Bernard H, Fruth A, Prager R, Spode A, Wadl M, Zoufaly A, Jordan S, Stark K, Krause G; the HUS Investigation Team. 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]
http://www.nejm.org/doi/full/10.1056/NEJMoa1106483

Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, Peters G, Karch H. Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect Dis 2011, published online June 23, 2011 DOI:10.1016/S1473-3099(11)70165-7
http://www.sciencedirect.com/science/article/pii/S1473309911701657

RKI: Zur aktuellen Häufung von EHEC-Infektionen und HUS-Fällen in Deutschland. Epid Bull (Supplement) 2011; 21:1-2
http://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2011/21__11__Supplement,templateId=raw,property=publicationFile.pdf/21_11_Supplement.pdf

RKI: Zur Entwicklung der Erkrankungszahlen im aktuellen EHEC/HUS-Ausbruch in Deutschland. Epid Bull 2011; 22:199-202
http://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2011/22__11,templateId=raw,property=publicationFile.pdf/22_11.pdf

RKI: Zur Entwicklung der Erkrankungszahlen im aktuellen EHEC/HUS-Ausbruch in Deutschland - Update. Epid Bull 2011; 23:207-209
http://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2011/23__11,templateId=raw,property=publicationFile.pdf/23_11.pdf

RKI: Zur Entwicklung der Erkrankungszahlen im aktuellen EHEC/HUS-Ausbruch in Deutschland - Update. Epid Bull 2011; 24:215-217
http://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2011/24__11,templateId=raw,property=publicationFile.pdf/24_11.pdf

RKI: Intensivierte Surveillance während eines großen EHEC-/HUS-Ausbruchs in Deutschland. Mai-Juni 2011. Epid Bull 2011; 25:225-229
http://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2011/25__11,templateId=raw,property=publicationFile.pdf/25_11.pdf