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EHEC Outbreak 2011

Investigation of the Outbreak
Along the Food Chain



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and many unnamed helpers who made their contribution towards overcoming the EHEC outbreak.

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1 Foreword

In spring 2011, the theme EHEC had consumers, businesses and authorities holding their breath not only in Germany but throughout Europe. The EHEC outbreak between May and July was the biggest bacterial outbreak of *Escherichia coli* in Germany since World War II. Ultimately, fenugreek seeds contaminated with the enteroaggregative EHEC strain O104:H4 imported from Egypt were identified as the highly likely cause of the outbreak.

Generally speaking, EHEC pathogens are well-known food safety hazards. Incidents of infections caused by this pathogen occur worldwide. Roughly 900 cases are reported every year in Germany alone. Enterohaemorrhagic *Escherichia coli* (EHEC) is a very robust bacteria, capable of surviving in the environment for many weeks and which is infectious to humans even in very minute quantities. Due to yearly re-occurrences of EHEC infections in Germany, the BfR decided in January 2011 to publish advice for consumers on protection against EHEC, with special emphasis on hygiene practices, which prevent the contamination and spread of EHEC bacteria. Hygiene practices in the kitchen were especially emphasized as it seems that consumers, due to the belief that food items in Germany are of such high standard, were neglecting customary hygienic practices.



**Prof. Dr. Dr. A. Hensel,
President BfR**

The latest outbreak in the early summer of 2011, however, involved the very unusual EHEC strain O104:H4, which had only very rarely occurred previously, and only in humans, and about which only very little was known prior to the outbreak. This made the investigation of the outbreak a huge challenge. To make matters worse, the infection produced extremely severe symptoms in the affected persons and the control of the outbreak became a challenge even for the very high state of the art medical technology in Germany.

When the outbreak began in May 2011, no established test existed in Germany to determine the EHEC strain O104:H4. A specific recognition system of this kind was only published at the end of May 2011 by the National Reference Laboratory for *E. coli* at the Federal Institute for Risk Assessment together with experts from the French Food Agency ANSES. This method was then made available to the examination laboratories of the German federal states. During the search for the cause of the outbreak, however, no microbiological evidence of the EHEC bacteria O104:H4 could be detected in the seeds and sprouts examined as suspected infection source.

By evaluating outbreak clusters, i.e. locations with frequent occurrences, as well as available delivery lists and data on distribution channels of foods, it was possible to ascribe the cases of illness and local outbreaks in Germany to sprouts that originated in a horticultural farm in Lower Saxony. A search was then made to establish where the contamination of the sprouts and possibly even the sprout seeds with EHEC could have occurred. By tracing seed deliveries backwards and forwards, it was ultimately possible to isolate the source of the contamination, with high probability, to fenugreek seeds from Egypt.

During the EHEC outbreak, local monitoring authorities, regional and national consumer protection ministries, European authorities and the WHO all took action. The task force deployed to combat the EHEC crisis proved to be so successful that the Federal Ministry of Food, Agriculture and Consumer Protection envisages it being further developed into a permanent crisis management tool.

The BfR made an important contribution towards investigating the outbreak and providing information to other authorities, the media and the general public. It had responsibility for carrying out risk assessments, providing scientific support to national and regional authorities in the investigation of the outbreak, making decisions on national measures, ensuring the exchange of scientific information on the European level as well as communicating risk data.

The main working results of the BfR in connection with the EHEC outbreak in 2011 have been compiled in this scientific paper.

A handwritten signature in blue ink, consisting of two distinct parts. The first part is a stylized, cursive 'A' followed by a horizontal line. The second part is a more complex, cursive signature that appears to be 'Hensel'.

Prof. Dr. Dr. Andreas Hensel
President Federal Institute for Risk Assessment

2 Introduction and Synopsis

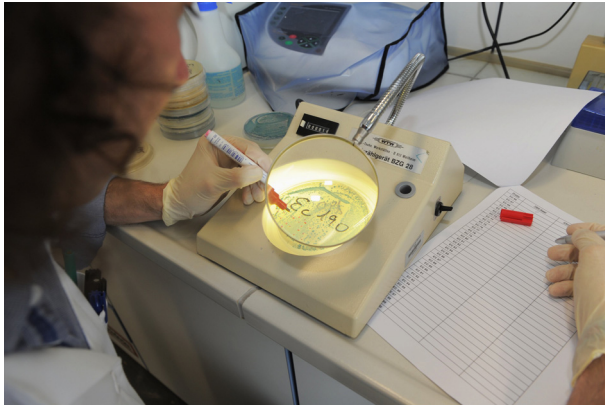


Fig 1: BfR employee counting *E. Coli* colonies on a growing medium with a food sample

In the early summer of 2011, the largest outbreak to date of enterohaemorrhagic *Escherichia coli* (EHEC) was recorded in Germany. EHEC are *Escherichia (E.) coli* bacteria which form cell toxins. These so-called Shiga- or verotoxins can cause severe, under certain circumstances even fatal, illnesses in humans. An EHEC infection usually results in slight to severe diarrhoea. Haemolytic-uraemic syndrome (HUS) can be contracted as a result of the infection. It is a disease which manifests itself in acute kidney failure, coagulopathy and destruction of the red blood cells

The pathogen of serotype O104:H4, which appeared in May, June and July 2011, showed a pronounced pathogenic potential. In relation to the number of cases of haemolytic-uraemic syndrome (HUS) associated with the outbreak, a serious illness with a high lethality rate, it was the largest outbreak of this kind described anywhere in the world. The authorities involved consider the outbreak to have been caused by fenugreek seeds imported from Egypt which were used in a horticultural farm in Lower Saxony and by private individuals for the production of sprouts. Where and how the seeds came in contact with the outbreak pathogen could not be ascertained at the time of completion of this scientific paper.

The pathogen of serotype O104:H4, which appeared in May, June and July 2011,

The EHEC O104:H4 strain is a peculiarity. By means of DNA sequence analysis, it was determined that the outbreak strain has considerably more in common with enteroaggregative *E. coli* (EaggEC) than with conventional EHEC. For this reason, it is also referred to as enteroaggregative EHEC O104:H4 or EaggEC O104:H4. This means that this strain is a recombinant of an enteroaggregative and an enterohaemorrhagic *E. coli* which had never previously been isolated in animals or from foods and which had only been detected in humans before. According to the latest scientific knowledge, it is not to be assumed that EHEC O104:H4 has any major significance for the contamination of agricultural areas.

During the outbreak, the Federal Institute for Risk Assessment (BfR) scientifically assessed each currently valid consumer safety situation and issued recommendations to the responsible regional and national authorities, as well as the involved commercial operators and consumers. The National Reference Laboratory (NRL) for *E. coli* located at the BfR, conducted the majority of the microbiological outbreak examinations and developed and evaluated the examination methods necessary for this purpose in cooperation with an international partner (ANSES, France). In addition to this, the BfR was involved in the Task Force EHEC established at the Federal Office of Consumer Protection and Food Safety (BVL), as well as the European Task Force, thus making an active contribution towards investigating the outbreak in Germany and Europe. The scientific assessments and recommendations derived from them were examined continuously and adjusted to reflect the latest level of knowledge on the basis of each current data and information situation.

An overview of the chronological sequence of the outbreak is given below (Chapter 3). Thereafter, the methodical processes used to identify the food that triggered the outbreak are described (Chapter 4). Despite extensive tests, it was not possible to produce microbiological evidence of *Escherichia coli* (EHEC) of serotype O104:H4 in suspect foods. There were various reasons to suspect freshly consumed salad ingredients (cucumber, tomato, lettuce, sprouts) as a possible vehicle of infection. This in turn resulted in the necessity for systematic tracing of the suspect foods all along the supply chain in order to identify the possible source

of the outbreak. Within the course of data collection, it was ascertained that the available methods of electronic data recording of supply relationships would have to be expanded and adapted for the purpose of epidemiological investigation into the outbreak. To this end, the BfR prepared adapted software solutions for the collection and quantitative evaluation of supply data. The processing of data and analysis of complex supply data with this specially developed tool enabled the epidemiological verification of the causative food and the likely source.

The tasks of the National Reference Laboratory for *E. coli* (NRL *E. coli*) are outlined in Chapter 5. Within the scope of its tasks the NRL *E. coli* did not only perform fine typing of suspect *E. coli* isolates as a service for the diagnostic laboratories of the federal states but also conducted analysis of food samples. Despite this, it was not possible to isolate the outbreak strain EHEC O104:H4 from the more than 8,000 vegetable, sprout and seed samples examined nationwide. Detection was only possible in samples with secondary contamination originating from households with patients.

Chapter 6 comprises three BfR health statements on the EHEC outbreak, thus constituting the chronology of the extensive risk assessments made by the BfR. The scientific assessments and recommendations derived from them were examined continuously and adjusted to reflect the latest level of knowledge on the basis of current data and information situation. Continuous updating in the course of a crisis is an expression of good scientific practice.

The relevance of sprouts and germ buds as well as seeds for sprout production in the EHEC O104:H4 outbreak in May and June 2011 was described in BfR Statement No. 023/2011 of 05 July 2011.

In this statement, the BfR assumes that the outbreak of EHEC O104:H4 illness in Germany is attributable to the consumption of contaminated sprouts. It is highly probable that the outbreak pathogen was introduced to sprout production via imported fenugreek seeds. A causative introduction to the horticultural farm in Lower Saxony via water, humans, animals or pests is unlikely in the view of the BfR because the outbreak strain could not be detected in any of the samples despite extensive testing on the premises. This conclusion is also supported by the results of the European Task Force which show a connection between the German and French outbreaks through the use of contaminated sprout seeds. Furthermore, the BfR is recommending that gastronomy and catering businesses carefully consider whether they should serve raw sprouts and seedlings to consumers as long as contaminated seed batches are still in circulation which could be used for the production of sprouts and seedlings. The BfR is also advising consumers against the consumption of raw sprouts and seedlings for the same reason.

In its updated statement, No. 031/2011 of 26 July 2011, the BfR commented on the relevance of EHEC O104:H4 in fenugreek seeds which are processed into other foods than sprouts and germ buds.

In this statement it is explained that possible risks and/or dangers emanating from foods in which contaminated fenugreek seeds were processed depend in essence on the treatment and processing methods employed. It is ascertained that only thermal treatment methods of fenugreek seeds (e.g. heating to 72° C for two minutes at the core in a moist milieu), if necessary combined with high-pressure methods or irradiation, are suitable for safely killing the germ as it is possible that the pathogen can also exist inside the seeds.

A third statement on the EHEC outbreak, No. 049/2011 of 23 November 2011, was prepared at the same time as this scientific paper. In this statement, an updated analysis was made on the basis of the information on the measures introduced in Germany and the EU, from which recommended courses of action were derived.

With explicit mention of the existing uncertainties, the responsible authorities were provided with a rational action and decision-making basis, thus formulating recommendations for commercial operators and consumers.

The occurrence and spread of EHEC in agricultural production are dealt with in Chapter 7. Drinking water, commercial fertilisers, animal by-products and fermentation residues from organic waste treatment are discussed here as potential sources of EHEC and the valid regulations are presented. It should be noted here that the fundamental possibility exists that zoonotic agents and other pathogenic germs can exist in organic fertilisers, especially if farmyard manure (e.g. solid dung, liquid manure and slurry) and other organic substances are used as the basic materials, thus possibly constituting a health hazard for humans and livestock. It has been established in the meantime, however, that the EHEC O104:H4 strain is a recombinant of an enteroaggregative and an enterohaemorrhagic *E. coli* which has never previously been isolated in animals or from foods and which had only been detected in humans before. According to the latest level of available knowledge, therefore, it is not to be assumed that EHEC O104:H4 has any major significance for the contamination of agricultural matrices.

The final Chapter 8 outlines the measures taken within the scope of Risk Communication with regard to press and public relations work and on a European level. The results of a representative population survey give an indication of the risk perception, as well as the information needs and requirements, of the general public.

3 Chronology of the Outbreak



Fig. 2: Top: BfR personnel sampling sprouts and seeds. Bottom left: Preparing samples at the NRL *E. coli*. Bottom right: Fenugreek seeds are the source of the disease outbreaks

An overview is given below of the chronological sequence of the EHEC outbreak events in the early summer of 2011. The key data relating to the outbreak are listed in Table 1.

In May 2011, doctors and hospitals in Hamburg in particular reported an increasing number of EHEC and HUS cases and fatalities. The Robert Koch Institute (RKI) notified the national government authorities responsible for consumer protection about the medical reports they were receiving from the health offices of the federal states.

When epidemiological examinations suggested a causal connection between the consumption of salad and contraction of the illness, the BfR and RKI recommended that consumers refrain from eating raw tomatoes, cucumber and green salads. The EHEC pathogens found by the authorities in Hamburg on Spanish cucumbers caused quite a stir all over Europe (one tabloid ran the headline “Death Comes from Spain”). When they were examined at the BfR, however, they turned out not to be the same pathogens as the ones found on the patients who had contracted the illness.

In its search for the cause of the disease, the Consumer Protection Ministry in the federal state of Lower Saxony followed a new trail which led to a supplier of sprouts in Lower Saxony, whereupon they issued a recommendation not to eat sprouts. The BfR, BVL (Federal Office of Consumer Protection and Food Safety) and RKI also advised against the consumption of raw sprouts and revoked the previous consumption recommendation. The official recommendations had a tangible effect on consumer behaviour.

Initially, the doubt surrounding sprouts in salad as the cause of the disease remained. Many sources, including biogas plants, fertilisation, sewage sludge, irrigation systems, drinking water and terrorism, were named as possible hypotheses for the contamination of plant-based foods. Ultimately, France also reported an increase in EHEC cases after the consumption of sprouts. Once the traceability results of the suspect sprout products from France and Germany were then brought together on a European level, they pointed towards certain batches of fenugreek seeds imported from Egypt as the source.

Consequently, the World Health Organization (WHO), European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) advised all European consumers not to eat raw sprouts. The BfR published the scientific risk assessment of the EHEC outbreak in regard to the relevance of sprouts and germ buds as well as seeds for sprout production as of 05 July 2011. The EU Commission obliged all member states to take measures to trace the suspect batches of fenugreek seeds. Once the actions taken by private companies and the federal states had sufficiently reduced the risk potential for the German market, the BfR and other federal authorities restricted their recommendations to the consumption of raw fenugreek seeds from Egypt and sprouts produced from them.

The RKI declared the end of the EHEC outbreak on 26 July 2011. At this point in time, no new cases of illness with an obvious link to the outbreak had been reported for three weeks. Overall, the outbreak accounted for 3,842 cases of illness (855 cases of HUS, 2,987 cases of acute gastroenteritis). 53 persons (35 HUS patients and 18 with gastroenteritis) died of the infection (Source: Robert Koch Institute, 2011).

Tab. 1: Chronology of the EHEC Outbreak 2011

Early May	Several persons in Hamburg contract bloody diarrhoea/EHEC and HUS.
21 May	RKI notifies BfR and BVL of rise in the number of HUS and EHEC cases reported to the RKI by the authorities in the federal states of Hamburg and Schleswig-Holstein.
22 May	The wave of disease reaches its peak in terms of the beginning of diarrhoea symptoms, with 161 new EHEC infection cases and 63 new HUS cases on one day.
24 May	RKI receives the first reports of deaths in connection with the infection. Results of epidemiological analytical studies (questioning of EHEC patients by RKI and Hamburg authorities) indicate plant-based foods (tomatoes, cucumbers and green salads) as the source of the diseases.
25 May	BfR and RKI issue a joint statement advising against the consumption of raw tomatoes, cucumbers and green salads in northern Germany (BfR Statement No. 014/2011).
26 May	The Hamburg Hygiene Institute finds EHEC pathogens in Spanish cucumber. Hamburg informs the general public of evidence of EHEC pathogens on cucumber from Spain and reports to EU Com and all member states via the Rapid Alert System for Food and Feed (RASFF).
30 May	BfR, National Reference Laboratory, detects during lab diagnosis of the findings from Hamburg that the EHEC pathogens are different from those in the infected patients.
31 May	BfR and ANSES develop a rapid test to identify EHEC contamination in foods.
05 June	Lower Saxony advises against the consumption of sprouts. Basis is the evaluation of goods flows which can be traced back from infected patients to a sprout supplier in Lower Saxony. BfR declares the next day that it will help Lower Saxony to investigate the indications.
10 June	Bacteria of the type O104:H4 are discovered on sprouts from Bienenbüttel. BfR, BVL and RKI advise against the consumption of raw sprouts and revoke the previous recommendation regarding cucumbers, tomatoes and salads (BfR Press Release 16/2011).
12 June	BfR advises against consumption of home-grown and raw sprouts.
24 June	France reports an increase in EHEC infections after the consumption of sprouts (Bordeaux) via RASFF.
26 June	EU COM commissions EFSA with the investigation with the involvement of BfR and BVL. Traceability results from Germany and France are brought together.
29 June	EFSA and ECDC publish a risk assessment on the outbreak in France. Common source of the outbreaks in Germany and France appears to be fenugreek seeds imported from Egypt.
30 June	BfR publishes preliminary risk assessment on the significance of fenugreek seeds for sprout production in connection with the EHEC outbreak in Germany (BfR Statement No. 023/11). Based on BfR statements, the federal state responsible for monitoring the German importer orders the return of several batches of fenugreek seeds from Egypt.
01 and 05 July	WHO, followed by EFSA and ECDC, advises European consumers not to consume raw sprouts.
05 July	BfR publishes a comprehensive risk assessment on the relevance of EHEC O104:H4 in sprouts, germ buds and seeds for sprout production in the outbreak of May/June 2011. BfR confirms that Egypt is the probable source of the EHEC pathogen.

Continuation of Tab. 1: Chronology of the EHEC Outbreak 2011

06 July	EU COM obliges member states to take traceability measures and imposes a ban on the import of certain seeds and beans from Egypt until 31 October 2011.
11 July	BfR publishes a scientific risk assessment on the relevance of EHEC O104:H4 in fenugreek seeds which are processed into other foods than sprouts and germ buds (BfR Statement No. 025/2011). BfR initiates a BVL survey of the results of the measures taken in the meantime by the federal states in order to estimate such aspects as the residual risk potential of cross-contamination.
21 July	BfR, BVL and RKI limit previous consumption recommendations to raw fenugreek seeds from Egypt and the sprouts produced from them (BfR Press Release 023/2011). The more extensive consumption recommendations of other authorities, such as EFSA, ECDC, ANSES, remain in place initially.
26 July	RKI announces that no new cases of illness have been reported by the federal states for three weeks. RKI declares the end of the EHEC outbreak in Germany.

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4 Methodical Procedure for Backward and Forward Traceability

4.1 Introduction

Despite extensive tests, it was not possible to produce microbiological evidence of *E. coli* (EHEC) of serotype O104:H4 on suspect foods (BfR Statement No. 023/2011 of 05 July 2011). On the other hand, there were various reasons, including the statistically evaluated results of patient surveys conducted by the Robert Koch Institute (RKI), to suspect freshly consumed salad ingredients (cucumber, tomato, lettuce, sprouts) as a possible vehicle of infection (Frank et al., 2011a,b; RKI, 2011; Task Force EHEC, 2011). This in turn resulted in the necessity for systematic tracing of the suspect foods all along the product chains, starting from selected clusters of reported cases, in order to identify the possible source of the outbreak, thereby enabling the investigation of the outbreak in the absence of microbiological confirmation. Within the course of data collection, it was ascertained that the initially available methods of electronic data recording of supply relationships would have to be expanded and adapted for the purpose of investigating the epidemiological outbreak. In addition to this, a visualisation of the information by conventional means (flipcharts and pinboards) was no longer possible due to the complexity of the supply network (number of knots and connections) and large number of outbreak clusters and suspect foods. For this reason, a modified data recording tool and new evaluation concept capable of displaying and analysing large data quantities were developed using network analysis techniques. This visualisation method turned out to be an efficient means of communication for the experts at the institutions involved in the investigation of the outbreak and also for public relations work. It was then possible to build up additional analyses, in particular various risk assessments and more extensive epidemiological examinations, on the basis of the evaluation concept. The developed solution also provides interfaces in the form of exportable Excel tables, for example, which can be used for more detailed analysis using other software products and applications.

Together with a restaurant cohort study conducted by the RKI, the result of the use of this technique led to the conclusion that sprouts from a horticultural farm in Lower Saxony were with a high degree of probability the causative vehicle of infection for the observed increase in cases of illness in Germany (Buchholz et al., 2011; RKI, 2011; Task Force EHEC, 2011). An expansion of this approach on a European level in the EFSA Task Force was equally successful. When it was reported on 24 June that a group of patients in France had also contracted bloody diarrhoea (EFSA, 2011) caused by the same EHEC-O104:H4 strain as in Germany, it was possible to make an epidemiological connection between the two outbreaks using the newly developed computer tool and database. In this way, it was possible to identify fenugreek seeds from Egypt as the likely source of the outbreaks in Germany and France (EFSA, 2011).

The data recording and evaluation methodology used in the investigation of the outbreak is presented below.

4.2 Methodology

Irrespective of specific outbreak events, traceability in general is structured as follows: starting off on the health side, the outbreak clusters are identified which are suitable from an infection epidemiological point of view for patient surveys and other examinations to explain the outbreak. Beginning with one or more outbreak clusters and working backwards via the various intermediate states – such as suppliers – of the supply chain, an attempt is made to identify a possible common source of the outbreak clusters (Fig. 3). When a source is suspected, the distribution from there can be followed forward along the supply chain to establish whether the connection to the outbreak clusters can still be confirmed after detailed information has been acquired (e.g. production batches). Forward tracing is also used to

identify additional, as yet unknown, outbreak clusters or critical points (e.g. intermediate dealers with widespread trade networks, retailers, large restaurants or canteens). Complete determination of delivered quantities also makes it possible to show the whereabouts of contaminated foods in the supply chain. In addition to this, the method supports the development of hypotheses regarding an introduction location and/or route, thus disclosing the cause of the contamination (see Fig. 3). Backward and forward tracing mutually complement each other.

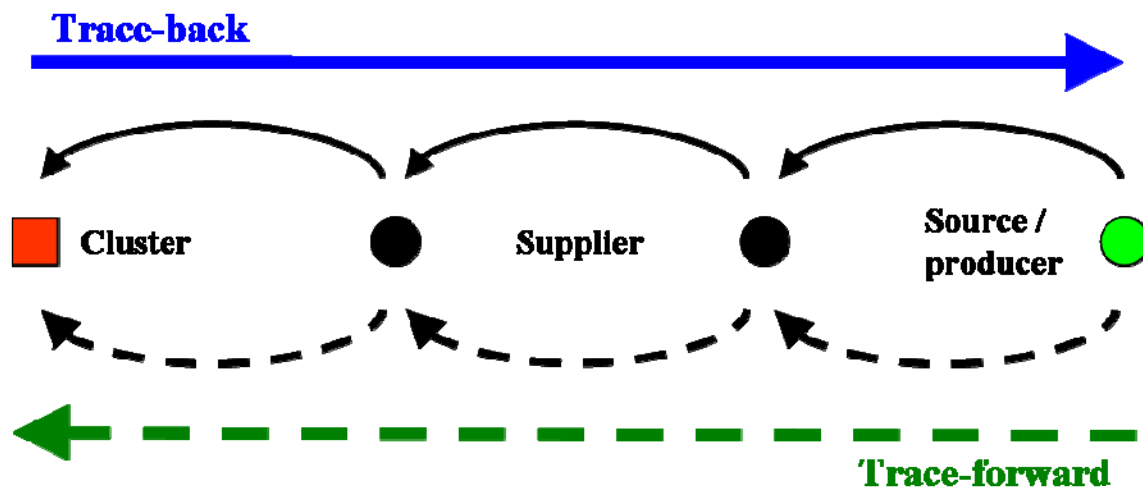


Fig. 3: Schematic presentation of tracing strategies

(1) Identification of “infection clusters” (RKI), (2) Backward Tracing: cluster → common producer/source (blue), (3) Forward Tracing: producer/source → other clusters (green)

In the outbreak in question, the food supply chains were analysed backward (upstream), beginning with selected outbreak clusters, and then forward (downstream), i.e. starting from a horticultural farm in Lower Saxony which had become the focus of attention. During the investigation of the outbreak, a data structure was defined for backward and forward tracing and a practicable procedure was developed for data collection.

4.2.1 Foods List

As the evaluation of all foods consumed in selected outbreak clusters would have led to a quantity of data which could not have been handled in a short period of time, a pre-selection of the foods to be tested was made on the basis of case control studies (RKI, 2011), experiences with EHEC outbreaks and interviews with restaurant chefs. At the end, the list comprised 91 foods (see Appendix, Tab. 2), including all sprout varieties, fresh herbs, green salad and fruit vegetables, such as cucumbers and tomatoes, which could have been used as ingredients for “salad platters” or “garnishings” and about which questions were asked during data collection.

4.2.2 Legislation and Information Sources for Tracing

The prerequisite for successful tracing is the availability of the corresponding supply data to enable evaluation, and the existence of an appropriately structured database.

EU legislation (Regulation [EC] No. 178/2002, Article 18) requires that food companies collect and file information on all of their suppliers (one step back) and all of their customers (one step forward). The implementation of this regulation is covered by the Food and Feed Code (LFGB) in Germany (Federal Law Gazette BGBl, 2005).

As with foods of animal origin, the traceability of vegetables, fruit and plant seeds is covered by Regulation (EC) No. 178/2002. Unlike the centralised livestock databases (“Central Database on Animal Identification and Registration” – HI-TIER: www.hi-tier.de), however, no such detailed centralised database exists (or is mandatory) for the origin and transport of further processed foods or for fruit and vegetables. Within the scope of QS schemes, data is collected by trade and industry on a voluntary basis for the purpose of traceability. Data of this kind were transmitted to the responsible authorities for use during the EHEC outbreak in 2011. Some of the businesses involved in the outbreak did not participate in data collection schemes, however, with the result that the EHEC Task Force had to resort to the collection and evaluation of bills of delivery and invoices with varying depths of information. The required information was obtained by the local authorities directly from the businesses (e.g. producers, suppliers, restaurants, hotels, supermarkets) usually in the form of bills of delivery. In certain individual instances, well-organised company-internal databases were available.

In addition to this, it was necessary to identify the relevant information for the outbreak from a scientific point of view. In the following step, a database had to be developed which was tailor-made for the specific information requirements and which enabled quality assured data entry and data analysis in a crisis situation. A general problem was that the obtained information came from sources of very different quality. In addition to this, it was only possible to have the data entered by personnel with ad hoc training due to the complexity of the supply chain data.

Batch-specific tracing poses several additional problems. On the one hand, batch numbers, product designations and article numbers change along the supply chain while on the other hand, the composition of products can change as it is possible that the product is processed or mixed with other products. This means that in every examined business, the correct allocation of the batch to the product and the batches to their respective ingredients is a demanding investigative task which requires close technical coordination of the data entry and harmonisation of the occurring detailed questions.

4.2.3 Tools for Structured Data Recording and Analysis

Until the EHEC outbreak in 2011, there were no tools for the structured recording of the necessary information. Prior to the establishment of the EHEC Task Force, an Excel table was in use at the Federal Office of Consumer Protection and Food Safety (BVL) in which the information on supply chains reported by the federal states was collected and administered. This table proved to be unsuitable for the batch-specific tracing of foods, however, whereupon a new data format and new data analysis tools had to be developed in a short space of time. These tools were developed by BfR and EFSA staff members within the scope of the EHEC Task Force and constantly expanded and improved.

4.2.3.1 Development of a Modified Excel Table for Structured Data Recording

To enable practicable backward traceability, it was necessary to develop a data structure and software which satisfies several requirements:

1. It must be easy to operate after a short instruction period.
2. In order to guarantee rapid data transfer, it must be possible to send the data via e-mail.
3. It must be possible to import the data into a centralised relational database to serve as a basis for the analyses.

The Excel format was retained for data recording because the program is available at all of the authorities involved with the collection of data on site and can be used by the personnel employed there. The newly developed Excel table is divided into five different blocks (see diagram in Fig. 4). Each line of the Excel table comprised the information on the delivery of a product from the pre-supplier (C) through the supplier (B) to the customer (A) (in compliance with Regulation [EC] No. 178/2002: one step back and/or one step forward from Focus Business B). The advantage of the newly developed structure was that all of the data required for backward as well as forward tracing could be collected with one single inquiry to a specific business. In this way, batch-specific tracing was guaranteed overall and the completed Excel tables could be imported into a special relational database. Plausibility testing was also possible by questioning all of the businesses in a supply chain (outgoing goods at Business B are incoming goods at Business A etc).

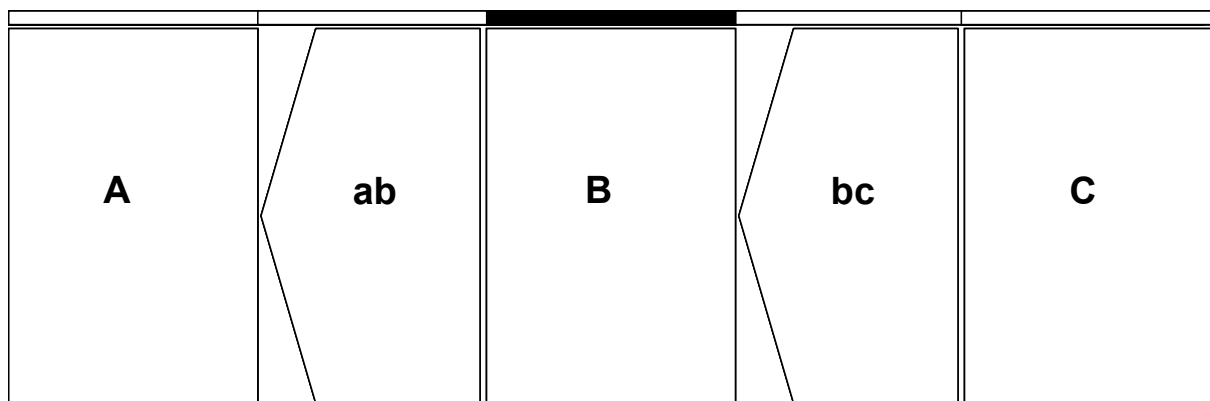


Fig. 4: Diagram of the Excel table for data recording

Block "B" is the business in focus. Block "C" stands for the supplier to "B" while Block "A" is the customer of Business B. The "ab" block contains information on the product delivered from Business B to A, while the "bc" block lists its ingredients as supplied by Business C. The arrows show the direction of the goods transport.

The available data can be entered into the Excel table by the authority responsible for tracing. An inquiry is then sent to the local food safety and health authorities along with the Excel table for completion of the missing data. The completed Excel table with all of the relevant data for backward and forward tracing is then ready for import into the relational database.

4.2.3.2 Relational Database for Structured Data Analysis

The use of a relational database – several tables linked via relationships – offers the following advantages: (1) transfer into a standardised and clearly structured data format, (2) consistency and plausibility testing during data import to correct error-prone data recorded per Excel table, (3) determination of supply chains in sections (one step forward and one step back for every business) and subsequent compilation of the entire supply network, (4) structuring and analysis of the entire supply network via data querying and (5) export of the data query in a standardised format for more extensive analysis in other software environments.

A software environment in HSQLDB (Hyper Structured Query Language Database) was used for the relational database which offers all of these options and which is established at the BfR. It is still the case that the number and sequence of the Excel tables are of no consequence for import into the database. Overall, the database consists of five relationally linked tables based on the Excel structure for data recording (see Fig. 5).

The current database structure is in principle suitable for products of all types (food, toys etc). It can be modified quickly in regard to the data fields, however, if for instance highly

processed products, such as convenience foods, are involved in an outbreak and additional information (degree and method of processing etc) is necessary according to food, food safety and nutrition experts.

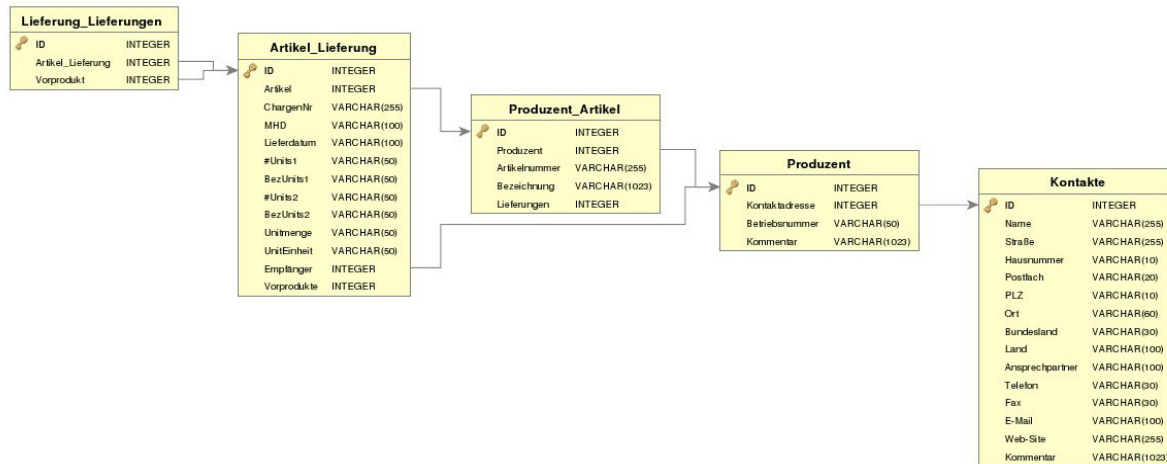


Fig. 5: Database Structure: The database consists of five tables connected relationally with one another

4.2.3.3 Analysis and Visualisation of Data

As explained at the beginning, initially a central task was the visualisation of the supply network. As a visualisation of this kind can become confusing very quickly when depicting several hundred supply relationships, the appropriate database filters were used. Product name and product number filters were of interest here. It is possible to (1) evaluate all supply relationships with a direct or indirect relationship to the “filter” in question, (2) define as many filters as desired and (3) link these with the logical attributes “AND” or “OR” to enable a visualisation for different questions.

This simplifies the primary task of tracing, i.e. the identification of common interfaces, because the various points in the network (e.g. the outbreak clusters) and desired filter (e.g. batch number) are defined to begin with. The database then produces a list of common points within the network and their connections to one another. All filtered data is exported in a standardised data format for further analysis and visual display with other software.

The visualisation of the networks (BfR Statement 023 of 05 July 2011) was made in two ways: with the R-package “network” (Butts, 2008, <https://statnet.org>, see Fig. 6) and graphviz visualisation software (www.graphviz.org, Weiser et al. [in prep.]). A further visualisation option, geographic projection on a local district level, was realised in Google Earth (www.google.de/intl/de/earth, Weiser et al. [in prep.]). This was achieved by generating a file in an appropriate format which can be interpreted by Google Earth.

Finally, the intended purpose of the supplied products was also analysed especially for the EHEC outbreak 2011 (BfR Statement 049 of 23 November 2011). To this end, however, it was necessary to research additional information after the event (internet presentation of consumer-oriented companies: “Which products are being sold to ultimate consumers?”) which were not recorded in advance.

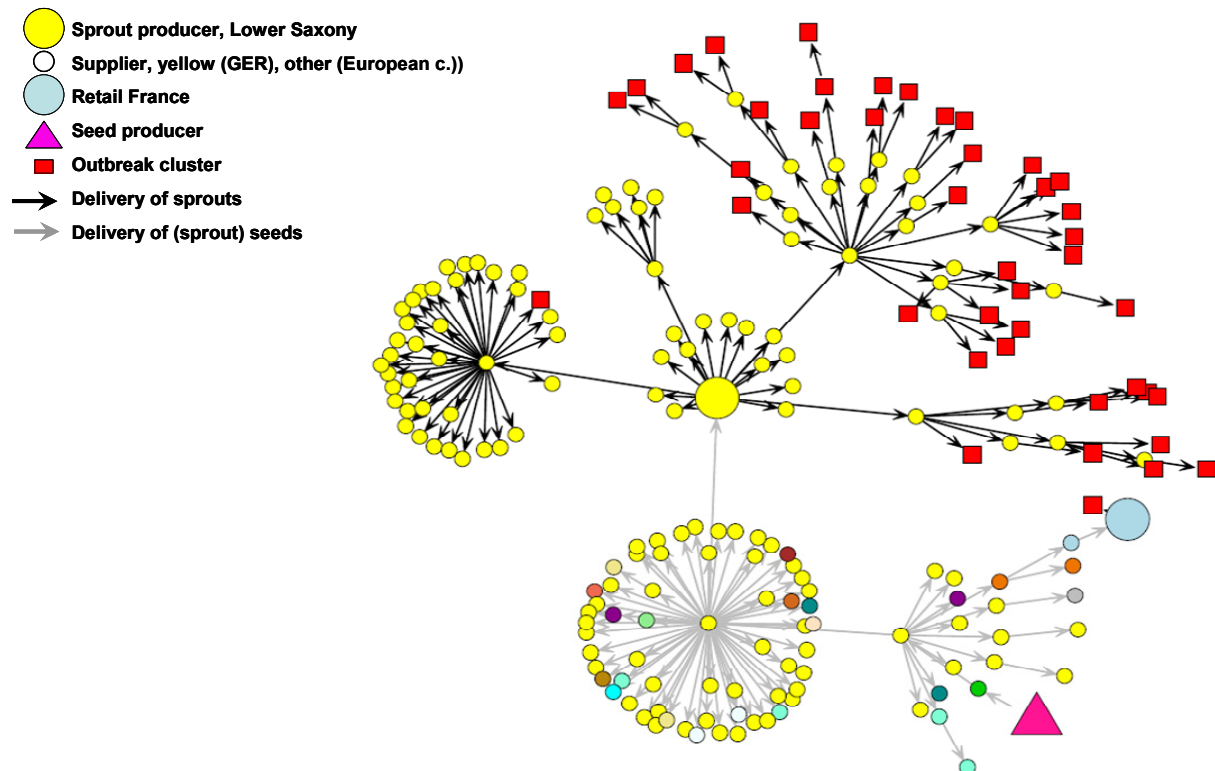


Fig. 6: Combined network presentation of all relevant fenugreek seed and sprout deliveries. Supplier network at EHEC-O104:H4 outbreak 2011, combined supply chain downstream and upstream: all businesses which came in contact with the suspect batch via seeds or produced sprouts are shown here. (Prepared with the R-package “network”: Butts, 2008, <https://statnet.org>)

4.3 Conclusion

The tracing analysis outlined here is a method of epidemiological outbreak investigation. With large, multifocal outbreaks, this procedure is based on the identification of outbreak clusters as the starting points for tracing. As a result, critical junction points of the distribution network can be recognised for in-plant inspections or microbiological sampling. Clarification of the EHEC outbreak in 2011 through microbiological evidence of the causative pathogen along the product supply chain was not possible. For this reason, the outbreak investigation was based solely on epidemiological evidence. In this way, the epidemiological methods for identifying the vehicle in food (especially through the recipe-based restaurant cohort study; RKI, 2011) proved their value in combination with the analysis of tracing data.

In retrospect, the complete collection and structured recording of all necessary detailed data relating to supply relationships can be singled out as the most difficult task of tracing. The concept described here – Excel table/relational database/network analysis – has proven during the EHEC outbreak in 2011 to work quickly and effectively, especially considering that it was developed and implemented in very short time. The data suppliers were able to provide the required information in a timely manner because Excel is widely available and convenient to use. A new relational database structure which supports plausibility checks, data correction and integration with analytical software was developed to compensate for the disadvantages of an Excel-based exchange of information. The entered data is checked automatically for correctness and plausibility and analysed immediately.

In summary, the concept of data recording and analysis for the tracing of supply chains described here played a major role in the successful identification of the sources of the EHEC outbreak in 2011. It can be used for all types of food and consumer-oriented products. Efforts should be made in the near future, however, to develop a data management system which

can realise the immediate electronic availability of trading data while simultaneously guaranteeing the confidentiality of data so that crises of this kind can be solved even more quickly and efficiently. In addition to this, the data processing systems should be adapted to each outbreak situation under consideration of scientific aspects.

4.4 References

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4.5 Appendix

Tab. 2: Foods list for batch-specific tracing from outbreak clusters

List of foods to be considered	
ADV No.	Designation
Sprouts/Seedlings	All sprouts
250213	Soya sprout
250214	Wheat germ
250215	Lentil sprout
250216	Mustard sprout
250217	Lucerne sprout
250218	Sunflower sprout
250219	Mung bean sprout
250220	Cress sprout
250221	White radish sprout
	Red radish sprout
	Adzuki bean sprout
	Alfalfa sprout
250227	Grain sprout
250228	Rye sprout
250229	Barley sprout
250230	Maize sprout
ADV No.	Designation
260316	Cress
	Watercress
250119	Cress/Garden cress/Nasturtium
Herbs	All herbs
530101	Ginger
530103	Zedoary
530104	Galangal
530105	Calamus
530106	Lovage root
530200	Spices
530201	Basil
530202	Wormwood
530203	Savory
530204	Borage
530205	Dill
530206	Tarragon
530208	Lovage leaf
530209	Marjoram
	Oregano
530211	Pimpernel
530212	Rosemary
530213	Lemon balm
530214	Sage
530215	Thyme
530216	Hyssop
530217	Grand wormwood
530219	Chervil
530220	Rue

Continuation of Tab. 2: Foods list for batch-specific tracing from outbreak clusters

530221	Blue fenugreek
530222	Parsley
530223	Chives
530224	Leaf celery
530226	Coriander
530227	Lemon grass
250149	Mint
Small leaves	All small leaves
250114	Spinach
260317	Dandelion
250152	Sorrel
250154	Wild garlic
250142	Rocket
250158	Fennel leaves
250132	Nettles
250116	Celery root leaves
250117	Parsley leaves
250121	Orache
250127	Turnip greens
Lettuces	All lettuces
250101	Garden lettuce
250102	Lamb's lettuce
250103	Mixed salad leaves
250104	Romaine
250105	Chicory
250106	Endive
250108	Dandelion
250120	Swiss Chard
250123	Radicchio
250126	Iceberg lettuce
250130	Frisee lettuce
250134	Oak leaf lettuce
250135	Batavia lettuce
250128	Sugar loaf lettuce
250137	Lollo rosso
250138	Lollo bianco
250157	Pak choi
Onion/Leek	
250131	Spring onion
250207	Shallot
250208	Onion
Others	All Others
250115	Ribbed/stalk/root celery
250202	Kohlrabi
250212	Fennel
	White radish
	Red radish
	May turnip

Continuation of Tab. 2: Foods list for batch-specific tracing from outbreak clusters

Fruit Vegetables	
250301	Tomato
250305	Cucumber
250309	Courgette

5 Work performed at the National Reference Laboratory for *E. coli* including Verotoxin-producing *E. coli*

5.1 Laboratory examinations at the time of the EHEC-O104:H4 outbreak

5.1.1 Tasks of the NRL *E. coli*



Fig. 7: Staff of the National Reference Laboratory for *E. coli* at the BfR

The normal range of tasks of the NRL *E. coli* includes the differentiation and typing of Shiga toxin-producing *E. coli* strains which are isolated within the scope of the examination activities of the federal state laboratories. In addition to this, the NRL for Antibiotics Resistance conducts the determination of resistances with commensal *E. coli* within the scope of its annual monitoring programmes. These examinations are conducted on the basis of samples (usually strain isolates) submitted by the examination laboratories of the federal states.

Unlike the labs for official monitoring, the NRLs of the BfR are not geared towards the routine examination of foods with a high sample throughput. In addition to the services they perform for external labs, they are set up to develop and provide detection and sample processing methods for monitoring, to organise and conduct interlaboratory comparison tests and to accompany and support the investigation of special problems in the laboratory.

In the course of the EHEC outbreak, it was quickly recognised that in order to support the examination laboratories of the federal states, tasks had to be taken on by the BfR which extended far beyond the customary range of performances of the NRL. In addition to an increased number of submissions for differentiation, typing and determination of the antibiotics resistance of isolates, the examination of plant and animal-based foods, water and seed samples for Shiga toxin-forming *E. coli* (STEC) was also necessary. The sample receipt quantities are shown graphically in Figure 8.

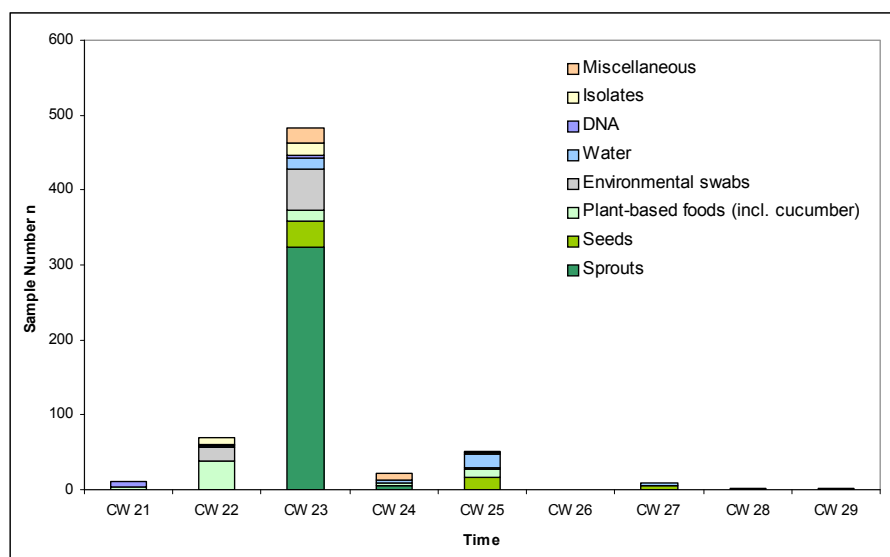


Fig. 8: Sample Receipt Quantities by Calendar Week (21 May to 24 July 2011)

An analysis of 980 sub-samples was conducted for the examination and diagnosis of 652 food, environmental and other individual samples. These multiple approaches were necessary in order to increase detection sensitivity, but they also resulted from several examination methods being applied parallel to one another.

As the submitted samples consisted of a large number of different matrices (see Table 3), it was also necessary to optimise sample protocols.

Tab. 3: Type and Extent of Examined Samples

Sample Type	Sample Qty. n
DNA	14
Plant-based foods (incl. cucumber)	73
Isolates	27
Seeds	58
Sprouts	329
Environmental swabs	77
Water	41
Miscellaneous*	33
	652

*The category "Miscellaneous" includes: animal-based foods, packaging materials fertiliser and animal faeces.

In light of the volatile nature of the circumstances surrounding the EHEC outbreak, it is understandable that lab results were expected as quickly as possible and that there was a certain lack of understanding among all of the institutions involved in the crisis with little or no knowledge of lab-specific matters as to why the number of tests per time unit could not be increased indefinitely. A restriction of the number of samples is not only caused by limited personnel resources, however, it also has to do with the infrastructure and equipment available at the laboratories. The occupancy of equipment (e.g. shakers, incubators), production of growth media, delivery status in the procurement of necessary agents, growth media etc all contributed towards the fact that the number of tests that could be conducted per day had to be limited.

In addition to this, different lengths of time are required for the various detection methods, and repeat and confirmation tests are required with suspect and positive samples. This means that these examination processes take considerably longer than screening processes with negative findings. The following list provides an overview of the amount of time required by the various test methods.

Test Duration:

1. Approx. 48 hours (2 days) are required for the screening of plant-based foods including sprouts for EHEC O104:H4 with a negative result. This includes the following work stages:
 - i. Preparatory work
 - ii. Pre-enrichment of the pathogen in a liquid medium
 - iii. Enrichment of the pathogen in a solid or liquid medium
 - iv. DNA extraction
 - v. Screening-real-time PCR to detect the pathogen (O104wzx gene [specifically for serotype O104] and Shiga toxin-2- [stx2-]gene)
2. In addition to the 48 hours (2 days) outlined above, a further 1-3 days are required for the screening of plant-based foods including sprouts for EHEC O104:H4 with a positive result. This includes the following additional work stage:

- vi. Confirmation-real-time PCR, ELISA to detect the Shiga toxin
 - vii. Microbiological work for the cultivation and isolation of the pathogen
 - viii. Confirmation of the pathogen per PCR and other molecular biological methods
 - ix. Determination of the serotype
3. The times listed above also apply to the screening of seed samples, but the swelling process can be of varying duration. If the seed also has to be allowed to germinate, the examination can be extended by 2-4 days (depending on the seed type).

At the time of the outbreak, work was done on the optimisation of sample processing and detection of *E. coli* O104:H4 parallel to the testing of the submitted samples. The testing of seed samples in particular was a novelty for which no sample processing regulation was available initially, even though it was urgently required by the testing laboratories.

5.1.2 Synopsis of the Results of the Tests during the EHEC-O104:H4 Outbreak

652 food, environmental and other individual samples (980 sub-samples) were processed, examined and diagnosed. 645 of these samples proved to be EHEC-O104:H4-negative. Other Shiga-toxin-2-forming *E. coli* were detected in various isolates, however. Seven samples whose origin is described in Table 4 were identified as EHEC-O104:H4-positive. Confirmation was made by means of real-time PCR (*O104wzx*, *stx2*, *aggR*, *terB*, *fliCH4*) and through serotyping and microbiology.

Tab. 4: Detection of EHEC O104:H4 in Food and Environmental Samples

Sample (Number)	Origin
Cucumber (1)	The household of a person infected with EHEC O104:H4
Smoked salmon (1)	Food contaminated by human excretions (catering personnel) – source of an EHEC-O104:H4 satellite outbreak in Hesse
Salmon, cooked (1)	same as above
Bell pepper (1)	same as above
Sprout mixture (1)	Produced in the incriminated vegetable farm in Lower Saxony, found in the household of persons infected with EHEC-O104:H4
Children's toys (1)	The household of persons infected with EHEC-O104:H4
Toilet seat (1)	same as above

In order to prove the genetic relationship of the positive isolates with the index outbreak strain provided to the NRL by the RKI, as well as other human isolates of patients infected with EHEC-O104:H4, the isolates were separated in accordance with a standardised pulse-net protocol in Pulsed-Field Gel Electrophoresis (PFGE) and their similarity determined. Figure 9 shows that the PFGE patterns of the seven isolates isolated from food and environmental samples match up completely with the PFGE patterns of the index strain and the other patient isolates. Two concurrently tested comparative isolates with no relation to the 2011 outbreak showed completely different PFGE patterns.

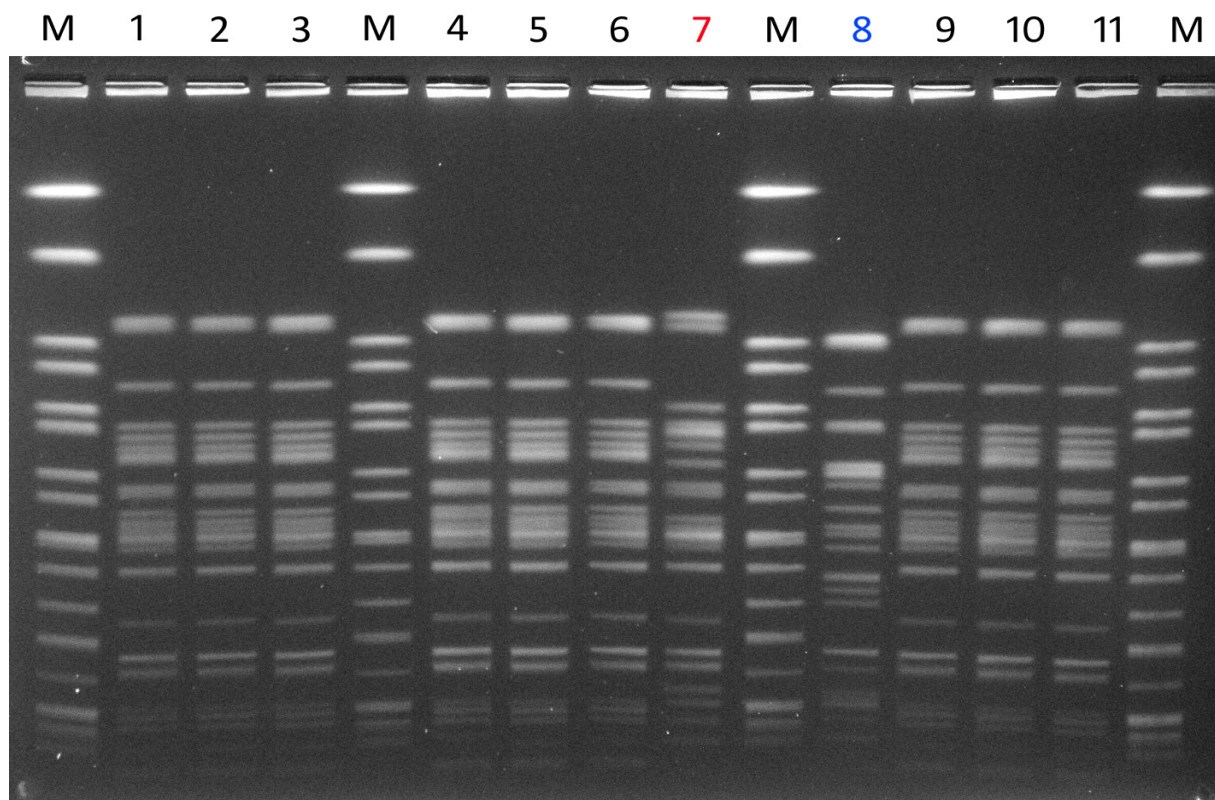


Fig. 9: Genotyping of O104 isolates of human and non-human origin by means of XbaI-PFGE

PFGE pattern of aggregative EHEC-O104:H4 isolates from patient and food samples. All aggregative EHEC O104:H4 isolates from 2011 (humans and food) show the same PFGE pattern (genetically identical).

Track 1 4) Patient isolates: RKI Index Strain, Berlin, Cologne, Magdeburg; 5) Isolate from a contaminated cucumber; 6) Isolate from contaminated sprouts; 9) Bell pepper isolate; 10) and 11) Isolates from salmon samples. The strains separated into Track 7 (aggregative EHEC-O104:H4 strain from a stool sample of a child who took ill in Cologne in 2001) and Track 8 (STEC-O104:H21 strain from a meat sample) differ significantly from the O104:H4 outbreak strain of 2011.

5.1.2.1 Seed Samples

The O104:H4 pathogen could not be detected in any of the seed batches examined.

The following problems had to be considered when examining the seed samples:

- Although the seed batches used are highly suspect on the basis of the epidemiological pre-examinations, because EHEC O104:H4 has never before been isolated there is no unequivocally positive comparative sample. Accordingly, a negative result does not necessarily mean that the EHEC-O104:H4 strain is not present.
- In addition to the contamination of the surface of the seed, internal contamination (i.e. contamination during the growth of the plant intended for seed production) is also possible.
- It has to be assumed that the contaminated seed particles within each batch are not evenly distributed and that they form “nests” which are randomly distributed. The strategy for drawing samples and the sample quantities used in the examination have to be optimised.
- It must be assumed that the pathogen only exists on/in seeds in very small numbers (literature data only available to date for other pathogens) and that it is in a state of dormancy, thus making cultivation more difficult. This is confirmed by a publication by P. Aurass, R. Prager and A. Flieger in *Environmental Microbiology* (2011).

- The problems outlined above were confirmed in a ring trial conducted by the EU Reference Laboratory for *E. coli* (Rome, Italy) to detect STEC/EHEC (not EHEC O104!) in naturally contaminated seeds intended for sprout production in which the NRL *E. coli* of the BfR also participated. None of the eight participating laboratories (including the EU Reference Laboratory itself) was able to verify the results achieved by the EU Reference Laboratory during pretesting.

Further research and development work is required in this area.

5.1.2.2 Water Samples

During the examination of water samples, the problem occurred that with several samples, the EHEC O104:H4 pathogen could not be isolated from the sample despite positive real-time PCR so that the samples were evaluated as negative. The question as to whether the pathogen was present in these samples and could not be isolated has still not been clarified. The NRL *E. coli* is currently working on combinations of microbiological and molecular genetic detection systems which should help to improve the detection of these pathogens in complex microbial background.

The examination of water samples also requires further research and development work.

5.2 Development of Real-time PCR Methods for the specific detection of EHEC including *E. coli* of the serogroup O104:H4

In cooperation with ANSES (Dr. Patrick Fach, Laboratory for Study and Research on Food Quality and Processes [LERQAP]), a micro-array was developed on the basis of GeneDisc® array technology prior to evaluation at the NRL *E. coli* (Bugarel et al., 2010). This micro-array serves for genetic identification of 12 O-types and 7 H-types of Shiga toxin-producing *E. coli* (STEC), including most of the clinically relevant enterohaemorrhagic *E. coli*- (EHEC-) serotypes as well as the enteroaggregative haemorrhagic *E. coli* O104:H4 which occurred recently.

The following genes were selected for the determination of the O-antigens: *rfbEO157*, *wzxO26*, *wzxO103*, *wbd1O111*, *ihp1O145*, *wzxO121*, *wzyO113*, *wzyO91*, *wzxO104*, *wzyO118*, *wzxO45* and *wbgNO55*); and also for the following H-types: *fliCH2*, *fliCH7*, *fliCH8*, *fliCH11*, *fliCH19*, *fliCH21* and *fliCH28*. All PCR systems showed a high specificity and concordance with the serological determination of the O:H-antigens.

The micro-array also showed a high specificity for EHEC-associated virulence factors, including Shiga toxins 1 and 2 (*stx1* and *stx2*), intimin (*eae*), enterohaemolysin (*ehxA*), serine proteases (*espP*), catalase peroxidases (*katP*), Type II secretion system (*etpD*), subtilase toxin (*SubA*), adhesin (*saa*) and Type III effectors in the genomic pathogenicity islands OI-122 (*ent/espL2*, *nleB* and *nleE*) and OI-coded 71 (*nleF*, *nleH1-2* and *nleA*). This array constitutes a valuable approach for the identification of STEC strains with a high potential for human virulence.

The GeneDisc cyler and developed GeneDiscs for the determination of O104-relevant gene sections such as *O104wzx*, *fliCH4*, *aggR*, *Stx2* and *terB* were made available for the duration of the outbreak examinations by the company Pall-GeneSystems. In this way, it was possible to conduct 36 tests for these five characteristics from examination material within 80 minutes, thus making the processing of the large quantity of samples that accrued during the outbreak considerably easier.

5.3 Development of a fast, reliable method for the recognition and isolation of enterohaemorrhagic *E. coli* of serogroups O26, O104, O111, O118, O121, O145, O157 and enteroaggregative haemorrhagic *E. coli* O104:H4 from ready-to-eat salads and sprouts

5.3.1 Problem

Infections in humans through Shiga toxin-forming (STEC) and enterohaemorrhagic *E. coli* (EHEC) are a global problem. In addition to foods of animal origin, plant-based foods also play a major role. In Germany, plant-based foods are only rarely examined and no data on this is currently available. Official methods also exist for the identification and characterisation of STEC/EHEC in accordance with Art. 64 LFGB. When eaten raw, plant-based foods pose a risk for an STEC/EHEC infection. Outbreaks of STEC/EHEC attributable to contaminated plant-based foods have already been reported from various countries.

Within the scope of a research project, 120 mixed lettuce/sprout samples from the retail trade in Berlin were examined for their microbiological pollution and contamination with pathogenic *E. coli* in the period from 2009 to November 2011. All of the products showed high microbial contamination (aerobic mesophile bacterial count 10^6 to 10^7 per gram). A large percentage of them had enterobacteriaceae (10^5 to $>10^6$ per gram). The guideline value of the German Society for Hygiene and Microbiology (DGHM) for *E. coli* (1×10^2 colony-forming units [cfu]/g) is seldom exceeded. After enrichment, however, *E. coli* was found in more than 50 % of the examined samples. This shows that this bacterium occurs frequently in lettuce samples, but in relatively small quantities. Cultures cultivated from the 120 lettuce samples showed positive findings with the real-time PCR for STEC of 1.6 %. Due to the high level of contamination with other *Enterobacteriaceae*, however, the isolation of pathogenic *E. coli* from lettuce samples proved to be very difficult. After enrichment for 24 hours, other *Enterobacteriaceae* are available in quantities 1,000 times greater than those for *E. coli*.

For this reason, methods were developed in 2010/2011 which permit a specific enrichment and isolation of pathogenic *E. coli* from plant-based foods intended for raw consumption. These methods were evaluated and published (Tzschope et al., 2011). A method for the detection and isolation of STEC/EHEC based on the methods described for plant-based foods in the Official Collection of Examination Methods in accordance with Art. 64 LFGB is in preparation.

5.3.2 Material and Methods

5.3.2.1 Real-Time PCR Method for the Detection of STEC and EHEC

MGB (minor groove binder) probes developed at the NRL *E. coli* for real-time PCR to detect the EHEC-typical virulence characteristics *stx1*, *stx2*, *eae* and *ehxA* were used. These had proven to be highly specific and sensitive in lab-internal evaluation. These MGB detectors were compared with the TaqMan detectors *stx1*, *stx2* and *eae* recommended for the CEN/ISO (European Committee for Standardisation) method.

5.3.2.2 Block Cycler PCR Method

Representative samples were compared in order to compare the sensitivity and specificity of real-time PCR methods and classical block cycler PCR (*Stx1* and *Stx2* detection).

5.3.2.3 Enrichment of EHEC from Lettuce Samples

The methods (L 00.00.92 and L 07.18.1) for STEC/EHEC detection from minced meat and milk (24 hour enrichment) published in the collection of official examination methods in accordance with Art. 64 LFGB (previously Art. 35 LMBG) proved to be unsuitable for detection from plant-based matrices during sampling within the scope of the research project. For this reason, alternative methods with a 6-hour enrichment were developed and evaluated.

5.3.2.4 Spiking Tests for the Sampling of Enrichment Methods, the PCR and the Isolation Methods

Ready-to-eat, pre-cut mixed salad was purchased in the retail sector and tested for its microbiological properties (limit and warning values in accordance with DGHM guidelines). Ready-to-eat salads were enhanced with defined quantities (1–10, 10–100, 100–1000 CFU) of EHEC O26, O103, O104:H4, O111, O118, O121, O145 and O157.

5.3.2.5 Isolation of EHEC from Lettuce Samples

Chromogenic indicator media which show typical signs of *E. coli* (TBX Agar, Chromagar *E. coli*) and/or EHEC (STEC Agar, *E. coli* O157 Agar and Chromagar O26/O157) were tested for their suitability and compared with one another.

5.3.3 Results

Re 3.2.2.1: Trial of various enrichment media and protocols (temperature, duration) for the optimum enrichment of *E. coli*. Implementation with spiked samples and natural *E. coli*-contaminated samples.

To develop an optimised enrichment and isolation process, defined quantities of EHEC were added to conventional lettuce samples before being homogenised in various enrichment media at various temperatures for different periods of time. Thereafter, dilution series of the enrichment culture were plated onto chromogenic media (see Methods) which were then incubated overnight at 37 °C and 44 °C. The plates were evaluated visually the next day (determination of the *E. coli* and STEC titres on chromogenic media) and DNA preparations were produced from elutriates of the plates. The DNA preparations were used as sample (target) DNA for the real-time PCR detection of the EHEC virulence characteristics *stx1*, *stx2*, *eae* and *ehxA*. Parallel to this, DNA from suspect EHEC colonies inoculated from the chromogenic media were also prepared and examined in the real-time PCR.

Real-time PCR detectors designed specifically for the O-antigen-coded genes (from the CEN/ISO method and own detector developments) were used to recognise the EHEC serotypes. The real-time PCR detectors (MGB probes) developed at the NRL *E. coli* were compared with detector systems which will be components of future CEN/ISO standards. Both detector systems proved to be of almost equal quality here, although the MGB probes showed slightly increased sensitivity (1 Ct value = doubling of sensitivity – Tab. 5).

In a comparison of real-time PCR and block cycler PCR, the real-time PCR proved to be more sensitive in the detection of slightly contaminated lettuce samples (1–10 CFU/10 g). Enrichment of lettuce samples in brilliant green bile lactose broth (BRILA) for 6 hrs at 37 °C has proven to be ideal for the detection of *E. coli* and EHEC in the PCR.

Tab. 5a: Comparison of Real-Time PCR Detectors for the Determination of EHEC from Lettuce Samples with EHEC-O111 Strains

Sample	Addition of CFU/10 g Lettuce EHEC O111	Stx1 CEN/ISO	Stx1 MGB/BfR	Stx2 CEN/ISO	Stx2 MGB/BfR	eae CEN/ISO	eae MGB/BfR
		Ct Values	Ct Values	Ct Values	Ct Values	Ct Values	Ct Values
2 0 37	0	0	0	0	0	0	0
2 0 44	0	0	0	0	0	0	0
2 7 37	100–1.000	17.62	17.55	16.70	17.55	17.40	16.66
2 7 44	100–1.000	20.23	19.26	19.51	18.37	19.77	19.26
2 8 37	10–100	22.98	22.76	22.07	21.20	22.09	21.63
2 8 44	10–100	22.82	22.55	22.23	20.97	22.46	21.75
2 9 37	1–10	23.91	23.84	22.92	22.20	23.22	22.48
2 9 44	1–10	23.76	23.29	23.01	21.73	23.36	22.67

Tab. 5b: Comparison of Real-Time PCR Detectors for the Determination of EHEC from Lettuce Samples with EHEC-O157 Strains

Sample	Addition of CFU/10g Lettuce EHEC O157	Stx1 CEN/ISO	Stx1 MGB/BfR	Stx2 CEN/ISO	Stx2 MGB/BfR	eae CEN/ISO	eae MGB/BfR
		Ct Values	Ct Values	Ct Values	Ct Values	Ct Values	Ct Values
4 0 37	0	0	0	0	0	0	0
4 0 44	0	0	0	0	0	0	0
4 7 37	100–1,000	23.95	23.21	23.21	23.09	23.56	23.69
4 7 44	100–1,000	17.73	16.81	16.47	16.73	17.50	17.62
4 8 37	10–100	27.08	26.43	26.08	26.43	27.01	27.01
4 8 44	10–100	22.58	21.79	22.68	21.69	22.36	22.49
4 9 37	<10	32.35	31.29	31.23	31.18	31.64	32.22
4 9 44	<10	28.96	28.20	28.30	28.04	28.27	28.09

Re 3.2.2.2: Sampling of various indicator media and incubation conditions for the optimum detection of (pathogenic) *E. coli* from lettuce samples

Two different indicator media were used for the generic detection of *E. coli* (*E. coli* Chromagar and TBX Agar) and three for the specific detection of STEC/EHEC (Chromagar STEC, Chromagar O157 and Chromagar O26/O157). The chromogenic media were tested for their specificity on 203 *E. coli* strains (STEC, EHEC and others). STEC Agar proved to be well suited here for detecting EHEC of the most important serogroups (O26, O111, O118, O145 and O157). On the other hand, however, not all EHEC-O103 and O121 strains could be cultivated on this medium. For this reason, combinations of STEC Agar and *E. coli* Chromagar/TBX Agar were used to enable the reliable isolation of STEC/EHEC on at least one of these media.

Dilutions from the enrichment cultures of the lettuce samples were plated on to the various indicator matrices and the plates incubated overnight at 37 °C and 44 °C. EHEC of the most important human pathogenetic groups (O26, O103, O111, O118, O121, O145 and O157) was clearly identified on one or more of these media. It was possible to severely reduce the natural background flora by plating the enrichment cultures on STEC Agar. The detection of EHEC through real-time PCR succeeded using DNA produced from elutriates of the covered indicator media. Incubation of the chromogenic media at 44 °C usually proved more advantageous than incubation at 37 °C mainly because there was a greater reduction of the disruptive natural background flora at 44 °C.

Re 3.2.2.3: Preparation of an optimum enrichment and isolation strategy from the results of Items 3.2.3.1 and 3.2.3.2

It was possible to prepare an optimum enrichment and isolation strategy for EHEC of the most important human pathogenetic groups from the results achieved in 2010. The procedure outlined in Items 3.2.3.1 and 3.2.3.2 permits detection and identification of EHEC from lettuce samples within 24 hours from the beginning of the enrichment of the lettuce samples. Table 6 shows the culture properties of the outbreak strain EHEC O104:H4.

Tab. 6: Culture Properties of the EHEC O104:H4 Outbreak Strain

Medium	Indication	Property
Enterohaemolysin Agar	Haemolysis	negative
Endo Agar	Lactose reduction	positive
Sorbitol-MacConkey Agar	Sorbitol fermentation	positive
Fluorocult Agar	Beta-glucuronidase	positive
CT-SMAC Agar	Tellurite resistance, sorbitol fermentation	positive/positive
CHROMagar STEC	STEC indicator agar	positive

5.3.4 Summary and Discussion

The method for detecting and isolating STEC/EHEC developed by the NRL *E. coli* at the BfR enables the routine testing of samples from fresh plant-based foods for STEC und EHEC. The methods previously described for the detection of STEC/EHEC in accordance with Art. 64 LFGB were conceived for minced meat and milk. Due to the higher microbial background level of plant-based foods and resultant difficulties with enrichment, they are not suitable for the detection of STEC/EHEC from plant-based foods.

The detection of STEC/EHEC through real-time PCR proved to be more specific and more sensitive than detection through conventional PCR. The real-time PCR detectors recommended in the CEN/ISO standard (*Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Shiga toxin-producing Escherichia coli [STEC] belonging to O157, O111, O26, O103 and O145 serogroups – Qualitative Method*) can be used for the detection of STEC/EHEC in line with the protocol developed here. In this way, the possibility exists of adapting the protocol to European standards. Although the MGB real-time PCR probes developed at the NRL *E. coli* showed a slightly higher sensitivity (Tab. 5), they are only available from one manufacturer for patent law reasons and are also more expensive than the conventional TaqMan probes described in the CEN/ISO method.

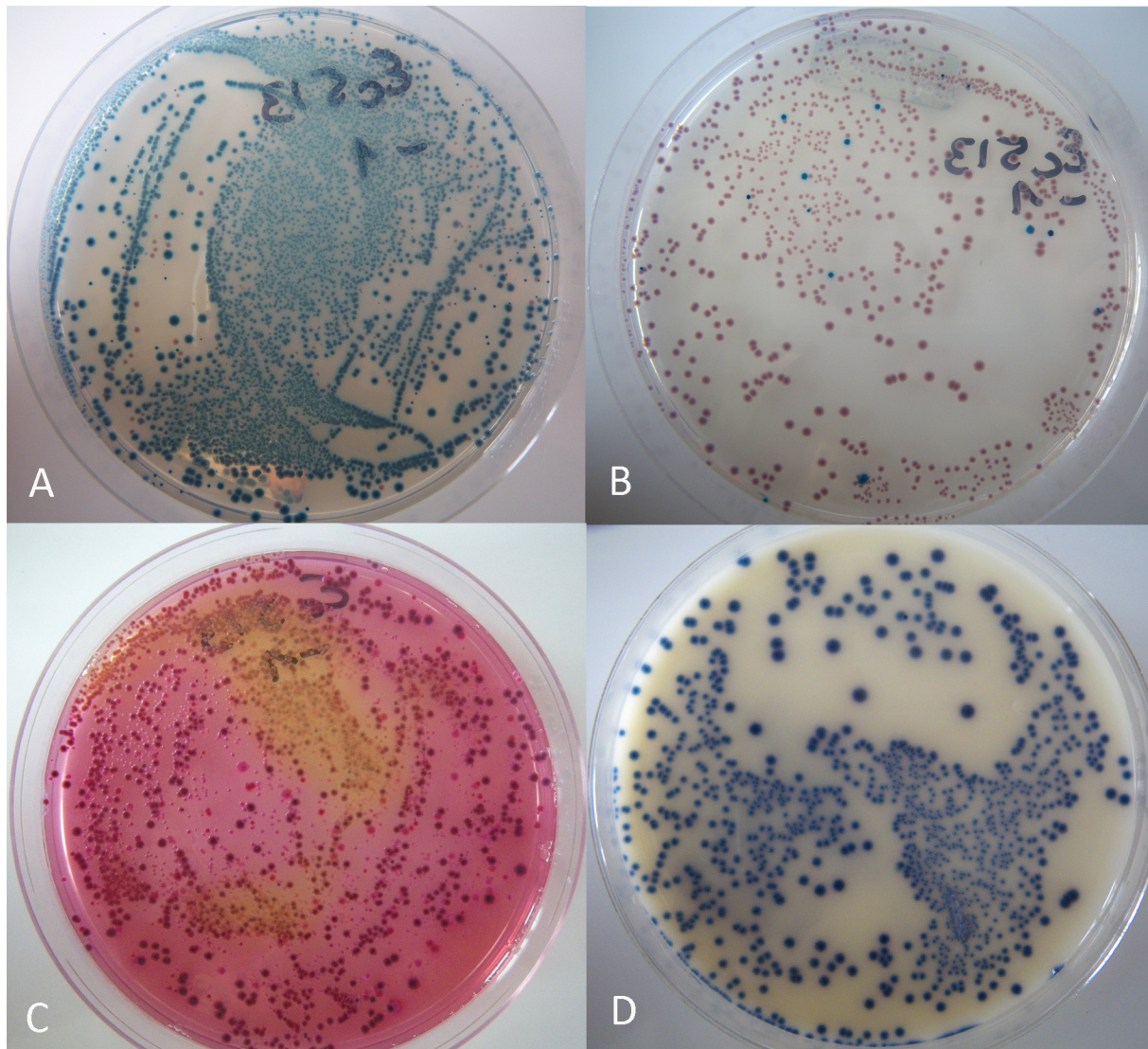


Fig. 10: Detection of the aggregative EHEC O104:H4 strain on chromogenic media from a sprout sample originating from the incriminated horticultural farm in Lower Saxony and taken from the household of persons infected with EHEC O104:H4

CHROMagar: STEC (EHEC O104:H4 = violet colonies, other sprout bacteria = blue colonies)

CHROMagar O104: specific enrichment of the EHEC O104:H4 outbreak strain (violet colonies) by adding cephalosporins in CHROMagar STEC

CT-SMAC Agar: Agar added X-Gluc. Sorbitol-fermenting, beta-glucuronidase producing EHEC O104:H4 appear purple, bacteria of the remaining sprout flora (other enterobacteriaceae) red.

ESBL Brilliance Agar: Growth of ESBL O104:H4 in deep blue colonies

From: Tzschoppe, Martin & Beutin (2011).

5.4 References

- Bugarel, M., Beutin, L., Martin, A., Gill, A., Fach, P. 2010. Micro-array for the identification of Shiga toxin-producing *Escherichia coli* (STEC) seropathotypes associated with hemorrhagic colitis and hemolytic uremic syndrome in humans. *Int J Food Microbiol* 142 (3), 318–329. Epub 2010 Jul 14
- Tzschoppe, M., Martin, A., Beutin, L. 2011. A rapid procedure for the detection and isolation of enterohaemorrhagic *Escherichia coli* (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. *Int J Food Microbiol* 2011 Oct 21. [Epub ahead of print]

6 Chronology of Risk Assessment



A total of eleven statements were published by the BfR on the EHEC outbreak (see appendix to Chapter 7, Risk Communication). The three following statements, which expand on the causal connection between the consumption of sprouts and the EHEC outbreak, constitute the detailed risk assessments of the BfR at the time indicated based on the guideline for health appraisals.

6.1 Relevance of sprouts and germ buds as well as seeds for sprout production in the current EHEC O104:H4 outbreak event in May and June 2011

Updated Opinion No. 23/2011 of BfR of 5 July 2011

The Federal Institute for Risk Assessment (BfR) has made a risk assessment on the basis of the data available on the relevance of EHEC O104:H4 in sprouts and germ buds as well as sprout seeds in the outbreak event in May and June 2011. BfR has based this assessment, amongst other things on the investigation results of the German EHEC Task Force and the European EHEC Task Force, which was set up by the European Food Safety Authority (EFSA). The clarification of the outbreak along the food chain focuses on laboratory diagnostics to detect EHEC O104:H4 in food and environmental samples as well as the trace back investigation of the supply and trade routes, in order to be able to identify the causal source of the outbreak and to take risk minimization measures.

According to the current findings, BfR assumes that the EHEC O104:H4 outbreak in Germany is attributable to the consumption of contaminated sprouts. The outbreak pathogen was very likely introduced via supplied fenugreek seeds into the sprout production. BfR believes that a causal input via water, humans, animals or pests into the horticultural farm in Lower Saxony is hardly probable, in particular because the outbreak strain was not detected in any of the samples taken despite intensive investigations.

The trace back investigation of seed supplies in Germany and other EU member states by the German authorities and the EFSA Task Force clearly shows that cases of disease which occurred in France in late June caused by EHEC O104:H4 are connected to the horticultural farm in Lower Saxony through the same seed batch produced in 2009. Furthermore, another fenugreek seed batch, produced in 2010, was used by the horticultural farm in Lower Saxony for sprout production in April and May 2011. According to information of EFSA/ECDC (European Centre for Disease Control) from 29 June 2011 these two seed batches were obtained through several intermediaries from Egypt.

Fenugreek seeds of the mentioned origin, which are used as single-variety or as blends for the production of sprouts and germ buds hence constitute a human health risk. This also applies to fenugreek seeds which are dispensed in very small packs to end consumers and are used for home-grown sprout production.

So far there is no specific indication suggesting that other seed types and batches were contaminated with the outbreak strain due to non-hygienic production conditions in the country of

origin or by cross-contamination between the intermediaries and recipients (e.g. cleaning, mixing and filling processes). This is nonetheless possible.

As long as there are still contaminated seed batches on the market and may be used for the production of sprouts and germ buds, restaurants and catering institutions are advised to carefully consider any serving of raw sprouts and germ buds to consumers. For the same reason, BfR advises consumers to continue to refrain from the consumption of raw sprouts and germ buds. Any seeds intended for sprouting in private households should be discarded with the residual waste.

Fenugreek seeds have already been used for a long time as spices and also as remedies. They can, therefore, be found in a large number of different products, including food supplements. However, there is so far no indication suggesting that apart from sprouts also other products produced from fenugreek seeds caused EHEC O104:H4 infections. This risk is assessed separately by BfR so that no provisional recommendations concerning these products are made in the following opinion.

BfR believes that in addition fenugreek sprouts and germ buds as well as seeds for their production should be controlled more intensely in the course of risk-based sampling. Moreover, the reinforced monitoring of human EHEC infections and HUS diseases should be maintained in order to be able to detect new outbreaks of EHEC O104:H4 at an early stage.

6.1.1 Subject of the assessment

Since early May 2011 there has been an increased occurrence of cases of disease with the so-called haemolytic-uraemic syndrome (HUS) and bloody diarrhoeas in connection with an infection by Enterohaemorrhagic *Escherichia coli* (EHEC) of the serotype O104:H4. The disease affects all federal states but in particular Northern Germany. Sprouts that are contaminated with the outbreak pathogen are considered as causal food vehicle.

With regard to the protection of the population against infections with the dangerous outbreak pathogen EHEC O104:H4, the Federal Institute for Risk Assessment (BfR), the Federal Office for Consumer Protection and Food Safety (BVL) and the Robert Koch Institute (RKI) recommended on 10 June 2011 to providently refrain, beyond the usual hygiene measures, from consuming sprouts and germ buds raw until further notice. Two days later, BfR extended this recommendation also to home-grown raw sprouts and germ buds.

The federal and *Laender* authorities have intensively worked on determining the possible input path for the contamination of sprouts with EHEC O104:H4 during the past weeks. As a result of the analysis of 41 outbreak clusters of disease accumulations as well as available data on delivery lists and distribution routes of food it was possible to attribute the associated diseases to sprouts from a horticultural farm in Lower Saxony. Early information from the competent authorities in Lower Saxony suggesting that seeds for sprout production could have been one of the causes of contamination of the sprouts, have so far not been corroborated by laboratory diagnostics. EHEC O104:H4 was not detectable in more than 900 samples of sprouts and seeds used for the production of sprouts. The detection succeeded only in a sprout mix from an opened package which was retrieved from the kitchen waste of one patient. This sprout mix contained sprouts and germ buds of fenugreek, a variety of lentils and radish.

Nonetheless, the results of epidemiological investigations of the Task Force EHEC established at BVL support the conclusion that the outbreak pathogen was introduced into the horticultural farm in Lower Saxony via seeds used for sprout production. Recent disease cases caused by EHEC O104:H4 in France at the end of June 2011, which are linked to the horti-

cultural farm in Lower Saxony through the same fenugreek seed batch used for sprout production, support this conclusion. Identical statements can be found in a risk assessment of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) of 29 June 2011 as well as the EFSA Technical Report of 5 July 2011.

On 24 June 2011 France reported about an accumulation of HUS-EHEC cases near Bordeaux with a disease onset between 15 and 20 June 2011. Thus far, in at least five cases EHEC O104:H4 was detected by laboratory diagnostics. According to tests carried out until now, the French and the German outbreak strains are genetically related and exhibit the same virulence and resistance profile.

The persons who became ill near Bordeaux consumed sprouts which were produced in a French children's camp from three different seed types. Only fenugreek sprouts were contained in the sprout mixture consumed in France and in the sprout blends of the horticultural farm in Lower Saxony which could be associated with EHEC O104:H4 disease cases in Germany. Also in one household in Lower Saxony, several persons fell ill after the consumption of home-grown sprouts from a seed blend which contained, inter alia, fenugreek seeds.

Due to the international significance of the EHEC outbreaks in Germany and France, EFSA set up a Task Force with the participation of BfR and BVL in late June 2011 which was to coordinate the further investigations on the clarification of the outbreak on an EU level.

The origin of the seeds used in the sprout production in France was determined and communicated to the member states in several alert notifications of the European Rapid Alert System for Food and Feed (RASFF). The backward tracing of the fenugreek seed batch used in France revealed that a certain seed batch produced in 2009 (Batch 48088) was also delivered by the same intermediary based in Germany also to the horticultural farm in Lower Saxony and was used in sprout production in the spring of 2011. A more concrete specification regarding the period of time it was used is not possible since the horticultural farm in Lower Saxony did not document this information in its plans for sprout cultivation. According to information by EFSA/ECDC of 29 June 2011, this batch was produced in Egypt. At the time that the authorities of Lower Saxony controlled the horticultural farm, this batch of fenugreek seeds had already been used and thus could not be sampled. However, sampling of this batch was possible in another company, but results of these tests for EHEC O104:H4 are still outstanding.

However, another batch of fenugreek seeds (Batch 8266) produced in 2010 was used for sprout production in the horticultural farm in Lower Saxony in April and May 2011. This batch was delivered by the same intermediary. According to information by EFSA/ECDC, this batch also originated in Egypt. Even though no disease cases have been associated with this batch outside of Germany and EHEC O104:H4 has not been detected in this batch either thus far, it is possible that this batch is also contaminated with the outbreak pathogen.

Based on the available EFSA/ECDC risk assessment of 29 June 2011, BfR drew attention to the potential health risk resulting from fenugreek seed batches in a BfR Opinion of 30 June 2011. Based on this Opinion, the German Federal State responsible for controlling the German importer has ordered the withdrawal of all batches of fenugreek seed that originated in Egypt if their best-before date has not yet expired or did not expire longer than six months time. The forward tracing of intermediaries in Germany has shown that the fenugreek seed batch produced in 2009 was also delivered from Germany to companies in 11 other countries.

Against this backdrop, BfR has assessed the results of the outbreak investigations carried out so far as of 5 July 2011. For improved readability, sprouts and germ buds will henceforth

be summed up in the term “sprouts” in this document. The assessment also includes the results of back and forward tracing of certain fenugreek seed batches carried out on EU level and which were published on 5 July 2011 in an EFSA Technical Report. Potential health risks through other products which were produced from or with fenugreek seeds have not been taken into account. For this purpose BfR is currently working on a separate risk assessment for these products.

6.1.2 Result

The joint recommendation on consumption by BfR, BVL and RKI of 10 June 2011 concerning sprouts is specified more precisely on the basis of the findings now available. Possible causes underlying the outbreak event have been narrowed down to a stronger extent in the meantime.

It has to be assumed that the EHEC O104:H4 disease outbreak in Germany is attributable to the consumption of contaminated sprouts, and that it is connected with the EHEC O104:H4 disease outbreak in France through the same fenugreek seed batch. BfR therefore concludes that it is highly probable that the outbreak pathogen was introduced to the sprout production through delivered fenugreek seeds. The causative entry via other vectors (e.g. water, humans, animals, pests) is considered improbable also because the outbreak strain could not be detected in the horticultural farm in Lower Saxony despite extensive testing. Only stool samples of three employees of the company, all of whom consumed sprouts produced there on a regular basis, were tested positive for EHEC O104:H4.

Based on the risk assessment of EFSA and of ECDC from 29 June 2011, it is clear that fenugreek seeds of the stated origin, which are used in single-variety or in mixtures for sprout production constitute a human health hazard. This also applies for those fenugreek seeds that are distributed to the final consumer in small packages to be used for home-grown sprout cultivation.

Thus far there are no specific indications that other seed varieties and batches were also contaminated with the outbreak strain due to unhygienic production conditions in the country of origin or that the treatment methods of distributors and recipients (e.g. cleaning, mixing and filling processes) caused cross-contaminations with the outbreak strain. Nonetheless, this can not be excluded.

Thus, BfR makes the following recommendations according to the present state of knowledge given the severity of the diseases in order to protect the consumer:

1. Recommendations for the competent authorities:

- The competent authorities are advised to completely identify the delivery routes of the two fenugreek seed batches which were used in April and May 2011 in the horticultural farm in Lower Saxony for sprout production and to withdraw these batches from the market. On the level of distributors and recipients, it should also be investigated furthermore whether cross-contamination of other seed types and batches by fenugreek seeds can be excluded in these facilities.
- The competent authorities should inform food companies about the two fenugreek seed batches which, based on the findings from Germany and on the EU level as a result of the trace back and trace forward, investigations could be contaminated with the outbreak strain EHEC O104:H4. This information should enable the food companies to possibly take measures of risk minimisation in respect of their own stocks and products produced by them.

- As part of risk-oriented sampling, fenugreek sprouts and seeds should be controlled more intensely.
 - The outbreak strain should be characterised more closely in regard to its properties including its viability and growth behaviour on seeds and in sprouts.
 - The enhanced surveillance of human EHEC infections and HUS diseases should be maintained to allow for an early detection of new outbreaks with EHEC O104:H4.
2. Recommendations for restaurants and catering institutions:
- BfR advises food companies in the restaurant and catering business (e.g. hotels, restaurants, canteens) to carefully consider any serving of sprouts for raw consumption to end consumers against the backdrop of the submitted assessment.
3. Recommendations for consumers:
- Due to the fact that it is possible that small packages of seeds intended for sprouting in private households could be contaminated with the dangerous EHEC pathogen, it is advised that seeds intended for sprouting are discarded with the residual waste.
 - Consumers are advised to continue to refrain from the consumption of raw sprouts, since it is not unlikely that sprout seeds contaminated with EHEC O104:H4 are still available on the market.
4. Basically, BfR advises that the general rules of kitchen hygiene should be observed unconditionally in order to prevent the spread of disease pathogens to ready-to-eat food.

6.1.3 Rationale

6.1.3.1 Risk Assessment

6.1.3.1.1 Hazard Identification

6.1.3.1.1.1 Enterohaemorrhagic and Enteroaggregative *E. coli*

Escherichia coli (*E. coli*) occur naturally in the bowel of humans and animals. Certain types of *E. coli*, such as the so-called enterohaemorrhagic *E. coli* (EHEC) or enteroaggregative *E. coli* (EAggEC) cause gastrointestinal diseases in humans. Since EHEC occur also in the bowel of ruminants and are excreted with faeces, they can be transmitted directly or indirectly (e.g. via food) from animals to humans and cause diseases. The typical EAggEC have, by contrast, not yet been described in animals. A transmission of EAggEC can occur via smear infections from humans to humans. The pathogen can also reach food at their preparation or production and be spread in this way.

A characteristic feature of EHEC is the property of forming Shiga toxins (*stx1* or *stx2*) and to attach via a specific protein (Intimin) in the intestines of its hosts. The terms STEC (for Shiga toxin forming *E. coli*) or VTEC (for Verotoxin forming *E. coli*) are therefore used as synonyms for *stx1* or *stx2* forming EHEC. By contrast, EAggEC forms normally no Shiga toxins and attaches through adherence factors (adhesions) to the intestinal wall.

Because of the possibly severe course of disease EHEC are amongst the most relevant causes for food-borne bacterial infections.

6.1.3.1.1.2 Characteristics of EHEC O104:H4 (outbreak strain)

In the current EHEC outbreak event, the outbreak strain of the serotype O104:H4 was clearly identified as cause for the disease. EHEC O104:H4 are designated in the reference collection of HUS associated EHEC isolates of the university clinic Münster also as "HUSEC041". The outbreak strain is however different from HUSEC041, amongst others, in its macro restriction pulse field gel electrophoresis (PFGE) patterns and its equipment with virulence factors.

By DNA sequence analysis it was determined that the outbreak strain has essentially more commonalities with the EAggEC than with the conventional EHEC. The outbreak strain is on the sequence level 93 % similar to a human EAggEC strain from Central Africa which has already been characterised. The EHEC-specific feature of the outbreak strain is the *stx2* gene. The outbreak strain is obviously a recombination of two pathogenic *E. coli* types (EHEC *eae*, *stx* and EAggEC), but it does not carry the typical *eae* (attaching and effacing *E. coli*) gene of classical EHEC.

The outbreak strain EHEC O104:H4, which belongs to the multilocus sequence type (MLST) ST678 and the phylogenetic group B1, exhibits altogether the following EHEC and/or EAggEC specific features:

EHEC features:

- Shiga toxin 1 (*stx1*): negative
- Shiga toxin 2 (*stx2a*): positive
- Intimin (*eae*): negative
- Enterohaemolysin: negative

EAggEC features (EAggEC virulence plasmid):

- ABC-transporter protein gene (*aatA*-PCR): positive
- master regulator gene of virulence-plasmid genes (*aggR*-PCR): positive
- secreted protein dispersin gene (*aap*-PCR): positive
- AAF/I-fimbral subunit-gene (*aggA*-PCR): positive
- AAF/I-fimbral operon-gene (*aggC*-PCR): positive
- Enterogastr. *E. coli* heat-stable enterotoxin (*EAST-1*) gene (*astA*-PCR): negative

Concerning the resistance phenotype, all isolates of the outbreak strain so far showed resistance to the beta lactam antibiotics of the groups acylamino-penicillin and cephalosporins. They were, however, sensitive to the carbapenems. In addition a resistance to tetracycline, nalidixic acid, streptomycine and trimethoprim/sulfamethoxazole was detected.

The outbreak strain also proves to be an extended spectrum beta lactamase (ESBL) producer. By means of molecular detection methods (PCR) an extended spectrum beta lactamase (ESBL) of the CTX-M-15 type with the upstream insertion sequence *ISEcp1* and a beta lactamase of the type TEM-1 were detected in all isolates. CTX-M-15 is the most frequent ESBL type for nosocomial ESBL *E. coli*, which has only been detected so far for a few isolates from animals. The resistance genes *bla*CTX-M-15 and *bla*TEM-1 are located on a conjugative plasmid (Inc11 Replicon, approximately 90 kbp).

According to the current state of knowledge the outbreak strain does not behave differently from the HUSEC041 reference strain in terms of its ability to form biofilms, its tellurite and mercury resistance and its acid tolerance. Under laboratory conditions it has already been confirmed that the outbreak strain can attach to surfaces in the form of biofilms.

6.1.3.1.1.3 Occurrence of EHEC O104:H4

Occurrence in humans

Until the beginning of the outbreak in Germany in May 2011, only a few sporadic cases of *stx2*-positive/negative EHEC O104:H4 have been described so far in literature. For example, ECDC reports about an infection of a person from Finland in 2010 who apparently contracted the infection during a trip to Egypt. Concerning another case in France in 2004, details on the disease (including the place of infection) are not known according to the ECDC report. Moreover, an isolation of this serotype is described in the literature for a patient with HUS in Korea in 2005 as well as for two cases (both with HUS) in Germany in 2001.

Occurrence in food

The occurrence of the serotype O104:H4 in food had not yet been described in Germany and the EU until the outbreak event. EHEC O104:H4 was detected for the first time in Germany within the course of the current outbreak investigation in and on food, respectively. The detection was made in a cucumber sample and a sample of sprouts which had been sampled at different locations from the kitchen refuse of persons infected with the outbreak pathogen.

Furthermore, EHEC O104:H4 was detected in three food samples (salmon raw and cooked, pepper) which were obviously contaminated by an employee of a party service during the incubation period.

However, STEC/VTEC of other serotypes have already been detected in food for many years. In Germany STEC/VTEC are observed within the scope of food-business operators own checks, controls of the official authorities, as well as in the course of zoonoses monitoring programs. In the course of the controls of the official authorities, STEC/VTEC are detected particularly in fresh meat as well as raw meat preparations, hence also in game meat. The detection rates were between 3 and 4 % in 2009. But also in stabilised meat products and milk samples (raw milk disposed at farm level, tank bulk milk) and dairy products (soft cheese made from raw milk) STEC/VTEC were detected.

A detection of STEC/VTEC from the group of the ten most frequent serovars in humans succeeded in the following sample materials in 2009: beef (O26), game meat (O128), minced meat (O55, O91, O103), stabilised meat products from beef (O157) and soft cheese made from raw goat milk (O26).

Within the EU detections of STEC/VTEC in food of plantal origin (vegetables, fruit) were also reported. This always concerned non-O104:H4 strains.

Occurrence in animals and in the environment

The outbreak strain EHEC O104:H4 had not been observed in animal stocks or in environmental samples prior to the onset of the outbreak event within the EU. None of the *E. coli* differentiated isolates at the National Reference Laboratory for *E. coli* (NRL *E. coli*) at the BfR belonged to this serovar. Hence, within the course of notifications of zoonoses reporting the serovar was so far not reported.

In cattle and sheep other serotypes of STEC/VTEC which do frequently occur in humans (O26 and O103) were identified in 2009.

EHEC O104:H4 was detected in Germany for the first time within the scope of outbreak investigations in an environmental sample. The detection was made via PCR analysis once in a water sample from a stream of flowing water in the federal state Hessen and might be related to discharges from a waste water treatment plant in the vicinity. In water samples taken at a later point of time from this flowing water stream this laboratory diagnostic result could not be repeated.

Concerning the resistance of the outbreak strain in the environment hardly anything is known so far. However, at present it cannot be excluded that EHEC O104:H4 strains can survive for a longer time in the environment.

According to the current state of knowledge it must generally be assumed that the outbreak strain with its detailed described genetic features has its reservoir in humans since this *E. coli* type has so far not been found in animals. Up to present, there are no indications whatsoever that the outbreak strain has overcome the species barrier human to animal. However, it cannot be excluded that the outbreak strain was able to colonise also animals secondarily, e.g. through the uptake of contaminated water. At present, it seems that the pathogen multiplies in humans and reaches the environment, e.g. the waste water, after release through faeces. It has to be assumed that for effective multiplication of the pathogen, it must again colonise humans.

6.1.3.1.1.4 Diagnostics of EHEC O104:H4

The detection of EHEC in humans infected with the pathogen is carried out normally via the laboratory diagnostic examination of a faeces sample. The goal of this laboratory diagnostic is the isolation of the pathogen together with the detection of the toxin gene by means of polymerase chain reaction (PCR) from bacteria colony run-off or faeces enrichment and/or toxin detection by enzyme-linked immunosorbent assay (ELISA) from the *E. coli* culture. The serotyping and (molecular biological) detailed characterisation of isolates follows. As a rapid differentiation method of the outbreak strain from all other EHEC a specific multi target PCR is available, with which four specific genes for EHEC O104:H4 can be detected simultaneously.

In food and/or in environmental samples the detection of EHEC is generally difficult because of the accompanying flora and the complex (biological) background matrix. Here, too, diagnostics targets the pathogen isolation with simultaneous toxin gene and toxin detection. A specific analytical method for the identification of the outbreak strain was developed and evaluated by the NRL *E. coli* together with experts of the French Food Safety Agency ANSES. This detection method was made available to the investigation laboratories of the official authorities of the federal states responsible for official controls of food, as well as the food business operators.

Since in particular the cultivation and detection of EHEC in food of plantal origin is difficult, the NRL *E. coli* provided additional specific enrichment protocols with subsequent detection of the pathogen by means of specific EHEC O104:H4 PCR. Concerning the sensitivity and detection limits of this method, only conditionally valid statements can be made for the time being. The detection limit of the pathogen in food of plantal origin (including sprouts) is stated by the NRL *E. coli* with significantly less than 10 genome copies per 25 gram sample. However, for the examination of seeds it is not yet possible to make any reliable statement, *inter alia*, because not enough is known whether pathogens can also occur within the seeds.

6.1.3.1.2 The Hazard Potential in the current Outbreak Event

Since early May 2011 there has been a frequent occurrence of the so-called haemolytic-uraemic syndrome (HUS) and bloody diarrhoea in connection with infections by enterohaemorrhagic *Escherichia coli* (EHEC) of the serotype O104:H4. The disease concerns all federal states but in particular Northern Germany. The prevailing number of diseases is connected to an exposure in Northern Germany. Foreign patients with HUS (more than 40 cases) or EHEC (more than 70 cases) have so far been reported from several member states of the European Union, Switzerland, Norway, Canada and the USA whereby a connection to Germany is known for most of the patients.

The majority of diseases caused by EHEC occur as non-bloody mostly watery diarrhoea. For part of the patients a haemorrhagic colitis develops with spasmodic stomach pains, bloody stool and partly fever. However, the infection can proceed also inapparent and be hence unnoticed. A feared complication is HUS. The full picture of HUS is characterised by acute renal failure to anuria, haemolytic anaemia (bloodlessness) and thrombocytopenia (lack of blood platelets). Typically HUS is often preceded by bloody diarrhoea. This severe complication occurs in about 5 to 10 % of the symptomatic EHEC infections. There is often a short-term dialysis obligation, more rarely an irreversible renal function loss with chronic dialysis occurs. During the acute phase the lethality of HUS is at approximately 2 %. The lethality for the current disease outbreak is at 0.4 % (EHEC infections) and 3.3 % (confirmed and suspected HUS).

Within the course of the current outbreak by serotype O104:H4 frequently neurological symptoms were observed among the clinically diseased persons; this is possibly due to the fact

that it is rather an enteroaggregative strain with the additional property of EHEC to form Shiga toxin.

Moreover, significantly more patients (25 %) developed an HUS in this outbreak than usually. In accordance with the Infection Protection Act (IfSG) 3,202 cases with an EHEC infection and 845 cases with HUS (691 confirmed cases and 154 HUS suspicions) were reported to the Robert Koch Institute (RKI) until 01 July 2011, 10 am. 48 of the reported patients died from the consequences of the diseases. This concerns one of the worldwide largest described outbreaks of EHEC infections and/or HUS so far and the largest outbreak in Germany. Female persons are affected to a larger extent by the current outbreak.

According to RKI the earliest beginning of disease of EHEC with diarrhoea was 1 May, the latest with the detection of EHEC O104:O4 was 26 June 2011 (data situation 01 July 2011, 10 am). Between 1 and 12 May one case to 18 cases with EHEC infections were reported per day. After that date the number of cases increased continuously to a maximum of 164 cases, with onset of disease on 22 May. Since then there has been a continuous decline in the number of EHEC cases.

For HUS, too, the earliest onset of the disease with diarrhoea was 1 May, the latest with the detection of EHEC O104:O4 was 26 June 2011 (data situation 01 July 2011, 10 am). Between 1 and 8 May zero to two persons became ill per day. On 9 May the number of diseased increased to seven cases and then rose continuously up to a maximum number of so far 62 cases on 21 May. Since then a continuous decline of HUS case numbers has been observed.

As of 01 July 2011 the last date of onset of disease for all EHEC or HUS cases was 27 June 2011.

The incubation time averages usually for EHEC infections to approximately two to 10 days (on average three to four days), whereby these data are essentially based on investigations on EHEC of serogroup O157. In the current outbreak event a median incubation time of eight days (interquartile interval 7-9 days) is assumed. In this outbreak, the symptoms of EHEC-associated HUS diseases begin in the median five days (interquartile interval 4-6 days) after the onset of the diarrhoea (data as of 18 June 2011).

The infectious dose of the known outbreak pathogen EHEC O157 is very low and is below 100 germs. No information is available about the infectious dose of the current outbreak strain; it can, however, be assumed that it is very low as well.

Contagiousness exists as long as EHEC bacteria are detected in faeces. Information on the average duration of germ excretion varies significantly from several days to several weeks, whereby most of the knowledge is available for the serogroup O157. Concerning this, an excretion duration for children of more than a month for cases without clinical symptoms can be expected. In how far these results apply also to EHEC O104:H4 must still be examined. A corresponding study of RKI has started but no results are available so far.. An excretion of pathogens beyond the disease stage is hence at least possible and must be assumed for a part of the infected patients.

In order to determine the cause for the outbreak, RKI carried out several inter-related epidemiological studies in co-operation with the health and food safety authorities on the federal and regional level since 20 May 2011. The analysis of the first two case control studies has revealed that patients concerned had consumed significantly more frequently raw tomatoes, cucumbers and green salads than healthy study participants. A supplementary case control study for canteen customers led to the result that the consumption of food from a salad counter was significantly associated with the disease. Hence, the first studies gave a initial

clear indication of raw vegetables as a possible source, but did not allow for any narrowing down to specific types of vegetables, so that further studies were initiated, which resulted in a statistically relevant relationship between sprout consumption and the risk of contracting the disease.

On 24 June 2011, France reported about an accumulation of HUS/EHEC cases near Bordeaux with an onset of the disease between 15 and 20 June 2011. As of 28 June 2011 15 adults contracted EHEC/HUS in this outbreak. In five cases EHEC O104:H4 was detected by laboratory diagnostics so far. According to the examinations performed to date the French and German outbreak strains are genetically related and show the same profile of virulence and resistance determinants. 11 cases attended an event at a camp for children on 8 June 2011. Nine of these cases have so far been questioned on their food consumption. During this event they have consumed sprouts with a cold soup (gazpacho) which had been self-grown in the children's camp from seeds (fenugreek, mustard, rocket salad). Further possible exposures are being investigated within the scope of a cohort study.

6.1.3.1.3 Exposure

The goal of exposure assessment is on the one hand the identification of the food involved as a cause and, on the other hand, to show the source of contamination and introduction pathways which are relevant for the characterisation of the risk and the derivation of recommendations for action.

For this purpose the results of the EHEC Task Force set up on 3 June 2011 at BVL were used. This Task Force includes experts of several federal states, BfR, RKI and BVL as well as technical experts from the European Food Safety Authority (EFSA) and the European Commission. The Task Force aims at identifying the food responsible for the EHEC outbreak (phase 1), hence the source(s) of the EHEC pathogen was to be shown and recommendations to eliminate this/these source(s) were to be deducted (phase 2), in order to be able to stop the outbreak.

6.1.3.1.3.1 Identification of the Incriminated Food Vehicle

In several case control studies, findings relating to the consumption of sprouts were determined. Already during the first intensive questioning of patients from Hamburg on 20 and 21 May 2011 a large number of animal and vegetable food including sprouts had been taken into account. During this explorative questioning only three of 12 patients mentioned the consumption of sprouts. For that reason a connection between the diseases of this outbreak and the consumption of sprouts was not taken into account in the initial case control study in conformity with internationally recognised guidelines. In a deepening case control study initiated on 29 May 2011, 27 HUS patients from Lübeck, Bremerhaven and Bremen were individually allocated to three healthy persons on the basis of their age, gender and place of residence. Six (25 %) of 24 patients mentioned that they had consumed sprouts during the assumed infection period, compared to seven (9 %) of 80 non-diseased for whom such information was available.

With a "recipe-based restaurant cohort study" the cause of the outbreak could then be narrowed down epidemiologically with a high probability to the consumption of sprouts. With this approach (as of 10 June 2011) five groups (travel groups, clubs etc.) with a total of 112 participants of whom a total of 19 contracted bloody diarrhoea after a joint restaurant visit were examined for their consumption at the restaurant. In this connection the restaurant visitors were not only questioned but based on the order lists and invoice data it was determined what menus the members of the travel groups had ordered. At the same time the kitchen of the restaurant concerned was questioned in detail how exactly each menu had been pre-

pared and the amounts of the different ingredients in the different menus were ascertained. In addition photos of the travel groups were evaluated to prove the food and garnishes on the plates. This information was evaluated in a cohort approach which allows a retrospective calculation of the relative disease risk for restaurant guests. The current analyses showed that customers who had consumed sprouts in their menu had an 8.6-fold higher risk of getting bloody diarrhoea or EHEC/HUS confirmed by laboratory detection than customers who did not have this food in their menu. Moreover, it could also be shown by this means that of all the cases covered by this study 100 % had sprouts in their menu.

The Task Force EHEC set up at BVL pursued a comprehensive trace back strategy, based on the intensive investigations of the federal *Laender* particularly concerned by the EHEC outbreak (Lower Saxony - NI, Schleswig-Holstein - SH, Mecklenburg-West Pomerania – MV, Hamburg – HH and Hesse - HE).

Based on the information already generated by the *Laender* of five well defined outbreak clusters, the supply relations of the food consumed by persons who contracted the disease at the five outbreak locations were initially analysed and the flows of goods were traced on the basis of delivery notes (Trace back). An outbreak cluster was defined by the Task Force as an accumulation of at least one case of disease (EHEC or HUS) at one place of exposure if there were strong indications that the infection could only have been contracted at this location. This was for instance the case if members of a travel group in which there had been several cases of disease had only taken a common meal in one restaurant. Places of consumption of individual cases were only considered as worthy for further investigations if one single place of exposure in North Germany was to be considered, for instance tourists from Denmark had only eaten at a certain motorway service area while travelling through northern Germany.

The analyses of the supply relations and flows of goods led to a horticultural farm in Lower Saxony which had already been in the focus of the investigations of the public authorities in Lower Saxony. The initial suspicion was based on findings by laboratory diagnostics made by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) in respect of sprouts produced by this farm. The positive ELISA findings could not, however, be verified by confirmation tests.

On this basis the EHEC Task Force pursued in addition a combined trace back/trace forward investigation strategy of the trade relationships proceeding from the suspected horticultural farm in Lower Saxony. Trace forward means the discovery and documentation of distribution channels in the direction of the consumer, whereas with trace back the distribution channels beginning with the consumption place in the direction of the producer of the good are considered.

The following results were determined by the EHEC Task Force as of 22 June 2011:

- The distribution routes based on batch-specific information for two sprout blends¹ with commonalities in terms of sprout sorts which occurred in both blends of a horticultural farm from Lower Saxony, lead via two nodes to all five priority outbreak clusters (Figure 11). All five outbreak clusters had received at least one of the two above mentioned sprout mixtures (germ sprout resp. spicy blend). Fenugreek germs and lentil germs were identified as common germ varieties.
-
- Overall 41 outbreak clusters identified by human epidemiology, localised in the six federal states mostly affected by the outbreak (NI, HH, MV, SH, HE and NW) could be interconnected via supply relationships of sprouts of the horticultural farm in Lower Saxony

¹ Germ sprout or mild blend (contains alfalfa germs, fenugreek germs, lentil germs, adzuki bean germs) and spicy blend (contains radish germs, fenugreek germs, lentil germs)

(Figure 12). These findings receive a particular strength of evidence because of the fact that the selection of the 41 clusters was made independently from the hypothesis of dissemination by sprouts.

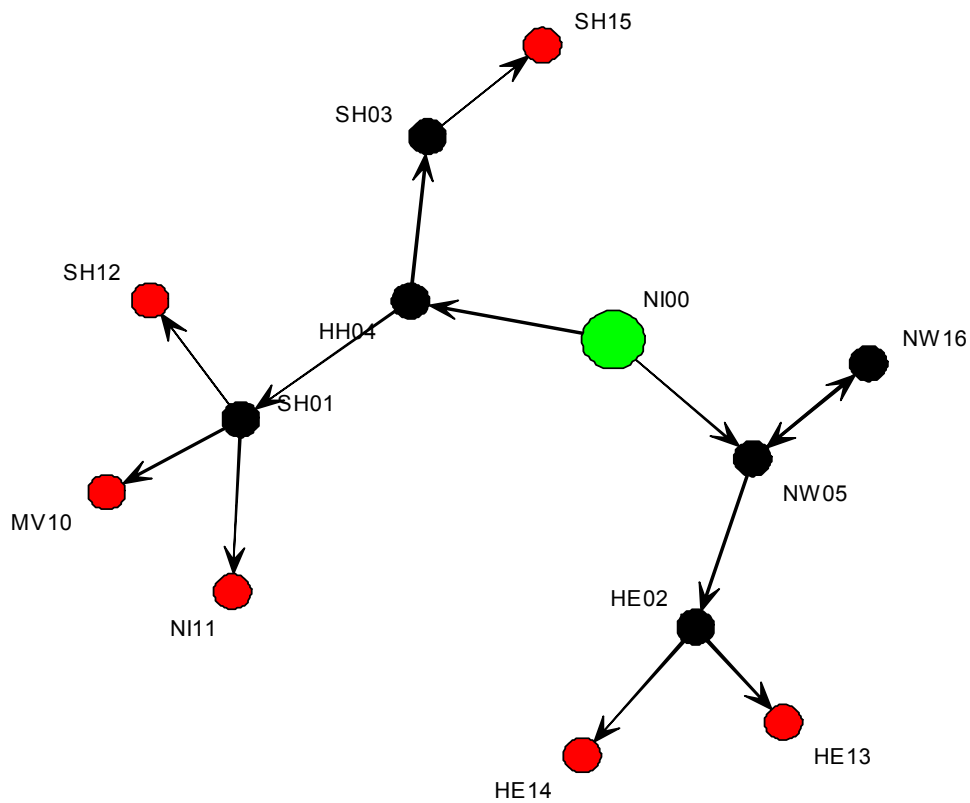


Fig. 11: Results of the trace back based on the five outbreak clusters (red) by means of specific batch information (HE13 and HE14 are evaluated as one cluster)

¹ Germ sprout or mild blend (contains alfalfa germs, fenugreek germs, lentil germs, adzuki bean germs) and spicy blend (contains radish germs, fenugreek germs, lentil germs)

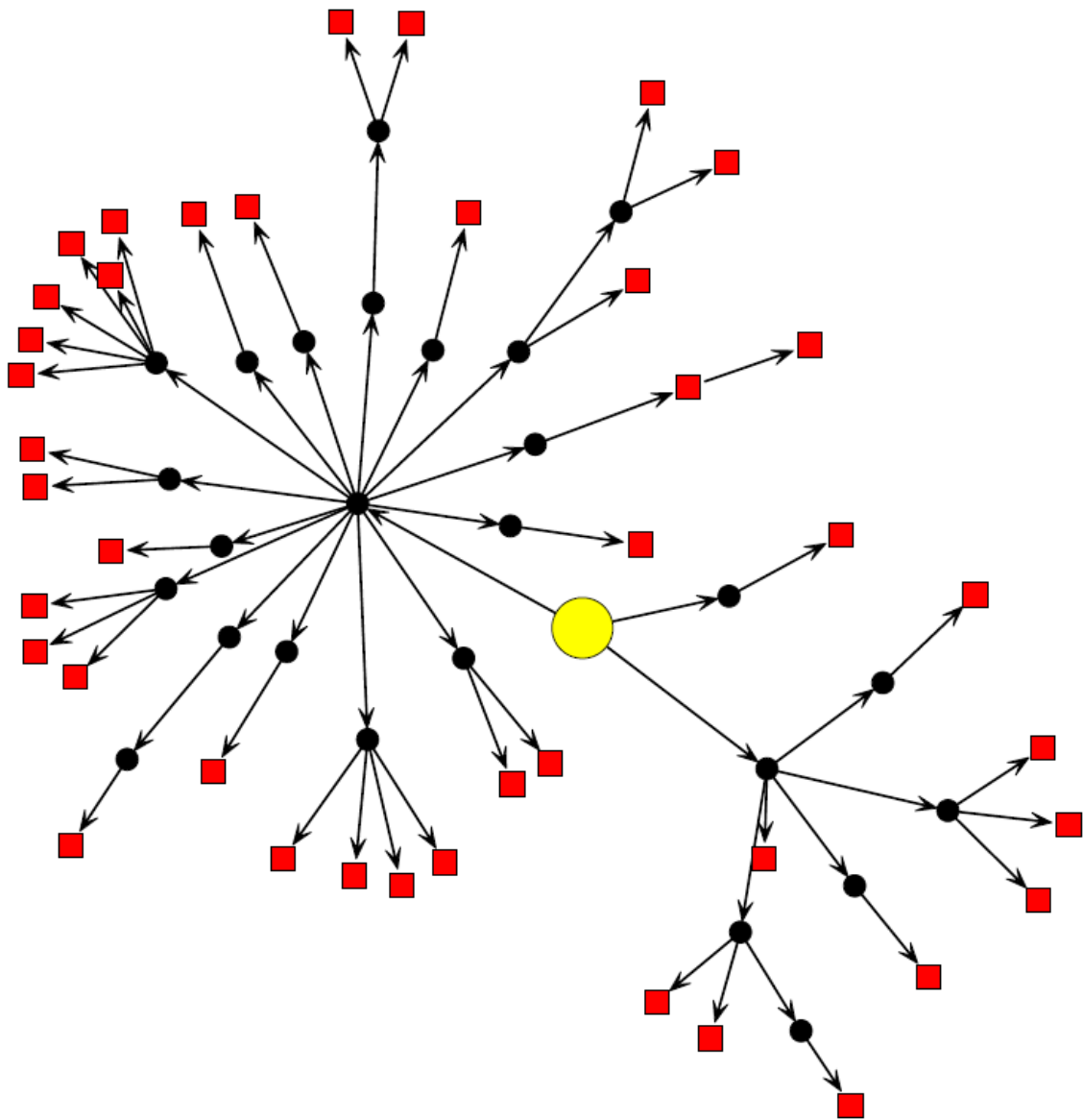


Fig. 12: Results of the combined trace forward/trace back strategy. Supply relationships of the horticultural farm in Lower Saxony (yellow) lead to all 41 outbreak clusters associated with sprouts (red). Wholesalers and intermediate dealers (black)

6.1.3.1.3.2 Findings on the Suspected Horticultural Farm in Lower Saxony

After narrowing down the food vehicle to sprouts from a suspected horticultural farm in Lower Saxony, intensive investigations and examinations were initiated on site. Furthermore, it was examined whether an outbreak of this size could be plausibly explained based on the production volume of the farm.

The horticultural farm in Lower Saxony is an establishment of primary production, which is registered for organic production. As major products of the farm vegetables grown at the field and sprouts are indicated. According to information provided by the owner 90 % sprouts (approximately 20 different sprout varieties) and 10 % field vegetables (from own production and purchased from a greengrocer from the region) are marketed, both based on organic and partly vegan guidelines. Whereas the sprout distribution is carried out through intermediaries mainly, fruit and vegetables are marketed on a weekly market in the region. Additionally a regional organic food store is supplied.

The farm is regularly audited by a control agency approved for this purpose according to the requirements of the EU regulations on organic food. According to the competent veterinary surveillance authority the farm has a quality management system, which, however, does not meet the requirements of the Codex Alimentarius HACCP concept

The sprout production is carried out based on conventional production methods in an area in which protective clothes is worn. The germination of the sprouts is at about 20°C ambient air. The entire water in the production area is recovered from the farm's own well system.

The identified operational production procedure with a very humid environment and mesothermal conditions in the growing recipients is to be assessed as particularly favourable for survival and/or growth of EHEC during sprout production.

For the purpose of packaging and further cold storage the sprouts are removed from the production area. From some sprout varieties with different weights, different germ sprout mixtures are composed (including a spiced blend and germ sprout/mild blend). The sprouts are exclusively distributed to the customers if packaged in different package sizes and with a best before date of 10 to 14 days.

The different seed types for sprout production are sourced from several wholesalers in Germany and abroad (as a rule in several 25 kg bags of one batch) and are stored partly for several months. An overview of the batches of the different seed types, which went into production immediately before and during the assumed exposure period (mid/end-April to mid-May 2011) or until the last placing on the market of the sprouts on 3 June 2011, was made available by LAVES. Records on the exact time when specific seed batches for sprout production were used did not exist in the horticultural farm. An official closing of the farm was ordered orally by the competent authorities in Lower Saxony on 5 June 2011.

6.1.3.1.3.3 Results of microbiological investigations of sample from the horticultural farm in Lower Saxony

Both, the competent authorities and the investigation offices in Lower Saxony (LAVES) and the NRL for *E. coli* have carried out extensive microbiological analyses of samples taken at the horticultural farm in Lower Saxony. The following sample types were examined: ready-to-consume sprouts, non-germinated seeds, germinated seeds, various environmental samples as well as sample materials from pets. The results of the laboratory diagnostics examinations carried out at the NRL *E. coli* are summed up in Table 1.

Tab. 7: Results from the NRL *E. coli* (status: 27 June 2011)

Sample type	Negative	Outbreak strain O104:H4	Other STEC	Number of samples
Seeds, non-germinated	32	0	0	32
Seeds, germinated	30	0	0	30
Sprouts	295	0	3	298
Plants/vegetable	8	0	1	9
Water	10	0	1	11
Swab environment	55	0	0	55
Waste	1	0	0	1
Fertilizer	3	0	0	3
Pet	2	0	0	2
Outward packaging	11	0	0	11
Sum	447	0	5	452

In the samples from the horticultural farm in Lower Saxony examined by the NRL *E. coli* the outbreak strain could so far not be detected. However, it is striking that from five samples other STEC could be isolated.

LAVES examined more than 170 samples from the horticultural farm in Lower Saxony for EHEC O104:H4 but neither the outbreak strain nor other STEC were detected.

6.1.3.1.3.4 Estimation of Consumption portions

Based on the findings related to the supply quantities, it was checked whether the total number of persons infected in association with the outbreak primarily through food as estimated by RKI can be explained by the delivery quantity of potentially contaminated batches from the horticultural farm in Lower Saxony. If the number of consumption portions which can be produced from the corresponding delivery quantities of the farm is much lower than the number of patients, this would support the existence of another undiscovered infection source.

For a maximum estimate of the consumption portions, the delivery quantity of all sprout products of the horticultural farm in Lower Saxony, which were delivered by the farm between 19 April 2011 (the assumed earliest delivery date of a contaminated batch) and 3 June 2011 (the last placing on the market before the discontinuation of sprout production) was used as a basis. This time span can be considered as "risk period" for the delivery of contaminated sprouts. A minimum estimation could be based on the quantity of individual, identified batches such as the "germ sprout blend" and the "spicy blend". In order to estimate the number of consumption portions, the data of the 24-hour recall and weighing logs from the National Consumption Study II (NVS II) were used which had been collected by the Max Rubner Institute and are available to BfR for assessment purposes.

The possible number of portions, broken down by sprout varieties, for the products with known delivery quantities was calculated. At the analysis of the consumption data of NVS II the terms "germ buds" and "sprouts" were considered and aggregated as synonymous. Based on the 227 sprout portions of the 24-hour recall the mean portion size (median) is at 19 g/portion with a 5th percentile of 2 g and a 95th percentile of 53 g/portion. The 95th percentile for data which were collected by weighing log is at 100 g/portion. Even if the weighing logs offer the better data basis in terms of methodology because of the collected individual recipes and the accuracy of weighed quantities, the estimate involves major uncertainties because of the low case numbers (42 consumed sprout portions) and taking into account the results from the 24-hour recall it is rather to be classified as an over-estimation. For a realistic estimate of the number of consumption portions the median can be used, whereas the

95th and the 5th percentile can be used as the lower and upper limit for the estimate of numbers.

If one considers the "germ sprout blend", in which fenugreek sprouts were contained, 3,234 consumption portions might have been consumed raw on average (614 and 30,725 portions based on the 95th and the 5th percentile). For the "spicy blend", which likewise contained fenugreek sprouts, the mean value is 6,821 portions (1,159 and 64,800 portions based on the 95th and the 5th percentile). These two blends are of particular interest because of the epidemiological evidence for a contamination.

This consideration involves an uncertainty since no data are available as to which extent the delivered quantities were consumed heated or were consumed at all. For this reason, based on BfR expert knowledge, a hypothetical proportion of 50% is assumed for raw consumption of the delivered sprout quantities. This means that for the purpose of the estimation it is assumed that half of the delivered sprouts are consumed heated or not at all. Actually the percentage of these sprout varieties consumed raw might have been higher.

These results prove that the total number of known infections could be explained with a contamination in the delivered quantities of the "germ sprout" and/or "spicy" blends.

6.1.3.1.3.5 Influence of Consumption Habits

The current outbreak event is characterised by an unusual distribution of age and gender among the HUS patients. So far mainly adults, more women than men, are affected by HUS. Before the current outbreak mainly children contracted HUS in Germany. The observed differences could be explained through the different consumption habits and the associated exposures. Since sprouts are considered as the causal vehicle, health-conscious diet, in particular of women, may have resulted in an increased exposure of primarily this demographic group. Nonetheless the German consumption studies do not provide clear evidence that women eat sprouts more often or in higher quantities than men. Insofar no narrowing down of the hazard to certain demographic groups is possible.

6.1.3.1.3.6 Possible Sources of Contamination and Introduction Pathways of the Outbreak Strain to the Horticultural Farm in Lower Saxony

The identification of the source of contamination is important from the food safety perspective in order to identify possible other so far unknown sources of infection. Based on the epidemiological evidence for the suspected horticultural farm in Lower Saxony as a source of the outbreak event, two different hypotheses and their possible consequences must be considered concerning the source of contamination in the mentioned farm:

1. It is a point source, i.e. all cases of disease can be attributed directly or indirectly to the horticultural farm in Lower Saxony.
2. It is a source that was predominantly but possibly not exclusively introduced to the horticultural farm in Lower Saxony. This source of contamination could possibly find other exposure routes into the human population.

If one considers the horticultural farm as a point source (hypothesis 1), different sources of contamination and introduction pathways in this farm have to be considered:

The introduction to the farm occurred through contaminated humans (e.g. staff), water, seeds as point contamination, (i.e. a single contaminated bag was delivered) or another currently not known vehicle (e.g. pet, rodent pest, insect pest, packaging material). The further spread-

ing of the pathogen within the farm effected several production batches, i.e. the introduction event took place several times or during a certain restricted period of time.

Further vehicles could have contributed to the spreading within the farm such as water which was contaminated in the farm by humans and then was used for sprout production. Further possibilities are seeds which were contaminated on site, i.e. stocks were contaminated, or utensils contaminated by infected humans which were used over several production periods.

Within the course of hypothesis (1) the following aspects were not considered in addition although they can be of essential significance for the further spreading of the pathogen. This concerns the possible infection source of humans, possible sources of contamination in water outside the horticultural farm as well as possible sources of contamination for other vehicles outside the horticultural farm.

If the source of contamination for the horticultural farm in Lower Saxony is also relevant for other sprout producers (hypothesis 2), this would mean that the horticultural farm in Lower Saxony is not the only possible origin for the current outbreak event. Consequently, new outbreak events could emanate from other producer sources. The following sources of contamination and introduction pathways have to be taken into account:

The introduction to the production chain occurred through contaminated seeds whereby the contamination occurred at a producer or supplier as a point contamination.

The further spreading of the pathogen within the production chain then occurred

- with the entire batch of a seed type after blending,
- with different production batches of a seed type through cross contamination, or
- with different batches of different types of seeds through cross contamination.

It has to be generally stated that the process for sprout production favours germ multiplication. The process steps in the production process might also have contributed to a homogeneous mixing and spreading of the pathogen in one production batch. The fact that there cannot have been a major carry over between the production batches as well as a discharge to the environment can be derived from the unsuccessful detection of the pathogen, including in the entire sewage system.

For the various possible sources of contamination the present state of knowledge is described below.

Humans as source of contamination in the horticultural farm in Lower Saxony

According to the health authorities in Lower Saxony 15 persons, including the owners, work at the horticultural farm. Of these three female employees (Cases 1-3) developed diarrhoea symptoms in terms of an EHEC infection (start of disease: 6.5., 11.5. and 12.5.2011). For one of these female employees who also developed an HUS, EHEC O104:H4 was detected (Case 3). For the other two employees (Case 1, Case 2) faeces was originally not examined.

Within the course of the investigation of the environment by the competent health authority 13 of the 15 employees were examined so far by laboratory diagnostics of the Lower Saxony Health Office (NLGA) for the occurrence of an EHEC infection; two of these persons were positive with EHEC O104:H4 (Case 4, Case 5). These two persons had not mentioned any diarrhoea symptoms. However, Case 1 and Case 2 were tested negative in the latest stool

analysis. Therefore, it has to be assumed that there are five EHEC (suspected) cases among the employees of the horticultural farm.

All 15 persons were questioned by means of a standardised questionnaire on possible infection causes. The replies of the employees concerning their travel history (Germany and abroad) did not, however, provide any clear findings in view of the identification of the infection cause. Concerning the consumption of sprouts, the five Cases indicated a preference for certain sprout varieties (fenugreek, broccoli, garlic).

Generally humans can be considered as a source of contamination. However, all these persons consumed sprouts from the farm and in particular the suspected sprout blends (germ sprout or spicy blend). Therefore it might be possible that the excretors were infected by the consumed product like other cases. For the hypothesis that the pathogen was introduced primarily by staff, it remains furthermore unclear how the persons became infected. So far no conclusive infection source could be identified in the environment resp. could be deduced on a travel history.

In view of the suspected infection time of the diseased employees a causal introduction through these persons is hardly probable, although a secondary introduction through excretors cannot be excluded basically. However, the outbreak strain was not detected in the farm despite intensive sampling although two asymptomatic excretors worked there during the same period.

Water as source of contamination and/or introduction pathway at the horticultural farm in Lower Saxony

Generally speaking, it would be conceivable that the pathogen was introduced via water or water contributed to the further spreading of the pathogen. Within the course of previous outbreak events with other EHEC a surface contamination of vegetables by water was identified as an underlying cause. However, in such cases both the source of contamination into the water and the pathogen itself could be detected in water.

During various site inspections the irrigation and wastewater system in the horticultural farm in Lower Saxony was sampled and evaluated. In the report on the water hygiene aspects (status: 15.06.2011) different hypothetic ways were shown how the introduction and spreading in the water system could have taken place.

Although the outbreak strain EHEC O104:H4 is characterised as a particularly good biofilm producer, the detection has not been successful in any of the samples taken on site. The quality of the intensive sampling is supported by the fact that other EHEC were detected in a water filter. Water as source of contamination is therefore unlikely.

Other vectors as source of contamination in the horticultural farm in Lower Saxony

The pathogen was not detected in Germany prior to the outbreak event. Based on its properties, the reservoir of the pathogen is assumed to be in humans. For that reason the pathogen may theoretically also have been transferred through vectors (e.g. via bugs, rodent pests) to the farm. The origin could be an exogenous source in the environment such as human waste. However, an input through vectors suggests that the pathogen would be detectable in different areas of the farm. Despite extensive sample taking and investigations the detection was not possible, so that there is no corresponding evidence.

Seeds as possible source of contamination

Based on the assumption that the pathogen was introduced several times into production but was not able to establish itself permanently and was no longer detectable at the time of the investigation, it appears to be most likely that the pathogen was introduced through seeds for sprout production.

BfR assumes that the outbreak pathogen reached the horticultural farm in Lower Saxony via fenugreek seeds which were used for sprout production. This conclusion is supported by the finding that the recent EHEC O104:H4 cases in France were linked with the use of fenugreek seeds of the same batch as used by the horticultural farm in Lower Saxony. The conclusion is also supported by the risk assessment of 29 June 2011 of EFSA and ECDC, and the EFSA Technical Report of 5 July 2011.

At the time of the investigations on the outbreak cause all seed batches were sampled which went into production, taking into account the possible beginning of the exposure, the germination time and the consumption date. An exception was a batch of fenugreek seeds which was no longer available at the time of sampling at the horticultural farm. The trace back of the fenugreek seeds batch used in France has shown that the seed batch produced in 2009 (batch number 48088) was supplied through the same intermediary located in Germany which also had supplied the horticultural farm in Lower Saxony with fenugreek seeds (see figure 5). Detailed information on the period of use of this specific batch of fenugreek seeds is not available since the horticultural farm in Lower Saxony has no documentation on the respective batch used. At the time of the inspection of the horticultural farm by competent au-

thorities in Lower Saxony this specific batch of fenugreek seeds was not available any more, and thus it was not possible to collect a sample from this batch.

For the production of sprouts in April and May 2011 the horticultural farm in Lower Saxony used one other batch of fenugreek seeds (Charge 8266) which had been produced in 2010. This batch was purchased from the same intermediary. According to information from EFSA as of 29 June 2011 both batches of fenugreek seeds were purchased from Egypt.

So far no EHEC O104:H4 was detected in the batch of fenugreek seeds which had been produced in 2010 (batch No. 8266). No analytical results are available for the fenugreek seeds batch which had been produced in 2009 (batch No. 48088). However, negative analytical results can not proof the absence of the pathogen. An irregular distribution of bacteria and the connected issue of representative sampling was previously described in the scientific literature for the sampling of food and feed. This issue must be considered for the sampling of seeds intended for sprout production, too.

Potential contamination of the seeds on the premises of the horticultural farm is a possible hypothesis. However, the control and inspection visits conducted by the competent authorities of Lower Saxony, within the framework of the outbreak investigation, gave no indication that hygiene standards were not met by the horticultural farm. In addition, the link between the EHEC O104:H4 outbreak in Germany and France via the use of identical seed batches used for the production of sprouts, suggests that the contamination occurred prior delivery of the seeds to the sprout producers. When cultivating and harvesting seeds, a contamination from the environment cannot be excluded; hence, decontamination processes with safe elimination of pathogens are not available.

Following the conclusion that seeds are the most likely source of EHEC O104:H4, it has to be expected that other sub-quantities of the specific batch are also contaminated. Contamination of other product batches might have occurred during storage, transport, cleaning, bagging and further treatment of the products.

Therefore it is not unlikely that after sprout consumption, new cases of EHEC O104:H4 infections might occur in future, caused by introduction to other production sites. For that reason the supply relations for seeds used for sprout production were intensively investigated and considered by the German EHEC Task Force, based at BVL.

6.1.3.1.3.7 Results of the Trace Forward and Trace Back of Seeds

The methodology for trace back of seeds for sprouts is described in the EFSA technical report from the 5 July 2011 and in status report drafts of the German EHEC Task Force. Distribution channels for seeds used for sprouting are not yet fully illustrated. However, partial amounts of the batches in question were also delivered to other businesses. In this process, batch numbers were changed several times, making trace back more difficult.

Figure 13 summarises the status quo (27 June 2011) of the investigations conducted by the German EHEC Task Force with regards to seeds. This illustration does not distinguish between various types of seeds. In Figure 4 however, German distribution channels are depicted for distinct types of seeds and certain batches of seeds, with reference to the horticultural farm in Lower Saxony. Figure 5 illustrates the link between the outbreak cluster in Germany and France determined via trace forward and trace back investigation of distribution channels. The determination of distribution channels was conducted at European level for the batch of fenugreek seeds produced in 2009 (status quo 30 June 2011, data from the EFSA technical report from 5 July 2011). Results of the EFSA technical report with regards to the link of the German and the French outbreak is shown in Figure 6. Also based on data

published in the EFSA technical report, Figure 7 depicts distribution channels of the identified seed batch within Europe.

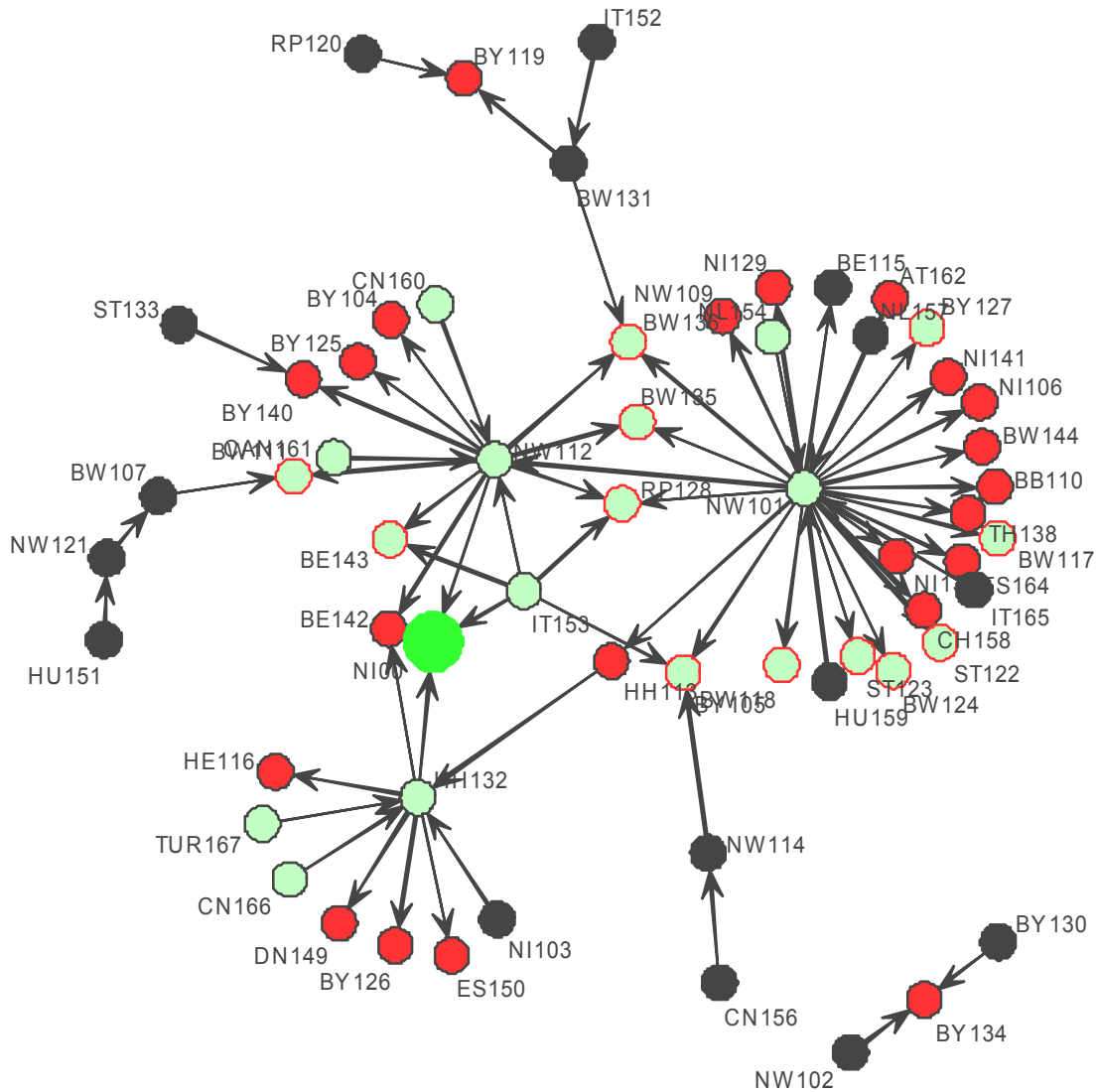
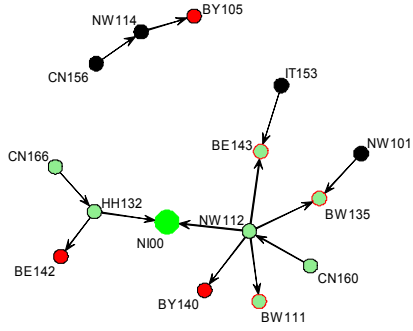
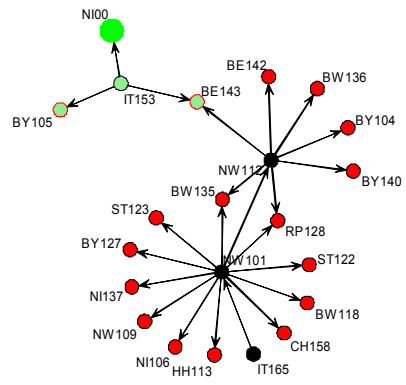


Fig. 13: Based on the trace back (combined trace forward/trace back strategy based on specific batch information) of the corresponding seed deliveries to the horticultural farm in Lower Saxony (NI00, large green dot) the determined distribution network (direction of arrow) to German sprout producers (red) combined for the seed varieties, adzuki, alfalfa, fenugreek, lentils, radish and daikon emerges. The supply chain points in light green are those through which/to which the same batches as those of the horticultural farm in Lower Saxony were transported/delivered

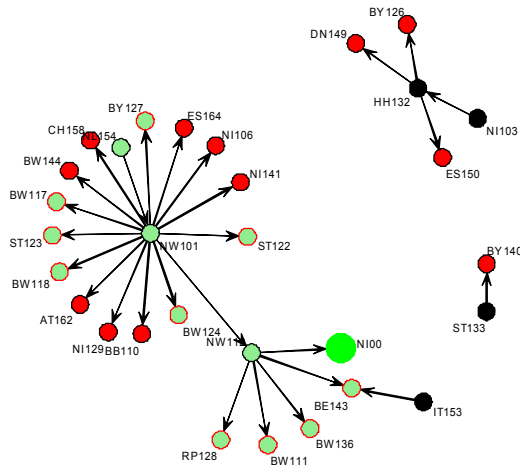
Sprout producers who received the same batches as the horticultural farm are shown in light green with red edge. The black dots show suppliers without relation to the batches of the horticultural farm.



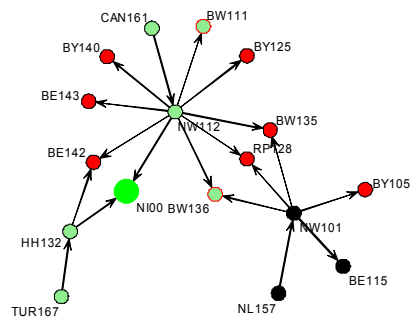
Adzuki



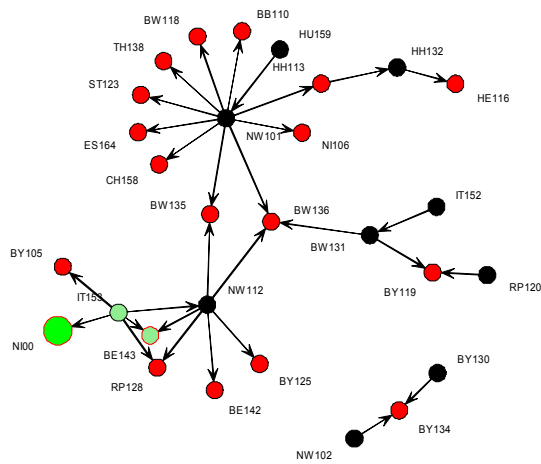
Alfalfa



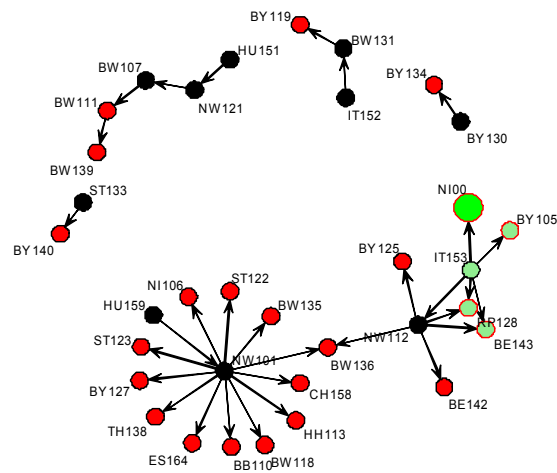
Fenugreek



Lentils



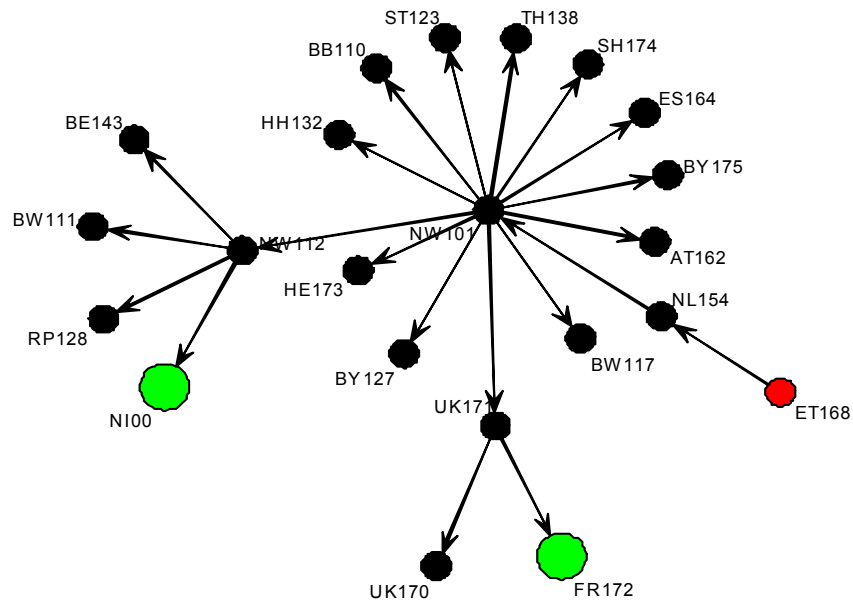
Radish



Daikon

Figure 14: Based on the trace back (combined trace forward/trace back strategy based on specific batch information) of the corresponding seed deliveries to the horticultural farm in Lower Saxony (NI00, large green dot) the determined distribution network (arrow direction) to German sprout producers (red), individually shown for the seed types adzuki, alfalfa, fenugreek, lentils, radish and daikon. The supply chain points in light green are those through which/to which the same batches as those of the horticultural farm in Lower Saxony were transported/delivered.

Sprout producers who received the same batches as the horticultural farm are shown in light green with red edge. The black dots show suppliers without relation to the batches of the horticultural farm.



Fenugreek batch from 2009

Figure 15: Results of the combined trace forward/trace back strategy as of 4 July 2011 of the fenugreek seed batch produced in 2009 (batch 48088), which was imported from Egypt according to EFSA (red)
This batch was supplied through the same node, the German importer (NW101) to both the sales outlet in France linked to the cases of disease (FR172, green) and to the horticultural farm in Lower Saxony (N100, green), partly through several intermediaries.

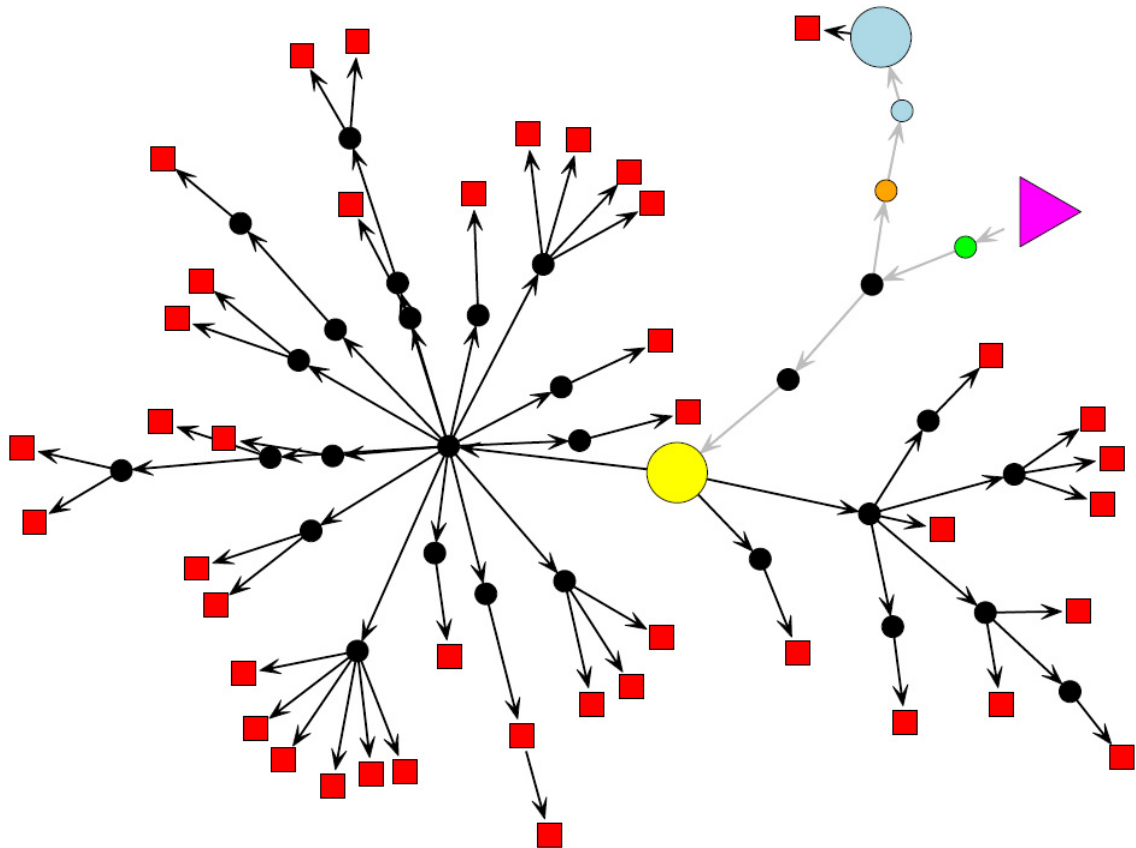


Figure 16: Visualisation of the connection between the German and the French EHEC outbreak with a joint source identified by the EFSA Task Force (magenta-coloured triangle), based on the EFSA Technical Report of 5 July 2011

Furthermore, the horticultural farm in Lower Saxony (yellow), the delivery routes in France (light blue) and the outbreak clusters (red) are shown.

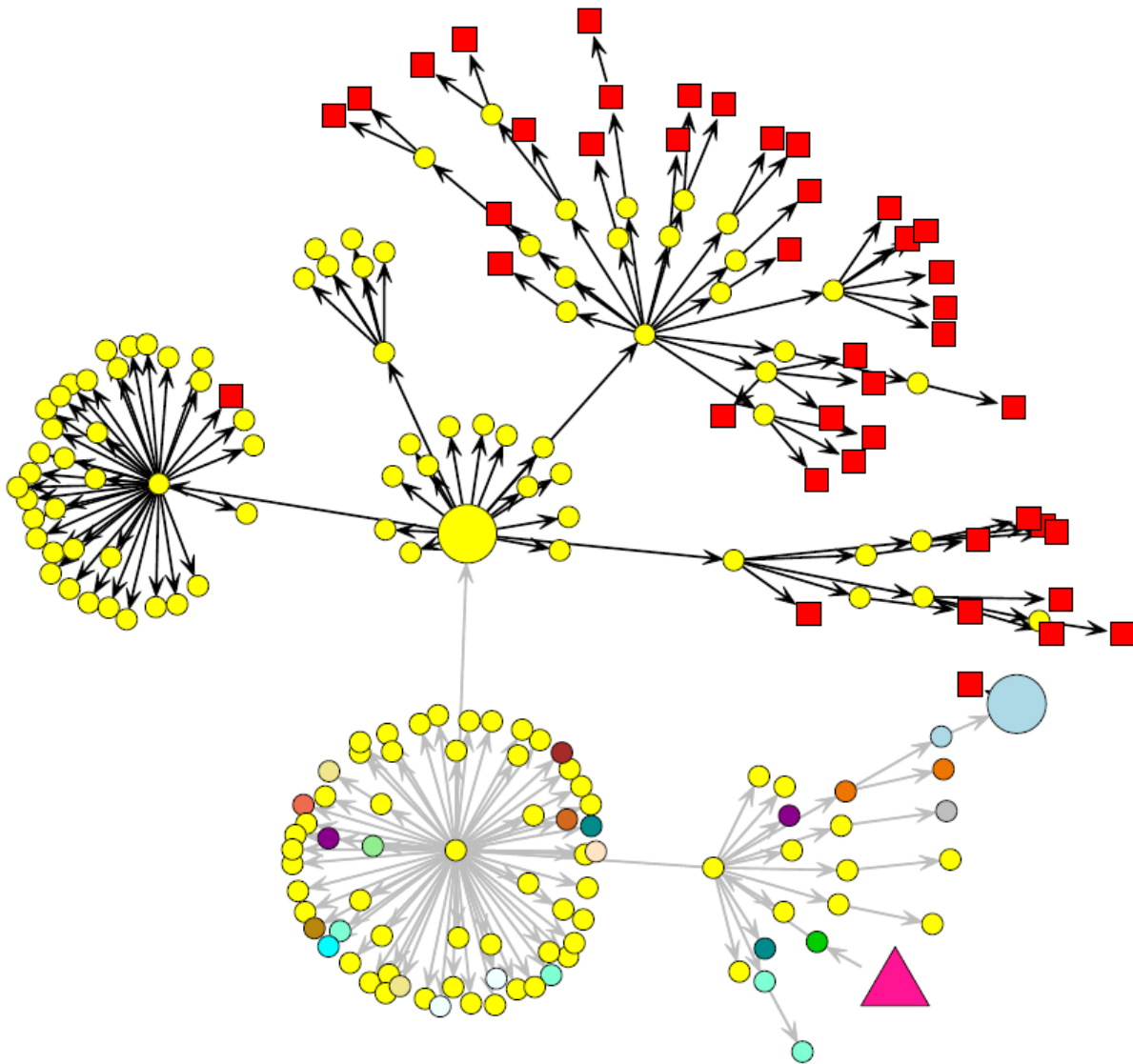


Figure 17: Visualisation of the connection between the EHEC O104:H4 outbreak in Germany and France based on the currently known European distribution network for an identified batch of fenugreek seeds (batch 48088)

The distribution network for this seed batch is based on the data compiled by the EFSA Task Force (EFSA Technical Report of 5 July 2011). The description of the symbols is the same as for Figure 6. Moreover, the intermediaries in other European countries are shown in different colours (Germany: yellow).

6.1.3.1.3.8 Investigation Results of the *Laender* on Samples of Sprouts and Seeds

Within the framework of the intensive investigation activities of the *Laender* on the EHEC outbreak event a total of 956 samples of sprouts as well as seeds for their production were tested for EHEC O104:H4 with a negative result (Communication by BVL, as of 27 June 2011). A microbiological confirmation of the conclusions drawn on the basis of epidemiological information is hence still pending. In order to reach this goal, based on the findings from the trace forward of fenugreek seed batches of the above-mentioned origin, samples of fenugreek seeds are still be taken on target and examined microbiologically.

6.1.3.1.4 Risk Characterisation

In the following chapter the consumer risk in connection with sprouts is characterised for two different situations. The risk in connection with other products in which fenugreek seeds are processed, is assessed separately by BfR. The risk of sporadic inputs of the outbreak pathogen EHEC O104:H4 by human secretors to other food chains is not considered.

Situation 1: Recommendation on consumption concerning sprouts is complied with

The situation during the period of the outbreak before the consumption recommendation of 10 June 2011 which advised to refrain from the consumption of raw sprouts and before the horticultural farm in Lower Saxony discontinued the production of sprouts, seems to be explainable from the current point of view. During this period there had been a steep increase in disease case numbers. The above-mentioned extent of the outbreak is primarily attributable to an exposure during this phase. The outbreak investigation which was carried out identified a horticultural farm in Lower Saxony which was involved with a high probability as a cause for the outbreak event in Germany. Whether the consumption of sprouts from other producers in Germany likewise caused diseases in Germany, is currently not known. The accumulation of cases reached epidemic dimensions so that it had to be described as a frequent damaging event, partly associated with very severe health damages. Following the measures taken by the public authorities (closing of the farm and consumption recommendation of BfR, BVL and RKI of 10 June 2011) the outbreak was obviously stopped. After the narrowing down to certain batches of fenugreek seeds as source of contamination, the responsible Land authority of the German importer officially ordered the withdrawal of the batches concerned on the basis of the BfR Opinion of 30 June 2011. The trace forward of implicit seed batches, the exclusion of possible cross contaminations at intermediaries and recipients of seed supplies and a complete return of seed batches will be continued. If the consumption recommendation concerning sprouts is complied with, there is at present with a high probability no longer any direct hazard.

Situation 2: Recommendation on consumption concerning sprouts is not complied with or cancelled

As already described above, there are many indications suggesting that the outbreak pathogen was introduced through contaminated fenugreek seeds to the horticultural farm in Lower Saxony and seeds of the same batches were also delivered to other sprout producers. For that reason the responsible Land authority of the German importer officially ordered the withdrawal of the batches concerned. The execution of these official measures continues. Moreover, there is a possibility that also other seed varieties and batches were contaminated with the outbreak strain due to non-hygienic production conditions in the country of origin or by cross-contaminations at intermediaries and recipients (e.g. during cleaning, blending and filling processes). Therefore, it cannot be excluded that material from the stocks of at least one contaminated seed batch is used by a further producer for sprouts. If the corresponding sprouts are consumed raw, another comparatively severe outbreak event could develop which is outside the scope of this assessment.

6.1.3.1.4.1 Assessment of the Severity of the Health Impairment

The health impairments are to be assessed as severe. It concerns a very severe clinical picture which can lead from bloody diarrhoea via renal failure with obligatory dialysis, severe neurological symptoms up to death. The period during which the health damage caused persists, leads to chronic courses (e.g. with permanent renal damage) or is reversible and which late sequelae can occur, cannot be assessed for the moment. Further fatalities cannot be excluded either.

6.1.3.1.4.2 Assessment of the Quality of Data

Trace forward and trace back

The quality of data for the delivery relationships of seeds is to be assessed as very good and the quality of those for sprouts as good. The data input based on delivery notes was done by trained members of the EHEC Task Force. Since the EHEC Task Force has not yet received all delivery data and the delivery relationships could, therefore, only be assessed incompletely, it must currently still be assumed that there is a certain uncertainty. This uncertainty in respect of the delivery relationships can, however, be considered as reasonable for the purpose of this assessment given the overall picture of the data situation.

It is recommended to completely finish the trace back and trace forward investigations of the supply chains for the two above mentioned fenugreek seed batches.

Microbiological investigation results

The quality of the microbiological investigation data for sprouts and seeds also depends on the sampling plan. The latter was carried out in accordance with the provisions of feed law. In the assessment of BfR it is not possible to indicate the statistical certainty for the sampling of EHEC O104:H4 in this sample matrix. This is due to the fact that 1) the analytical method is not validated for this purpose, 2) it cannot be assumed that there is a homogeneous distribution of the pathogen in the sample material and 3) it is not known in individual cases how many bags per seed batch were available in the depot of the sampled producers.

6.1.3.2 Other Aspects

6.1.3.2.1 Technology of Sprout Production especially considering Microbiological Aspects

The consumption of sprouts increased in Germany over the past years. Given their germination from the seeds, products of this kind can actually not be produced in a germ-free manner. In order to produce nonetheless a food with a hygienically high quality and a low microbial count, high requirements have to be made on the raw materials and processing technology. If these requirements are not met, there is not only a risk of microbial spoilage before the sprouts reach the consumer, but also a risk of contamination with pathogenic microorganisms. If bacteria or moulds get to the sprouts during storage, in the course of germination or during the subsequent treatment until consumption, they can survive there. Because of the moist warm climate, the germination phase offers bacteria or moulds the possibility of multiplying. This applies to the non-specific germ count and for pathogens such as pathogenic *E. coli*, *Listeria monocytogenes*, *Salmonella* spp. and moulds.

Fresh sprouts are increasingly also used as topping on breads or to upgrade salads and are consumed untreated or shortly blanched, only. The most well-known are the sprouts of mung beans which in general are often (erroneously) referred to as soya bean sprouts. But also the consumption of other varieties such as alfalfa sprouts (US name for lucerne sprouts) or sprouts of lentils, radish, peas (Green Pees), beans and garlic which are appreciated because of their mild aromas, is increasing.

There are several systems for growing sprouts at home. In most cases sprouts are grown in special growing recipients. Growing recipients are widely spread through the so-called organic trade. Since growing recipients constitute an ideal breeding place for microorganisms of all kinds, the production of sprouts requires high hygiene standards as far as intermediate cleaning and disinfection is concerned.

But also discounters and large retail chains offer fresh sprouts in their range which no longer have to be grown but can be consumed immediately. The products are mostly offered in so-called trays made of plastic or cardboard with a wrapping film or enclosed plastic trays without protective gassing or other identifiable preservations (antibacterial films, inlays). The best before date is stated with up to 14 days.

The production sequence of a producer of sprouts offers many possibilities of introduction for spoilage agents or pathogenic microorganisms. After the introduction of a germ a multiplication of the microorganism or its persistence can occur on every level of production. The extrinsic factors such as mesothermal conditions in the growing recipient as well as the intrinsic factors such as a high water activity (a_w value) favour the survival and growth of pathogenic *E. coli*. Technological procedures for the reduction of germs are not involved in the production process of sprouts.

6.1.3.2.2 Possibilities of Microbiological Process Control

The technological aspects of sprout production have been described above and show clearly that the substrate properties of the sprouts permit both spoilage microorganisms and also pathogenic microorganisms not only to survive but also to grow. It, therefore, appears to be necessary and appropriate to comply with the principles of good hygiene practice (GHP) at the production of sprouts and moreover to apply the HACCP concept.

Already back in 2003 the Codex Committee for Food Hygiene (CCFH) pointed out in Annex II of the Code of Hygienic Practice for Fresh Fruits and Vegetables, based on the experience in food-borne outbreak investigations, that for instance *Salmonella* spp., pathogenic *E. coli*, *Listeria monocytogenes*, and *Shigella* spp. could occur on sprouts. As a possible cause CCFH identified the production conditions for seeds which are primarily due to animal feed and agricultural demands.

Investigations by BfR in 2009 on the germ contamination of sprouts and ready-to-eat salad mixtures confirmed the assumptions of the Codex Committee for Food Hygiene. Samples of fresh, packaged sprouts at retail had a very high germ contamination at the end of the best before date. The result also showed that germs can already strongly multiply on packaged sprouts within only a few days.

With the Code of Hygienic Practice for Fresh Fruits and Vegetables updated by CCFH in 2010 the Committee underlined once more the significance of hygienic production conditions because there are so far no appropriate methods for seeds and for sprouts in order to prevent the possible occurrence of pathogenic microorganisms or at least reduce them. The proposals of the Codex Committee for Food Hygiene to ensure a hygienic production of sprouts not only include measures within the framework of Good Hygiene Practice (GHP) but also Good Agricultural Practice (GLP) in order to avoid a contamination of seeds for sprouts.

A hygienic production of sprouts requires first of all a hygienic production of seeds through the control of waste waters and biomass, the chemicals used and the harvesting machinery. Also the further treatment, storage and transport have to be designed taking into account hygienic aspects. Investigations in respect of pathogenic microorganisms can prove compliance with these demands.

Food-business operators own incoming checks for seeds which are intended for passing on to end consumers or the production of sprouts can contribute towards checking compliance with the requirements during seed production. However, the validity of spot checks involves residual uncertainties. The results of the investigations must be documented.

Not only staff has to comply with hygiene requirements, but also the production plants themselves. According to the rules of GHP, it is also necessary to provide sufficient possibilities for hand washing and hand disinfection in addition to constructional, personnel and structural demands on the production plant. Furthermore, for instance wearing of hygienic clothing, gloves, leak-proof aprons, mouth protection and hair net is necessary in the production rooms.

For production plants a layout is required with which cross-contaminations can be avoided on all levels of the production. During the production process of sprouts the quality of the water used is of major significance. In accordance with the "Code of Hygienic Practice for Fresh Fruits and Vegetables" seeds can be decontaminated before sprouting for instance also with lactic acid solutions, whereby it is currently not yet possible to make any statement about the efficacy of such an acid treatment as far as EHEC O104:H4 is concerned. Sprouting, harvesting and storage of sprouts require high hygienic standards. Even after the completion of the production of sprouts, the process can be verified through microbiological controls.

By means of documentation at the critical hygiene points during the production of sprouts, as referred to, exemplarily, deviations which are relevant in terms of hygiene can be identified and corrective measures can be initiated.

6.1.4 Conclusion and Recommended Measures

The trace back of seed deliveries in Germany and other EU member states by the German authorities and the EFSA Task Force has shown that certain batches of fenugreek seeds are related to the EHEC outbreaks in Germany and France; this is confirmed by the risk assessment of EFSA and the ECDC of 29 June 2011 as well as a technical report by EFSA of 5 July 2011. According to EFSA, these batches were imported from Egypt.

For that reason fenugreek seeds for sprout production are considered by BfR as the most likely source of contamination of the pathogen at the horticultural farm in Lower Saxony, although the results of the microbiological analyses have so far been negative.

Fenugreek seeds of the mentioned origin which are used as single-variety or in blends for sprout production hence can constitute a hazard for human health. This also applies to fenugreek seeds which are dispensed in very small packs to the end consumer and are used for sprout production in the consumer's households.

At present there are no concrete indications suggesting that also other seed varieties and batches were contaminated with the outbreak strain due to non-hygienic production conditions in the country of origin or by cross-contaminations at intermediaries and recipients (e.g. during cleaning, blending and filling processes). Nonetheless this is not unlikely.

Given the severity of the diseases, BfR has issued the following recommendations on risk minimisation based on the current state of knowledge for the protection of the consumers:

Between the fenugreek seeds of a certain batch produced in 2009 (batch 48088) and used for sprout production in the horticultural farm in Lower Saxony as well as in France and diseases caused by EHEC O104:H4 there is a striking epidemiological connection. The horticultural farm in Lower Saxony used in April and May 2011 another fenugreek seed batch (batch 8266) produced in 2010 of the same origin for sprout production, which was delivered through the same intermediary. BfR, therefore, draws the conclusion that the two fenugreek seed batches used by the horticultural farm in Lower Saxony constitute a possible source of contamination of the pathogen. For that reason the competent authorities are recommended to completely identify the delivery routes of these fenugreek seed batches and to withdraw

these batches from the market. Concerning intermediaries and recipients of these batches, it should also be investigated whether their treatment processes, such as cleaning or bagging of the seeds, exclude cross contamination of further seed varieties and batches.

1. Between the fenugreek seeds of a certain batch produced in 2009 (batch 48088) and used for sprout production in the horticultural farm in Lower Saxony as well as in France and diseases caused by EHEC O104:H4 there is a striking epidemiological connection. The horticultural farm in Lower Saxony used in April and May 2011 another fenugreek seed batch (batch 8266) produced in 2010 of the same origin for sprout production, which was delivered through the same intermediary. BfR, therefore, draws the conclusion that the two fenugreek seed batches used by the horticultural farm in Lower Saxony constitute a possible source of contamination of the pathogen. For that reason the competent authorities are recommended to completely identify the delivery routes of these fenugreek seed batches and to withdraw these batches from the market. Concerning intermediaries and recipients of these batches, it should also be investigated whether their treatment processes, such as cleaning or bagging of the seeds, exclude cross contamination of further seed varieties and batches.
2. Furthermore, the competent authorities should inform food companies about these two batches of fenugreek seeds which, according to the findings of the trace back and trace forward investigations carried out in Germany and on the EU level, could be contaminated with the outbreak strain EHEC O104:H4. This information should enable the food companies to possibly take measures of risk minimisation in respect of their own stocks and products produced by them.
3. Within the framework of risk-oriented sampling sprouts as well as seeds of fenugreek should be controlled more intensely.
4. Since EHEC O104:H4 is a new, highly pathogenic pathogen, it should be characterised in more closely in regard to its properties, including its viability and growth behaviour on seeds and in sprouts.
5. BfR advises food companies in the restaurant and catering business (e.g. hotels, restaurants, canteens) to carefully consider any serving of sprouts for raw consumption to end consumers against the backdrop of the submitted assessment.
6. Both, the outbreak in France and findings from the trace forward carried out in Germany and on the EU level in respect of certain fenugreek seed batches suggest that fenugreek seeds in very small packs, including in blends, intended for sprouting in private households could be contaminated with the dangerous EHEC pathogen. The raw consumption of the germinated sprouts or a spreading of the pathogen in the kitchen could cause new cases of disease. Since it is not unlikely according to the current state of knowledge that there are still contaminated sprout seeds in private households available, BfR recommends to refrain from the germination of the sprouts and to discard all available seed packs with the residual waste.
7. Consumers are advised to continue to refrain from consuming raw sprouts, since it is not unlikely according to the current state of findings that sprout seeds contaminated with EHEC O104:H4 are still available on the market.
8. Since sprout seeds contaminated with EHEC O104:H4 can currently still be on the market, an enhanced surveillance of human EHEC infections should be maintained during the coming months, so that possible new cases of disease after the consumption of sprouts can be early detected.
9. Given the hygienic aspects of sprout production it appears to be necessary and appropriate not only to comply with the principles of Good Hygiene Practice (GHP) during the production of sprouts but also to apply the HACCP concept. Good Agricultural Practice (GLP) as a basis for the hygienic production of sprouts is likewise to be included, documented and supported by microbiological analyses. On this basis the probability of identifying deviations with relevance in terms of hygiene at critical hy-

- giene points during the production of sprouts and initiating corrective measures can be improved.
10. It is possible that persons producing or preparing foods are infected with EHEC O104:H4 without feeling ill. For that reason compliance with the general rules of kitchen hygiene is very important in order to avoid the transmission of pathogens to ready-to-eat foods.
 11. A continuation of the outbreak investigation is important in order to fully identify the introduction pathways of EHEC O104:H4 into the fenugreek seeds and to subsequently recommend concrete measures in terms of Good Manufacturing Practice for seeds and sprouts.

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6.2 Relevance of EHEC O104:H4 in Fenugreek Seeds which are processed into other Foods than Sprouts and Germ Buds

Updated Opinion No. 031/2011 of BfR of 26 July 2011

The BfR updated its Opinion No. 025/2011 of 11 July 2011 as to considerably emphasize the characteristics when using dry heat only for the elimination of EHEC on fenugreek seeds.

There is a high probability that the cause for the EHEC outbreak event in Germany and France in May and June 2011 was attributable to contaminated fenugreek seeds and sprouts grown from them (see BfR Opinion "Relevance of sprouts and germ buds as well as seeds for sprout production in the current EHEC O104:H4 outbreak event in May and June 2011"). According to the current state of knowledge, sprouts from fenugreek seeds were involved in the outbreak event. However, the seeds are not only used for sprout production. Therefore, the Federal Institute for Risk Assessment (BfR) has assessed the relevance of EHEC O104:H4 in fenugreek seeds which are used in different food including food supplements. Whether these products can cause an infection if processed with contaminated fenugreek seeds is primarily determined by the preparation and processing methods. Depending on the producer and product, these methods can be very different and are, thus, not known in detail to BfR.

According to the current state of knowledge it has to be assumed that the outbreak pathogen is only present in a very low concentration in fenugreek seeds. The capacity of EHEC to survive in the seeds of fenugreek depends on the initial germ content and the treatment processes applied. Since it is possible that the pathogen also occurs inside the seeds only thermal treatment methods (e.g. heating to 72 °C in moist environment for two minutes in the core of the seed), if necessary in combination with high pressure processes or irradiation are suitable to safely eliminate the germ. A chemical treatment such as cleaning with chlorine water etc. is not sufficient in order to safely eliminate any EHEC bacteria which may be in the seed core. Similarly, the germ survives maturing, drying, salting and acidification of foods.

Fenugreek seeds can be found in a large number of different foods such as cheese, herbal teas, mustard, curry spices and food supplements. For technological reasons and for reasons of taste fenugreek seeds are usually heated prior addition to foods.

Against the backdrop of the severity of the disease caused by EHEC O104:H4, food companies should examine whether material from a possibly contaminated fenugreek seed batch may already have been used or whether their processes are suited for the safe elimination of the germ in and on the seeds. In case of doubt they should withdraw the manufactured products from the market.

BfR moreover advises all consumers to thoroughly heat fenugreek seeds e.g. by roasting in a pan before further processing in private households.

Herbal teas containing fenugreek seeds should be infused with boiling water and left to draw for at least 5 minutes like all herbal teas. Water from hot water dispensers is generally not suited for the preparation of herbal teas since it is not hot enough to safely kill bacteria (see also BfR Opinion "Temperierte Heißwasserspender für Kräuterteeaufgüsse nicht geeignet").

6.2.1 Subject of the Assessment

In May and June 2011 there had been an accumulated occurrence of cases of disease involving the haemolytic-uraemic syndrome (HUS) and bloody diarrhoea in connection with an infection caused by enterohaemorrhagic *Escherichia coli* (EHEC) of the serotype O104:H4. By means of DNA sequence analysis it was determined that the outbreak strain has essentially more similarities with enteroaggregative *Escherichia coli* (EAggEC) than with conventional EHEC. Therefore, the pathogen is designated as enteroaggregative EHEC O104:H4 in the present assessment. The disease event affects all *Laender* in Germany but in particular Northern Germany. Sprouts contaminated with the outbreak pathogen from a horticultural farm in Lower Saxony are considered as the causal food vehicle. The results of epidemiological investigations support the conclusion that the outbreak pathogen was introduced through supplied fenugreek seeds into sprout production even if there is still a absence of laboratory diagnostic evidence. This conclusion correlates with the results of other epidemiological investigations which suggest that seeds are mostly, if not always, the source of sprout-related outbreaks (Puohiniemi et al., 1991; CDC, 1997a; Mahon et al., 1997).

In June 2011, an outbreak occurred with the enteroaggregative EHEC O104:H4 in France, during which home-grown sprouts were determined as the underlying cause. Amongst others, fenugreek seeds were used for the production of the sprout blend. The trace back of the fenugreek seed batch used in France revealed that a certain fenugreek seed batch produced in 2009 was supplied through the same intermediary located in Germany also to a horticultural farm in Lower Saxony where it was used for sprout production in spring 2011. Furthermore, the horticultural farm in Lower Saxony used another fenugreek seed batch for sprout production which was produced in 2010 and was supplied through the same intermediary. According to a risk assessment of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) of 29 June 2011 these two fenugreek seed batches were imported from Egypt.

Based on the risk assessment of EFSA/ECDC of 29 June 2011 BfR drew attention to the possible health risk of certain fenugreek seed batches in an Opinion on 30 June 2011. Based on this Opinion the competent *Land* for monitoring the German importer ordered the withdrawal of all batches of fenugreek seeds from Egypt for which the best-before date had not yet expired or had not expired for more than six months. Trace forward investigation at intermediaries in Germany showed that the fenugreek seed batch produced in 2009 was supplied from Germany to operations in at least 14 other countries.

On 5 July 2011 BfR published a risk assessment on the relevance of EHEC O104:H4 in sprouts and germ buds as well as sprout seeds during the outbreak event in May and June 2011. At its assessment BfR relied, amongst other things, on the investigation results of the German EHEC Task Force and the European EHEC Task Force which had been set up by EFSA due to the cross-boundary relevance of the outbreak event.

EFSA submitted a technical report on the investigation results of the European Task Force about the flows of commodity involving the suspected seed batches on 5 July 2011. According to this report a total of 37 tonnes of fenugreek seeds were imported from Egypt to Germany between December 2009 and February 2011. Since the origin of the contamination of the seeds is at present unknown and the possibility of a contamination of other seed types and batches exist, on 6 July 2011 the EU Commission took measures to protect consumers. The Commission ordered the recall and the innocuous destruction of the fenugreek seed batches which were imported between 2009 and 2011 from Egypt and identified within the scope of the trace back investigations on the EU level. Hence, an import ban for certain seeds from Egypt until 31 October 2011 was ordered (Commission Implementing Decision of 6 July 2011, 2011/402/EU).

Fenugreek seeds can be found in a large number of different products, such as curry spices and food supplements. At present there is no evidence that apart from sprouts other foods manufactured from fenugreek seeds caused enteroaggregative EHEC O104:H4 infections. Nonetheless, the possibility has to be considered that few pathogens can survive under certain conditions in or on the seeds and can again multiply in the intestines of humans. Therefore, it has to be investigated whether other foods including food supplements can constitute a risk for human health if they contain a produce of fenugreek seed (whole seeds, seed meals and powders, seed extracts) from Egypt.

Against this backdrop, BfR has made an assessment concerning the processing of products from fenugreek seeds at the production of other foods which should complement the risk assessment of 5 July 2011 on the relevance of sprouts and germ buds². However, BfR is so far not aware of the extent to which fenugreek seeds of the batches withdrawn from the market have been processed into other foods than to sprouts.

6.2.2 Result

There is hardly any or almost no knowledge about the behaviour and survival capacity (tenacity) of enteroaggregative EHEC O104:H4 alone or as part of a biofilm in the environment on or in food. Therefore, the risk assessment including the deduced recommendations is based to a large extent on findings concerning the behaviour of other EHEC strains (e.g. EHEC O157:H7) assuming that enteroaggregative EHEC O104:H4 have a comparable tenacity. For the assessment of the tenacity of the outbreak strain O104:H4 it is necessary to conduct additional scientific studies.

In the scientific literature predominantly treatment procedures for sprout seeds are described which should ensure a 5 log germ reduction although in this matrix only low concentrations of pathogenic germs are expected. This is required for seeds for sprout production because the mesothermal and moist conditions during sprout growing enable an intensive germ growth to proceed.

According to the current state of knowledge it has to be assumed that the outbreak pathogen exists only in a very low amount in fenugreek seeds. Therefore, it is, from BfR's point of view, not necessary to make similar demands on the decontamination of fenugreek seeds which are processed into other products than into sprouts if no multiplying of enteroaggregative EHEC O104:H4 can occur after the addition of the seeds. Under this condition BfR believes that treatment methods which can lead to at least a 2 log reduction of the model germ EHEC O157:H7 in and on seeds provide sufficient security.

It is possible to use heating processes as they are standard for other foods. For reasons of taste and technological reasons fenugreek seeds are usually heated prior to their addition to foods. Heating to at least 72 °C for two minutes in the core or a temperature time combination with a similar effect is appropriate to eliminate the pathogen in the seeds as well as in most of the other foods. This remains valid for the elimination of the pathogen in seeds in a moist environment (e.g. by hot water steam treatment). When using dry heat only, temperatures around 70 °C require a heat treatment of several hours. Furthermore, BfR considers the irradiation of seeds, as allowed for spices according to the Food Irradiation Regulation, to be sufficient for this intended purpose.

² Published under
http://www.bfr.bund.de/cm/343/bedeutung_von_sprossen_und_keimlingen_sowie_samen_zur_sprossenherstellung_im_ehec_o104_h4_ausbruchsgeschehen_im_mai_und_juni_2011.pdf

Chemical treatment processes, including the use of chlorine solutions or the addition of sulphur dioxide (SO₂) are basically not appropriate to eliminate pathogens which are possibly present in the core of fenugreek seeds.

Against the backdrop of the severity of the diseases the following recommendations are given at present by BfR for risk minimisation purposes, based on the current state of knowledge for the protection of consumers even if there are so far no indications suggesting that other products than sprouts have caused infections with enteroaggregative EHEC O104:H4 in Germany:

6.2.2.1 Recommendations for Food Companies

Recipients of recalled fenugreek seed batches should take the necessary measures of risk minimisation concerning their own stocks and their produced products. For that reason they should examine whether their production processes are suitable to eliminate the pathogen in and on the fenugreek seeds so that the products do not pose any infection risk for humans and no introduction into the environment occurs. In case of doubt they should withdraw the produced products from the market. Moreover, they should clarify whether a cross-contamination of other commodities may have taken place during storage or processing of the seeds.

6.2.2.2 Recommendations for the Competent Authorities

The competent authorities should inform the recipients of fenugreek seed batches which, based on the findings of the trace back and trace forward investigations carried out in Germany and on the EU level, could be contaminated with enteroaggregative EHEC O104:H4 of the possible health risk which may emanate from the seeds as well as products made thereof. Subsequently, they should examine together with the company whether the initiation of measures for risk minimisation is necessary.

6.2.2.3 Recommendations for Consumers

Consumers should infuse herbal teas including those with fenugreek seeds with boiling hot water and leave to draw them for at least 5 minutes. Water from hot water dispensers is not appropriate for the preparation of herb teas.

Consumers are advised to intensively heat fenugreek seeds prior to further processing in private households, e.g. by roasting in a pan.

As a matter of principle, BfR recommends to continue to investigate the survival capability and growth behaviour of enteroaggregative EHEC O104:H4 within the scope of scientific tenacity studies.

6.2.3 Rationale

6.2.3.1 Risk Assessment

6.2.3.1.1 Enterohaemorrhagic and enteroaggregative *E. coli* as Possible Hazard

Escherichia coli (*E. coli*) are naturally occurring in the intestines of humans and animals. Certain types of *E. coli*, such as EHEC or EAggEC can cause gastro-intestinal diseases in humans. Since EHEC can also occur in the intestines of ruminants and is excreted with faeces,

it can be transmitted directly or indirectly (e.g. through food) from animals to humans and cause diseases. According to the current state of knowledge it has to be assumed that the reservoir for EAggEC is of human origin. A transmission of EAggEC can occur through smear infection from human to human. The pathogen can also get into food during preparation or production and be spread in this way.

So-called atypical EAggEC can be isolated from calves, piglets and horses. These strains lack, however, certain properties so that it is currently assumed that these animals do not represent a reservoir for human pathogenic, typical EAggEC (Uber et al., 2006). In 2004 a study was conducted in Great Britain during which 1,227 *E. coli* isolates from cattle, sheep and pigs were screened for a certain EAggEC typical feature. None of the isolates displayed this feature. However, the authors specified, that with the method applied not all EAggEC could be comprised and therefore it cannot be excluded that bacteria of this kind occurred among the investigated bacteria (Cassar et al., 2004).

A characteristic property of EHEC is the production of Shiga toxins (*stx1* or *stx2*) and to attach through a certain protein (intimin) to the intestines of its hosts. The terms STEC (for Shiga toxin producing *E. coli*) or VTEC (for Verotoxin producing *E. coli*) are therefore used as synonyms for *stx1* or *stx2* producing EHEC. However, EAggEC normally does not produce Shiga toxins and attaches through adhesion factors (adhesins) to the human intestinal wall where it can form biofilms. This property of forming biofilms has been described for both EHEC and EAggEC, including for abiotic surfaces.

EHEC also belong to the most significant causes for food-borne bacterial infections due to the possible severe course of the disease. Since the mid-1990s EAggEC have already been described several times as causes for food-borne outbreaks with acute and persisting diarrhoea (Okeke and Nataro, 2001). This *E. coli* variant is mainly known from regions with deficient hygienic conditions. However, such outbreaks have also taken place in developed regions with a higher hygiene standard. The largest outbreak known so far took place in Japan where more than 2,500 children at different schools contracted an infection most likely through the school meals. The suspect school meals in this outbreak included bread, noodles, noodle salad, milk pudding, roast vegetables and milk (Itoh et al., 1997).

In a further study in Brazil during which the contents of 100 baby milk bottles (prepared by mothers from a poor socio-economic background) were examined for *E. coli*, EAggEC could be detected in three samples in a concentration of 10^3 - 10^4 colony-forming units (CFU)/ml (Morais et al., 1997). Studies on the investigation of causes underlying travel diarrhoea, with Mexico as the country of origin for the infection, have shown that EAggEC could be isolated from desserts with an average concentration of 0.5×10^4 CFU/g (Vigil et al., 2009). Water from open wells was likewise related to outbreaks.

The pathogenic role and the transmission pathway of *E. coli* strains which possess both EHEC and EAggEC-specific virulence factors (*stx* production and enteroaggregative adhesion) is at present almost unexplored. Morabito et al. already assumed in 1998 that such recombined strains can be just as pathogenic for humans as classical EHEC strains.

6.2.3.1.1.1 Characteristics of EAggEC EHEC O104:H4 (Outbreak Strain)

During the outbreak event in May and June 2011 the serotype O104:H4 was clearly identified as cause for the disease.

By means of DNA sequence analysis it was determined that the outbreak strain had essentially more commonalities with EAggEC than with the conventional EHEC. The outbreak strain is on the sequence level 93 % similar to a human EAggEC strain from Central Africa

which has been already characterised. The EHEC-specific feature of the outbreak strain is the bacteriophage-encoded *stx2-* gene. The outbreak strain is obviously a recombination of two *E. coli* pathotypes (EAggEC and EHEC) which lacks the typical *eae* (attaching and effacing) gene for EHEC.

The outbreak strain exhibits a resistance to beta lactam antibiotics of the groups acylaminopenicillines and cephalosporins as well as to tetracycline, nalidixic acid, streptomycin and trimethoprim/sulfamethoxazol. Furthermore, an extended spectrum beta lactamase (ESBL) of the CTX-M-15 type and a beta lactamase of the TEM-1 type were detected in all isolates.

6.2.3.1.1.2 Occurrence of EAggEC EHEC O104:H4

Occurrence in humans

Until the beginning of the outbreak in Germany in May 2011 only a few sporadic cases of *stx2*-positive *E. coli* of serotype O104:H4 have been described in literature. For example, ECDC reports about the infection of a person from Finland in 2010 who supposedly acquired the infection during a trip to Egypt. Concerning another case in France in 2004, details on the disease (including the place of infection) are not known according to the ECDC report. According to the Centers for Disease Control and Prevention (CDC) there were two HUS cases in Georgia in 2009. An Isolation of this serotype is described in the literature for a patient with HUS in Korea in 2005 as well as for two cases (both with HUS) in Germany in 2001. It has only been reported for the isolates from Germany (2001), Finland (2010) and Georgia (2009) that these were enteroaggregative EHEC.

Enteroaggregative *E. coli* of the O104:H4 type without Shiga toxin genes are known from at least one major English case control study with patients suffering from infectious intestinal diseases (Wilson et al. 2001).

Occurrence in foods

The occurrence of the serotype O104:H4 in foods had not yet been described in Germany and the EU until the outbreak event. Enteroaggregative EHEC O104:H4 was detected in Germany for the first time within the course of the current outbreak investigation in and/or on food. The detection was made in a sample cucumber and a sample sprout which had been sampled at different locations from kitchen refuse from persons infected by the outbreak pathogen. Furthermore, EAggEC EHEC O104:H4 was detected in three food samples (salmon raw and cooked, pepper), which had obviously been contaminated by an employee of a party service during the incubation period.

However, STEC/VTEC of other serotypes have already been detected in food for many years. In Germany STEC/VTEC are monitored within the scope of food-business operators own checks, controls of the official authorities as well as in the course of zoonoses monitoring programs. In the course of the controls of the official authorities, STEC/VTEC were detected particularly in fresh meat as well as in raw meat preparations and also game meat.

Within the EU, detections of STEC/VTEC in food of plantal origin (vegetables, fruit) were also reported. This always concerned non-O104:H4 strains.

Occurrence in animals and in the environment

The outbreak strain EAggEC EHEC O104:H4 had not been observed in animal stocks or in samples from the environment prior to the onset of the outbreak within the EU. None of the isolates differentiated at the National Reference Laboratory for *E. coli* (NRL *E. coli*) at the

BfR belonged to this serovar. Hence, within the course of notifications on zoonoses reporting the serovar was so far not reported.

According to the current state of knowledge, it must generally be assumed that the outbreak strain with its detailed described genetic features has its reservoir in humans since this *E. coli* type has so far not been found in animals. At present there are no indications suggesting that the outbreak strain has overcome the human-animal species barrier. However, it cannot be excluded that the outbreak strain could also colonise animals secondarily, for instance through the uptake of contaminated water or feed. At present it seems that the pathogen multiplies in humans and reaches the environment, e.g. the waste water, after release through faeces. It has to be assumed that for effective multiplication of the pathogen, it must again colonise humans.

6.2.3.1.1.3 Tenacity of Enterohaemorrhagic and Enteroaggregative *E. coli*

Hardly anything is known so far about the resistance of the outbreak strain in the environment. However, at present it cannot be excluded that the enteroaggregative EHEC O104:H4 strain can survive for a longer period of time in the environment, e.g. in water. Concerning its survival capability in food hardly anything is known either. Scheutz et al. investigated the outbreak strain in 2011 in terms of its capacity to form biofilms and figured out that, as it is typical for EAaggEC strains, it is a moderate to good biofilm producer. Furthermore, there are indications that the pathogen has a high acid resistance.

EHEC bacteria including the serovar O157 have been intensively researched. The current assessment including the deduced recommendations is based to a large extent on knowledge concerning the behaviour of EHEC O157:H7 assuming that enteroaggregative EHEC O104:H4 exhibit a comparable tenacity. EHEC are resistant to dehydration, freezing and acidification, so that they can survive in the environment (soil, water, faeces) for weeks and months.

EHEC bacteria of the serovar O157:H7 have the capacity to colonize both abiotic (Saldaña et al., 2009) and biotic surfaces such as lettuce with biofilms (Takeuchi et al., 2000). In biofilms these bacteria are more resistant to cleaning agents than in free life forms (Stopforth et al., 2003). The increase in tenacity of the EHEC bacteria depends to a large extent on the food matrix and the accompanying biotic and abiotic factors. For instance, tenacity is intensified if the biofilm consists besides the EHEC bacteria of further bacterial groups and if the biofilm remains undisturbed during the first 48 hours (Stopforth et al., 2003). EHEC can also form biofilms on the surface of iceberg lettuce and cos lettuce within a few hours (Patel et al., 2011). For that reason salad mixtures to which fenugreek has already been added can remain contaminated with the pathogen even after the removal of the fenugreek.

Since the persistence of the pathogen in food depends on the matrix and the applied food technology, the assessment of the effect of the individual processes requires not only knowledge on the processes themselves but also precise details on the matrix. Especially for oleiferous products it is known that the tenacity of pathogens is significantly higher. In the same way longer survival is proved in biofilms. The pathogen is not sufficiently inactivated with processes such as maturing, drying and acidification (Mathusa et al., 2010). EHEC germs can also be insensitive to salt. Many EHEC strains can multiply facing salt concentrations of 4 to 5 % at ambient temperature (25 °C) and some strains even survive at 15 % salt concentrations for at least 24 hours likewise at ambient temperature (Olesen und Jespersen, 2010; Cheville et al., 1996).

Tenacity to heat, high pressure and irradiation

For reasons of taste and technological reasons fenugreek seeds are usually heated prior to their addition to foods. For EHEC O157:H7 D-values (time to kill 90 % of the microorganisms of a population) for foodstuffs such as meat and milk are known. These D-values are similar to those of other *E. coli* types in a temperature range of 57 to 64 °C for times between 270 and 9.6 seconds. The fat content and the drying of foods can, however, increase the D-value. For an at least 2 log reduction of EHEC in and on fenugreek seeds it is, therefore, necessary to use higher temperatures, e.g. in moist environment at least 72 °C for 2 minutes in the core of the seed or a similar temperature time combination in terms of the mechanism of action. As shown by studies of Beuchat and Scouten in 2002 on the heat resistance of *E. coli* O157:H7 on alfalfa seeds, when using dry heat only, temperatures around 70 °C require a heat treatment of around 5 to 10 hours depending on the a_w -value.

For seeds for sprout production mostly combinations of several mild reduction processes for bacteria are applied within the meaning of a hurdle principle in order not to impair the germination capacity of the seeds. Studies on the inactivation of EHEC O157:H7 through thermal treatments of sprout seeds have shown that only at an application of dry heat at 70 °C for 24 hours or at 70 °C for 6 hours followed by high pressure treatment (600 MPa) for 2 minutes at 35 °C a 5 log germ reduction could be achieved (Neetoo and Chen, 2011). According to the current state of knowledge a heat treatment at 50 °C for one hour followed by equally distributed gamma irradiation (2 to 2.5 kGy) is supposed to be appropriate for a 4 to 5 log reduction of EHEC O157:H7 for different sprout seeds.

According to the current state of knowledge it has to be assumed that the irradiation of the seeds, as it is authorised for spices according to the Food Irradiation Regulation, can reduce the concentration of EHEC by at least 2 logs.

Tenacity to chemical treatment processes

In the case of treatments of sprout seeds by chemical processes it has to be assumed that they are not appropriate to eliminate any pathogens contained in the seeds.

Sulphur dioxide used for the preservation of foods can cause a germ reduction. Sulphur dioxide (SO₂) is authorised as a preservative and antioxidant agent for different foods (E220). SO₂ is used, inter alia, for dried fruit, but also for instance for dried potato products or dried or deep-frozen white vegetables with product-specific maximum amounts being authorised. In wine production the use of sulphur dioxide is common. For instance, a germ reduction of EHEC O157:H7 by up to 5 logs was achieved in different sour apple juice products through the use of 50ppm SO₂ (Basaran-Akgul et al., 2009).

Ethanol as one of the most well-known antimicrobial substances likewise influences the survival of EHEC O157:H7. However, the order of magnitude of the germ reduction currently cannot be assessed.

Tests for the decontamination of foods contaminated with EHEC O157:H7 with 0.5, 1.0 and 1.5 % organic acids proved to be ineffective and underline the acid tolerance of this pathogen (Brackett et al., 1994). In the laboratory it can be shown that cultures with 3 x 10⁴ CFU/ml EHEC O157:H7 are stable after 24 hour incubation both at 4 °C and at 24 °C at pH 3.4 and pH 11. At pH 2 there is only a slight reduction (0.5 – 1 log) of the germ count (Miller and Kaspar, 1994). Tests with artificial gastric juice suggest that not only EHEC O157:H7 survive at pH 1.5 but also other pathotypes such as enteropathogenic *E. coli* are extremely acid tolerant (Arnold and Kaspar, 1995). Given this data situation and the capacity of the

outbreak strain to form biofilms it should generally be assumed that there is an increased insensitivity to chemical treatments.

Investigation results showed that treatments of sprout seeds with chlorine solutions, which contain, for instance 2 % chlorine from calcium hypochlorite do not yield a full elimination of EHEC germs (Fett et al., 2005). Inter alia, this observation is possibly due to the fact that these germs show a higher chlorine tolerance in biofilms. For bacteria in biofilms an about 100-fold higher chlorine tolerance is to be expected (Prof. Exner, University of Bonn, personal communication of 21 June 2011).

Since EHEC O104:H4 is a new, highly pathogenic germ, it should be characterised in more detail in terms of its properties, including its survival capacity and its growth behaviour in different matrices.

6.2.3.1.2 The Hazard Potential in the EHEC O104:H4 Outbreak Event

The infective dose of the known outbreak pathogen EHEC O157 is very low and amounts to less than 100 germs. No data are available about the infective dose of the enteroaggregative EHEC O104:H4 but it has to be assumed that it is likewise very low.

At present it has to be assumed that the pathogen does not have to multiply in the environment or in the products in order to infect humans. An efficient multiplying of the pathogen seems to occur in particular in the gastrointestinal tract of humans. This can also cause severe courses of disease.

In May and June 2011 there had been an accumulation of the so-called haemolytic-uraemic syndrome (HUS) and bloody diarrhoea in connection with infections caused by enteroaggregative EHEC O104:H4. The majority of the diseases caused by the outbreak pathogen occurred as non-bloody, mostly watery diarrhoea. In part of the patients a haemorrhagic colitis developed with spasmodic stomach pains, bloody stool and partly fever. However, EHEC infections can also have an unapparent and hence unnoticed course. A feared complication is HUS. The full picture of HUS is characterised by acute renal failure up to anuria, haemolytic anaemia (low blood) and thrombocytopenia (lack of blood platelets). Typically diarrhoea, often bloody, precedes HUS. This severe complication occurs in approximately 5 to 10 % of the symptomatic EHEC infections. It frequently leads to short-term obligatory dialysis and more rarely to an irreversible renal function loss with chronic dialysis. During the acute phase the lethality of HUS is approximately 2 %.

Within the scope of the outbreak caused by serotype O104:H4 also neurological symptoms were frequently observed in clinically diseased persons which might be attributable to the fact that the outbreak pathogen is an enteroaggregative strain with the property of EHEC to form Shiga toxins.

The incubation time for EHEC infections is usually 2 to 10 days (on average 3 to 4 days) whereby these data are essentially based on investigations on EHEC of serogroup O157. In the outbreak event caused by enteroaggregative EHEC O104:H4 a median incubation time of 8 days (interquartile interval 7-9 days) is assumed. During this outbreak the symptoms of EHEC-associated HUS diseases commenced in the median 5 days (interquartile interval 4-6 days) after the onset of the diarrhoea (data as of 18 June 2011).

For further information reference is made to the risk assessment of BfR of 5 July on the relevance of EHEC O104:H4 in sprouts and germ buds as well as sprout seeds in the outbreak event in May and June 2011.

6.2.3.1.3 Exposure Assessment

Different effects are attributed to fenugreek. Fenugreek seeds contain apart from protein approximately 6 to 10 % fat, saponines, bitter substances, mucilaginous substances and vitamins. The seeds have a slightly bitter taste which disappears after cooking or roasting.

Quantitative data on germ concentrations of pathogenic food germs on sprout seeds are limited. Quantitative analyses of seeds whose sprouts caused a disease after consumption resulted in germ counts in a range of less than 1 to 6 CFU/100 g seeds. During microbiological investigations within the framework of the intensive investigation activities of the *Laender* on the EHEC outbreak event, EHEC bacteria were only found in one of more than 900 investigated samples of sprouts and seeds for their production. Detection was merely successful in a sprout blend from an opened package which was collected from the kitchen refuse of a diseased person.

Experimental investigations have shown that some EHEC strains can also penetrate inside plants through the roots. For alfalfa the entrance of pathogenic and apathogenic bacteria into the inside of the seed has already been observed. It is assumed that bacteria get access to the plant through fissures in the lateral roots (Dong et al., 2003). For fenugreek seeds this is so far not yet known.

The survival capacity of EHEC bacteria in products from fenugreek seeds depends on the treatment processes applied. If fenugreek is used in the form of an extract, it has to be assumed that the existing bacteria concentration is reduced by extraction agents (e.g. ethanol) and heat effect. Whether this effect leads to a full elimination of possibly existing enteroaggregative EHEC O104:H4 cannot be assessed for the moment. However, for fenugreek seed powder a survival capacity is to be assumed, because during cleaning, grinding and blending of a fenugreek seed powder no increased temperatures are to be expected.

6.2.3.1.3.1 Processing of Fenugreek Seeds in the Food Production

Cheese

Fenugreek seeds are contained in some young and medium-aged semi-hard cheeses as an ingredient in order to provide them with a nutty flavour. Fenugreek seeds are usually heated prior to the addition to cheese for reasons of taste and technological reasons. Whether each cheese factory handles the production in this way and which temperatures are reached thereby, is not known to the BfR. Cheeses with fenugreek seeds are also produced by small cheese makers and distributed via the Internet. Young semi-hard cheese matures for a period of up to 5 weeks, medium-aged semi-hard cheese matures, however, for up to 3 months. It has to be assumed that the processes of cheese making do not have an influence on the survival of enteroaggregative EHEC O104:H4 possibly occurring inside the fenugreek seeds. But also the survival of pathogens adhering externally to the seeds is not unlikely since EHEC of other serotypes have been detected in semi-hard raw milk cheese (Zweifel et al., 2010).

Spice blends and condiments

Fenugreek seeds are used in a ground condition for the production of spice blends, mainly curry powder. In Indian curry spice blends fenugreek seeds are a standard component.

For the production of spices thermal processes such as hot water steam treatment or irradiation can be used for germ reduction purposes. Appropriate thermal inactivation processes include both extruder and vacuum processes. Extruder processes achieve a germ reduction of approximately 10^2 CFU/g in spices. With vacuum processes the contamination of spices can be reduced to germ counts of significantly below 5000 CFU/g. In both processes hot steam is used whose temperature varies according to the specificities of the product. Nonetheless it can be assumed that for industrial production processes for spices low concentrations of enteroaggregative EHEC O104:H4 would be eliminated.

For the irradiation of spices and dried aromatic herbs the average absorbed total dose may not exceed 10 kilogray in accordance with §1, para 2, No.1 of the Regulation for the treatment of foods with electron, gamma and x-rays, neutrons or UV rays (Food Irradiation Regulation – LMBestRV). This irradiation dose would be appropriate to kill enteroaggregative EHEC O104:H4 possibly occurring in low amounts.

Fenugreek seeds are also offered "pure" or as seed meal for seasoning and/or own production of spice blends.

Furthermore, fenugreek seeds are used for the production of certain mustard specialities. The production of mustard includes essentially grinding and mixing processes as well as a certain fermentation time in order to produce the typical mustard aroma. During the grinding processes temperatures of 50 °C may not be exceeded in order to preserve the volatile aromas. Traditionally produced mustard varieties contain usually no preservatives. If fenugreek seed meal is processed during mustard production it is not to be expected that the processes of mustard production will fully eliminate possibly existing enteroaggregative EHEC O104:H4. For that reason products based on fenugreek seeds which are further processed into mustard should be treated prior to their addition with an appropriate germ reducing process.

Fenugreek seed extract is also added to liquid spicy sauces (e.g. soya bean and fish sauces) for reasons of taste. It has to be assumed that the existing bacteria concentration is reduced by the extraction step. However, whether this effect leads to a full elimination of possibly existing enteroaggregative EHEC O104:H4, cannot be assessed at present. If a further heating to at least 72 °C occurs during the preparation of the spicy sauces, a survival of the pathogen is unlikely.

Tea

Products based on fenugreek seeds are also contained in certain breastfeeding teas (tea-bags). According to the current state of knowledge, it has to be assumed that infusing with boiling water and leaving the tea for at least 5 minutes could eliminate externally adhering EHEC germs to such an extent that no infection risk exists any more since the seeds themselves are not consumed.

Food supplements

There are many food supplements, e.g. in the form of capsules, which contain fenugreek seeds frequently combined with other components. Seed extract or powder is processed whereby the survival capacity of EHEC in seed powder is likely to be higher than in seed extracts. Given the large number of producers working in this field and the many production processes used, no general statement can be made on the survival capacity of EHEC in food supplements. According to knowledge available to BfR some products use "activated fenugreek"; for activation purposes the seeds are undergoing a special thermal treatment. However, frequently thermal processing of the seeds is deliberately waived at the production of these products. Especially for these products it must be assumed that existing EHEC bacteria can survive.

In the past cases became known in which a contamination of food supplements resulted in diseases such as salmonella in hemp-based products. Several producers explicitly mention a thermal treatment of their products and/or the individual ingredients.

6.2.3.1.4 Risk Characterisation

The consumer risk which emanates from differently treated products of fenugreek seed contaminated with the dangerous germ which were further processed into other foods than sprouts and germ buds is characterised below. This does not include the risk that EHEC bacteria can form biofilms on many different abiotic surfaces so that all utensils used while handling contaminated seeds and products made thereof can contribute to a continuous contamination.

Situation 1:

Fenugreek seeds contaminated with enteroaggregative EHEC O104:H4 were further processed into foods (apart from sprouts and germ buds) and were not previously treated or only treated insufficiently so that the pathogen was not fully eliminated.

It is possible that material from the stock of at least one contaminated fenugreek seed batch was used in a manufacturing plant for foods including food supplements. If contaminated fenugreek seeds have been processed without having been sufficiently treated in advance, depending on further technological effects, it has to be expected that the pathogen survives in cheese, mustard, teabags and food supplements.

The risk of an infection with enteroaggregative EHEC O104:H4 would exist, more particularly, for the direct consumption of these cheeses and mustard types as well as food supplements with fenugreek seeds and seed powders. Other disease outbreaks caused by enteroaggregative EHEC O104:H4 would be possible.

An infection after the consumption of food supplements and liquid spicy sauces from seed extract would, however, be hardly likely. After appropriate preparation of tea there would be no risk of infection in accordance with the current assessment.

Situation 2:

Fenugreek seeds contaminated with enteroaggregative EHEC O104:H4 were further processed into other foods than sprouts and germ buds and previously treated in such a way that the pathogen was completely eliminated.

If contaminated fenugreek seeds have been sufficiently treated before their further processing and if a recontamination with enteroaggregative EHEC O104:H4 would be impossible, one could assume that cheeses, mustard and food supplements with products based on fenugreek seeds do not pose any risk for human health.

6.2.3.1.4.1 Assessment of the Severity of the Health Impairment

The health impairment has to be assessed as severe. It concerns a very severe clinical picture which can lead from bloody diarrhoea via renal failure with obligatory dialysis, severe neurological symptoms up to death. The period during which the health damage caused persists, leads to chronic courses (e.g. with permanent renal damage) or is reversible and which late sequelae can occur, cannot be assessed for the moment. Further fatalities cannot be excluded either.

6.2.3.1.4.2 Assessment of the Quality of the Data

Processing methods

The current state of knowledge concerning the use of fenugreek seeds outside sprout production as well as the manufacturing processes for products which contain fenugreek seeds is low. The manufacturing processes can be very different depending on the producer and the products and thus are not known to BfR in detail.

Tenacity of the pathogen

The quality of the data referred to the outbreak pathogen is to be assessed as highly incomplete. Hence, EHEC pathogens and EAggEC were used as model germs to assess the possible risks. But also for these germ types data have to be considered as incomplete concerning the assessment of products and production processes. This uncertainty was considered accordingly during the assessment.

6.2.4 Conclusion and Recommended Measures

The trace back investigations by the German authorities and the EFSA Task Force of seed supplies to Germany and other EU member states have shown that certain batches of fenugreek seeds are connected to the EHEC outbreaks in Germany and France; this was confirmed by the risk assessment of EFSA and ECDC of 29 June 2011. According to EFSA, these batches were imported from Egypt.

Fenugreek seeds are not only used for sprout production but can be found in a large number of different products such as curry spices and food supplements. BfR is currently not aware whether material from the stock of at least one contaminated fenugreek seed batch has been used in a manufacturing plant for foods including food supplements.

There is so far no evidence that apart from sprouts also other products manufactured from fenugreek seeds have caused enteroaggregative EHEC O104:H4 infections. Nonetheless, the possibility must be taken into account that few pathogens survive under certain conditions in or on the seeds and can again multiply in the intestines of humans. For the period during which the pathogen is viable on or in seeds, in case of insufficiently treated seeds a transmission of the pathogen to humans via the seed itself or products thereof is possible.

There is hardly any or almost no knowledge about the behaviour and the survival capacity of enteroaggregative EHEC O104:H4 alone or as part of a biofilm in the environment, on or in foods and in view of a possible colonisation of animals. Therefore, the risk assessment including the deduced recommendations is based to a large extent on findings concerning the behaviour of other EHEC strains (e.g. EHEC O157:H7) assuming that enteroaggregative EHEC O104:H4 have a comparable tenacity. For the assessment of the tenacity of the outbreak strain O104:H4 it is necessary to conduct further scientific studies.

In the scientific literature predominantly combinations of different treatment procedures for sprout seeds are described which should ensure a 5 log germ reduction together with the preservation of the germination capacity, although in this matrix only low concentrations of pathogenic germs are expected. This is required for seeds for sprout production because the mesothermal and moist conditions during sprout growing enable an intensive germ growth to proceed.

From BfR's point of view it is not necessary to make a similar demand on the decontamination of fenugreek seeds which are processed further into other products apart from sprouts, as far as any multiplying of enteroaggregative EHEC O104:H4 after the addition of the seeds cannot occur. Under these conditions BfR considers treatment processes as appropriate for this purpose since they can reduce the model germ EHEC O157:H7 in and on seeds by at least 2 logs.

To this end, heating processes can be applied as they are standard for other foods, too. For reasons of taste and technological reasons fenugreek seeds are usually heated prior to their addition to foods. Heating to at least 72 °C for 2 minutes in the core or a comparable temperature time combination with a similar effect is appropriate to eliminate the pathogen in the

seeds as well as in most of the other foods. This remains valid for the elimination of the pathogen in seeds in a moist environment (e.g. by hot water steam treatment). When using dry heat only, temperatures around 70 °C require a heat treatment of several hours. Moreover, BfR considers that the irradiation of the seeds, as allowed for spices according to the Food Irradiation Regulation, is sufficient for this intended purpose.

Chemical treatment processes, including the use of chlorine solutions or the addition of sulphur dioxide (SO₂) are basically not appropriate to eliminate pathogens which are possibly present in the core of fenugreek seeds.

Against the backdrop of the severity of the diseases, on the basis of the current state of knowledge, BfR makes the following recommendations for risk minimisation purposes to protect consumers, even though there are so far no indications suggesting that in Germany other products than sprouts have caused infections with enteroaggregative EHEC O104:H4:

1. Recipients of recalled fenugreek seed batches should take the necessary measures of risk minimisation concerning their own stocks as well as their produced products. For that reason they should examine whether their production processes are appropriate to eliminate the pathogen in and on the fenugreek seeds so that the products do not pose any infection risk for humans and no introduction into the environment occurs. In case of doubt they are to withdraw the produced products from the market. The recipients of these batches should, moreover, clarify whether there could have been a cross-contamination of other commodities may have taken place during storage or processing of the seeds.
2. The competent authorities should inform recipients of fenugreek seed batches which, based on the findings of the trace back and trace forward investigations carried out in Germany and on the EU level, could be contaminated with enteroaggregative EHEC O104:H4 of the possible health risk which may emanate from the seeds as well as products made thereof. Subsequently, they should examine together with the company whether the initiation of measures for risk minimisation is necessary.
3. Food companies which process fenugreek seeds should review the risk analysis to be conducted within the framework of the HACCP (Hazard Critical and Control Points) concept in view of the EHEC O104:H4 outbreak and possibly adjust it. Since pathogenic germs can possibly penetrate the inside of plants through the roots, seeds should be treated anyway before further processing in such a way that possibly existing pathogens in the seed core can be safely killed.
4. Consumers should infuse herb teas including those with fenugreek seeds with boiling water and leave to draw them for at least 5 minutes. Since herb teas can be contaminated with pathogens, water from hot water dispensers is generally not appropriate for the preparation of herb teas. This has already been pointed out by BfR in an Opinion back in 2005.
5. BfR advises consumers to thoroughly heat fenugreek seeds before their further processing in the private household e.g. by roasting in a pan, in order to kill possibly existing pathogens on or in the seeds. Any seed meal and spice blends made thereof, derived from untreated fenugreek seeds should as a matter of precaution not be consumed but discarded with the household refuse.
6. As a matter of principle BfR advises to continue to research the survival capacity and the growth behaviour of enteroaggregative EHEC O104:H4 within the framework of scientific tenacity studies.

6.2.5 References

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6.3 EHEC Outbreak 2011: Updated Analysis as a Basis for Recommended Measures

BfR opinion No. 049/2011, 23 November 2011

The EHEC O104:H4 outbreak in Germany of the early summer 2011 is now over. The investigations in Germany and the European Union have been completed. It is thought that the outbreak was caused by fenugreek seeds imported from Egypt which were subsequently used to produce sprouts by a horticultural farm in Lower Saxony and by private individuals. Where and how the seeds came into contact with the pathogen leading to the outbreak could not be determined.

In both Germany and Europe, task forces were formed to identify the food that had caused the outbreak and to reconstruct the distribution channels of the suspected seed batches. The Food and Veterinary Office of the European Commission (FVO) conducted investigations in Egypt. Those investigations showed flaws in the production of seeds intended for human consumption. However, EHEC O104:H4 was not detected in the seeds from Egypt. It is to be assumed that the pathogen only exists on or in the seeds in very low germ counts and that they are unevenly distributed across the batches, meaning that they are difficult to detect. In consequence, a negative test result does not mean that EHEC O104:H4 was not present in the seeds.

Once the recall measures for suspected batches of fenugreek seeds from Egypt had been completed, the Federal Institute for Risk Assessment (BfR) undertook an analysis of the available information and on that basis made recommendations. Essentially, eating raw sprouts entails the risk of contracting disease. In its expert opinion published on 15 November 2011, the Panel on Biological Hazards of the European Food Safety Authority (EFSA) too concludes that sprouts pose a microbiological risk from a food safety viewpoint. The reasons for this are that seeds may be contaminated with pathogens and that the cultivation conditions for sprouts facilitate multiplication of pathogens. This is exacerbated by the fact that sprouts are often not at all or only lightly heated before consumption. As a result, consumers could potentially eat existing pathogens on the sprouts, for light heating is insufficient to eliminate them safely.

For this reason, strict hygienic standards must be observed when cultivating, storing, treating and transporting seeds used in the production of sprouts in order to minimise the risk of contamination with pathogens to the greatest extent possible. In addition, producers of sprouts are advised to use only seeds that have been cultivated specifically for this purpose. Equally, suitable germ-reducing measures should be taken before cultivation wherever possible, especially if the sprouts are intended for raw consumption.

The BfR points out to consumers that pathogens can be eliminated by cooking or frying the sprouts. Persons with a weak immune system should, to be on the safe side, therefore only eat sprouts after they have been sufficiently heated. In order to reduce contamination by germs, sprouts that are eaten raw should be washed thoroughly and consumed as quickly as possible. Germs cannot be safely eliminated by washing the sprouts, however. As a precaution, fenugreek seeds purchased before October 2011 should not be allowed to sprout. They should instead be used in thoroughly cooked dishes or disposed of as household rubbish.

6.3.1 Subject of the Assessment

Between May and July 2011, Germany saw a succession of cases of the haemolytic uraemic syndrome (HUS) and bloody diarrhoea following infection with enterohaemorrhagic *Escherichia coli* (EHEC) of serotype O104:H4. DNA sequence analysis showed that the

strain of bacteria causing the outbreak has much more in common with the enteroaggregative *Escherichia coli* (EAggEC) than with conventional EHEC. The pathogen responsible for the outbreak is therefore also called enteroaggregative EHEC O104:H4 or EAggEC O104:H4. In this assessment, the term “EHEC O104:H4” is used for enhanced readability.

Although infections occurred all over Germany, North Germany was most heavily affected. According to the Robert Koch Institute (RKI), a total of 2987 cases of bloody diarrhoea and 855 cases with HUS were attributed to the outbreak; 53 persons died as a result of the infection. Fenugreek seeds imported from Egypt which were used for sprout production both by a horticultural farm in Lower Saxony and by private individuals were, upon completion of the investigation into the outbreak, seen as the cause of the illness.

To protect the population from infection with the EHEC O104:H4 pathogen, the authorities recommended on 10 June 2011 that as a precaution consumers refrain from consuming raw sprouts and seedlings until further notice.

On 24 June 2011, France reported a cluster of HUS/EHEC cases near Bordeaux with the onset occurring between 15 and 20 June 2011. The results of the studies conducted revealed that the French and the German strain causing the outbreak are genetically related and exhibit the same virulence and resistance profile. It is therefore to be assumed that the EHEC O104:H4 strains isolated in connection with the outbreaks in Germany and France in the early summer of 2011 are identical.

The persons who diseased near Bordeaux had eaten sprouts produced in a French leisure centre for children from three different types of seeds. Only fenugreek seeds were present in both the sprout mixture eaten in France and in the sprout mixtures of the horticultural farm in Lower Saxony, which were linked to EHEC O104:H4 cases in Germany. In a household in Lower Saxony too several people became ill following consumption of self-cultivated sprouts from a seed mixture containing, among other ingredients, fenugreek seeds.

Due to the international significance of the EHEC O104:H4 outbreaks in Germany and France, a task force that included German representatives was established at the end of June 2011 at the European Food Safety Authority (EFSA) coordinating further investigations into the causes of the outbreak at the EU level. The international epidemiological reconstructive investigation concluded that fenugreek seeds which had been imported from Egypt were in all likelihood the cause of the EHEC O104:H4 outbreaks in Germany and France. Retracing of the fenugreek seed batch used in France showed that a specific batch of fenugreek seeds produced in 2009 had also, via the same German-based distributor, made its way to a horticultural farm in Lower Saxony where it was used for sprout production in the spring of 2011. In addition, the horticultural farm in Lower Saxony used an additional fenugreek seed batch, originally produced in 2010 and supplied by the same distributor, for sprout cultivation in April and May 2011.

On 5 July 2011, the EFSA presented a technical report on the investigation findings of the European Task Force about the flow of goods of suspected seed batches. According to this report, at least 37 tons of fenugreek seeds were imported into Germany from Egypt between December 2009 and February 2011. The European Commission took measures to protect consumers on 6 July 2011. The Commission ruled that the fenugreek seed batches imported from Egypt in the period 2009-2011 which had been identified as part of the reconstructive investigation at EU level must be recalled and destroyed in a non-harmful way. In addition, it imposed a ban on imports of certain seeds from Egypt (Commission Implementing Decision from 6 July 2011, 2011/402/EU) until 31 October 2011.

The affected food business operators are responsible for implementing this ruling, whereas supervision of these measures is incumbent upon the food safety authorities of the federal states concerned. Affected federal states collected data on the distribution and residual stocks and communicated investigation results via the Rapid Alert System for Food and Feed (RASFF) of the European Union. Furthermore, as part of risk-oriented operating controls, the possibilities of cross-contamination between the importer, the distributor and sprout producers were investigated.

In July 2011, neither the Federal Office of Consumer Protection and Food Safety (BVL) nor the BfR had received any indications from state authorities that cross-contamination had occurred for any other types of seeds. As a result, it was possible to update the consumption recommendation from 10 June 2011. To protect consumers from infection with EHEC O104:H4, the German federal authorities recommended on 21 July 2011 that as a precaution fenugreek seeds imported from Egypt as well as sprouts and seedlings cultivated from them were not to be eaten raw until further notice.

The EFSA withdrew its recommendation “not to cultivate any sprouts for domestic consumption and to only eat sprouts and seedlings after they have been sufficiently cooked” in a press release from 3 October 2011, after it had been informed by the European Commission of the completion of the retracing activities along the food supply chain in the EU member states.

To establish the actual infection source of the EHEC O104:H4 outbreaks in Germany and France, the Food and Veterinary Office of the European Commission (FVO) conducted an audit in Egypt between 21 and 25 August 2011. The FVO inspectors also assessed the production and processing conditions for seeds which were presumably the cause of the outbreak. The results of the audit carried out in Egypt revealed shortcomings in the production of seeds for human consumption which may be allowed to germinate. At the production sites for fresh legumes intended for direct human consumption, these shortcomings were not observed, however. The importation of fresh or chilled legumes, with the exception of seedlings, was therefore permitted again by means of a changed Commission Implementing Decision of the European Commission from 6 October 2011.

In the opinion of the European Commission, the measures taken by the competent authorities in Egypt do not minimise the risks observed to a sufficient degree. With its Commission Implementing Decision of 28 October 2011, the European Commission therefore extended the import restrictions for certain seeds and beans from Egypt from 6 October 2011 to 31 March 2012.

At the request of the European Commission, the Panel on Biological Hazards of the EFSA conducted a risk assessment over the last few months of the production chain for sprouts and seedlings in the EU. On 15 November 2011, the EFSA published its expert opinion “Scientific Opinion on the Risk Posed by Shiga Toxin Producing *Escherichia Coli* (STEC) And Other Pathogenic Bacteria in Seeds And Sprouted Seeds”.

Against this background, the BfR undertook an analysis of the available information on the recalled fenugreek seed batches from Egypt and on that basis recommended measures for the production and treatment of sprouts and seedlings. The opinion also complements the two risk assessments on the EHEC outbreak³ already published by the BfR in July 2011. For

³ Published at

http://www.bfr.bund.de/cm/343/bedeutung_von_sprossen_und_keimlingen_sowie_samen_zur_sprossenherstellung_im_ehec_o104_h4_ausbruchsgeschehen_im_mai_und_juni_2011.pdf
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enhanced readability, sprouts and seedlings are summarised as “sprouts” henceforth in this document.

6.3.2 Result

The EHEC O104:H4 outbreak of the early summer 2011 in Germany is now over. According to the RKI, it was the largest outbreak by EHEC infection in Germany so far and, with regard to the number of reported HUS cases, the largest outbreak of its kind reported anywhere in the world. Fenugreek seeds imported from Egypt which were subsequently used for sprout production both by a horticultural farm in Lower Saxony and by private individuals are, upon completion of the investigations, seen as the cause of EHEC outbreak. Where and how the seeds came into contact with the pathogen leading to the outbreak is not known. The pathogen causing the outbreak was not detected in the tested fenugreek seeds. However, a negative test result does not mean that EHEC O104:H4 was not present in the seeds.

The recall of suspected seed batches has significantly reduced the risk of consumers of contracting an EHEC infection following the consumption of raw sprouts made from these fenugreek seeds.

Irrespective of the EHEC outbreak which is now over, the consumption of raw sprouts generally involves a non-quantifiable risk of contracting a food-borne infection. The reasons for this are that seeds used may be contaminated with pathogens and that the cultivation conditions for sprouts facilitate the multiplication of existing pathogens. In addition, sprouts are not at all or only lightly heated before consumption, meaning that pathogens may survive. The Panel on Biological Hazards of the EFSA too concludes that sprouts pose a microbiological risk from a food safety viewpoint.

Based on the insights gained in the course of the investigation into the outbreak and on the current state of knowledge generally, the BfR makes the following preventive recommendations to ensure the protection of consumers from food-borne infections:

1. When cultivating, storing, treating, transporting and analysing seeds used in sprout production, at least the standards of the Codex Alimentarius (Annex 2 of the CODEX Code of Hygienic Practice for Fresh Fruits and Vegetables CAC/RCP 53/2003) should be observed.
2. Sprout producers are advised to use only seeds which were cultivated for this purpose and which comply with the above-mentioned standards of the Codex Alimentarius. Wherever possible, the seeds should be treated with suitable germ-reducing procedures before cultivation, especially if the sprouts may be intended for raw consumption. The procedures described in the literature must, however, be optimised for the seed types to be used before they are applied. The Panel on Biological Hazards of the EFSA recommends that the safety and effectiveness of the treatment procedures for seeds be evaluated and harmonized at EU level.
3. Sprout producers are advised to monitor critical points in the production process by means of microbiological checks at regular intervals, for example by testing intermediate products (e.g. germinated seeds 48 hours after germination) and swab samples from the production environment.
4. Food business operators who circulate seeds for the production of sprouts in private households should only use seeds which were cultivated for that purpose and which comply with the above-mentioned standards of the Codex Alimentarius. The BfR advises those circulating such sprout seeds to conduct microbiological tests on the received batches to establish whether they contain pathogenic germs and to supplement these tests by treating or having treated the seeds with suitable germ-reducing procedures prior to packing them in end user packaging.

5. As a precaution, it is recommended to consumers not to allow fenugreek seeds purchased before October 2011 to germinate. The seeds should instead be used in thoroughly cooked dishes or disposed of as household rubbish.
6. In addition, the BfR advises consumers producing their own sprouts only to use seeds which are marketed for sprout production by the producer.
7. Persons with a not fully developed or weak immune system (infants, pregnant women, the elderly and sick people) should, as a precaution, only ever eat sprouts after they have been sufficiently heated (boiling, frying).
8. To reduce germ contamination, sprouts that are to be eaten raw should be thoroughly washed and consumed as quickly as possible. Pathogens cannot be safely eliminated by washing the sprouts, however.
9. General rules of body and kitchen hygiene should be observed in order to avoid human-to-human transmission (smear infection) and contamination of foods with pathogens.

In terms of prevention of food-borne infections, the growth and survival of enteroaggregative EHEC should be researched in various food matrices including seeds and sprouts. This research should also probe the question what influence accompanying flora living on sprouts has on the growth and survival of pathogens. Moreover, research is needed on the detection of enteroaggregative EHEC in the "sprout production" food supply chain.

6.3.3 Rationale

6.3.3.1 Risk Assessment

6.3.3.1.1 Enterohaemorrhagic and Enteroaggregative *E. coli* as a Potential Hazard

Escherichia coli (*E. coli*) are naturally found in the intestine of humans and animals. Certain types of *E. coli* such as EHEC and EAggEC can cause gastrointestinal disease in humans. Since EHEC also exist in the intestine of ruminants and are excreted with the faeces, they can be transmitted, either directly or indirectly (e.g. via food), from animals to humans and thus cause disease. The current state of knowledge suggests that the reservoir for EAggEC is human. EAggEC can be transmitted from human to human via smear infection. The pathogen can also enter food during preparation or production and thereby be disseminated.

So-called atypical EAggEC can be isolated from calves, piglets and horses. However, these strains lack certain properties so that the current assumption is that these animals are not a reservoir for the typical EAggEC that are pathogenic to humans (Uber et al., 2006). In the year 2004, a study was conducted in Great Britain which tested 1227 *E. coli* isolates from cattle, sheep and pigs for a specific EAggEC-typical property. None of the isolates had the particular property. However, the authors stated that not all EAggEC are detected with the method used and that therefore the possibility of bacteria of this type being present among the tested bacteria could not be excluded (Cassar et al., 2004).

For EHEC, characteristic properties include the ability to form Shiga toxins (Stx1 or Stx2) and to adhere to the intestine of hosts using a specific protein (intimin). The terms STEC (for Shiga toxin-forming *E. coli*) and VTEC (for verotoxin-forming *E. coli*) are therefore used synonymously for Stx1 or Stx2-forming EHEC. In contrast, EAggEC do not normally form Shiga toxins and adhere to the human intestinal wall by means of attachment factors (adhesins) where they are capable of forming biofilms. This ability to produce biofilms has been described for both EHEC and for EAggEC and even for abiotic surfaces.

Not least due to the potentially severe illness, EHEC are among the most important causes for bacterial infections that are transmitted by food. Since the mid-1990s, EAggEC have repeatedly been described as the cause of food-related outbreaks with acute and persistent diarrhoea (Okeke and Nataro, 2001). This *E. coli* strain is known mostly from regions with

inadequate hygienic conditions. But even in developed regions with higher hygienic standards, such outbreaks have occurred. Thus the largest known outbreak so far took place in Japan where 2500 children were infected in different schools, most probably from a meal eaten at school. The suspected school meals leading to this outbreak consisted of bread, noodles, noodle salad, milk pudding, fried vegetables and milk (Itoh et al., 1997).

Another study conducted in Brazil which tested the contents of 100 baby milk bottles (prepared by the mothers themselves from weak socio-economic areas) for pathogenic *E. coli* found EA_ggEC in three samples in a concentration of 10^3 - 10^4 colony-forming units (CFU)/ml (Morais et al., 1997). Studies on the causes for travel diarrhoea with Mexico as the country of origin of the infection have shown that EA_ggEC were isolated from desserts in an average concentration of 0.5×10^4 CFU/g (Vigil et al., 2009). Water from public fountains too was associated with outbreaks.

The pathogenic role and the transmission routes of *E. coli* strains which have both EHEC and EA_ggEC-specific virulence factors (Stx production and enteroaggregative adhesion) are currently virtually unresearched. Morabito et al. surmised as early as 1998 that such recombinant strains may be as pathogenic to humans as conventional EHEC strains.

6.3.3.1.1.1 Characteristics of EHEC O104:H4 (Strain responsible for the Outbreak)

For the outbreak occurring between May and July 2011, the serotype O104:H4 was unequivocally identified as the cause of disease.

DNA sequence analysis showed that the strain of bacteria causing the outbreak has much more in common with EA_ggEC than with conventional EHEC. Thus at the sequence level, the strain responsible for the outbreak shows a 93 % similarity to a human EA_ggEC strain from central Africa whose characteristics are already known. The EHEC-specific feature of the outbreak strain is the bacteriophage-encoded stx2 gene. The outbreak strain is an EA_ggEC O104:H4 which has absorbed the Stx2-encoded bacteriophage and is capable of forming Stx. The strain lacks the *eae* (attaching and effacing) gene typical of conventional EHEC.

The outbreak strain exhibits resistance to beta lactam antibiotics of the groups acylaminopenicillines and cephalosporins as well as to tetracycline, nalidixic acid, streptomycin and trimethoprim/sulfamethoxazol. Furthermore, an extended spectrum beta lactamase (ESBL) of the CTX-M15 type and a beta lactamase of the TEM-1 type have been detected in all isolates.

6.3.3.1.1.2 Diagnosis of EHEC O104:H4

In humans, EHEC are generally detected on the basis of a laboratory analysis of a stool sample of persons infected with the pathogen. The goal of this laboratory analysis is to isolate the pathogen with the detection of the toxin gene by means of polymerase chain reaction (PCR) from washed-away bacteria colonies or enriched stool samples and/or toxin detection by means of Enzyme-Linked Immunosorbent Assay (ELISA) from the *E. coli* culture. This is followed by the serotyping and (biomolecular) characterisation of isolates. For quick differentiation of the outbreak strain from all other EHEC, both conventional multiplex PCRs (University of Münster) and real-time PCRs (developed by Anses/BfR) are available which allows simultaneous detection of four genes typical of EHEC O104:H4.

In food and/or environmental samples, the detection of EHEC is generally difficult on account of the accompanying flora and the complex (biological) background matrix. Here too the

diagnostics aim at isolation of the pathogen with simultaneous toxin gene and toxin detection. A special real-time PCR testing method for identifying the outbreak strain was developed and evaluated by NRL *E. coli* together with experts of the French Food Agency ANSES. This detection method was made available by the BfR to the diagnostic laboratories of the official food control administration of the states and operators of food businesses.

Since the cultivation and detection of EHEC is especially difficult in plant-based food, the NRL *E. coli* additionally provided specific enrichment protocols with subsequent detection of the pathogen by means of specific EHEC O104:H4 PCR. Only statements of limited validity can currently be made on the sensitivity and detection threshold of this method. Thus the NRL *E. coli* states that the detection threshold for the pathogen in plant-based foods (including sprouts) is well below 10 genome sections per 25-gram sample. For seed analysis, no reliable statement can currently be made. Partly the reason for this is that not enough is known about on whether pathogens can occur inside seeds.

Even an inter-laboratory test of the joint EU reference laboratory for *E. coli* (CRL, Rome, Italy) for the detection of STEC/EHEC (not EHEC O104!) in naturally contaminated seeds intended for sprout production demonstrated how difficult the detection of STEC/EHEC is in seed samples. The eight participating laboratories (even the CRL itself) were not able to verify the positive results obtained by the CRL in pre-tests. It was not possible to detect STEC/EHEC. It is thought that the *E. coli* strains only exist in very low concentration in or on the seeds and are unevenly distributed. In addition, Aurass et al. assume that the pathogen may be dormant thus making cultivation more difficult.

For this reason, it is advisable to supplement testing of seed batches by sampling and with microbiological tests during sprout production in order to increase the probability of detection of existing pathogens. Germinated seeds (48 hours after germination) provide a suitable sample material. Whether the probability of detection of EHEC O104:H4 in seeds can thereby be increased as well is not known as yet. Alternatively, the discharge water from the sprout cultivation containers can be tested. However, the Panel on Biological Hazards of the EFSA concludes in its expert opinion published on 15 November 2011 that due to the dilution effect, there are uncertainties as to whether this testing strategy is sufficiently sensitive. The taking and microbiological testing of swab samples from the production environment as well as regular personnel testing serve the purpose of identifying other potential contamination sources.

6.3.3.1.1.3 Occurrence of EHEC O104:H4

Occurrence in Humans

Until the beginning of the outbreak in Germany in May 2011, only a few sporadic cases of stx2-positive *E. coli* of serotype O104:H4 had been described in the literature. For example, ECDC reports on the infection of a person from Finland in 2010 who supposedly acquired the infection during a trip to Egypt. As regards another case in France in 2004, details on the disease (including the place of infection) are not known according to the ECDC report. According to the Centre for Disease Control and Prevention (CDC, Atlanta), there were two HUS cases in Georgia in 2009. Isolation of this serotype is described in the literature for a patient with HUS in Korea in 2005 as well as for two cases (both with HUS) in Germany in 2001. Only for the isolates from Germany (2001), Finland (2010) and Georgia (2009) was it reported that they were enteroaggregative EHEC.

In October 2011, EPIS (Epidemic Intelligence Information System of the ECDC) reported on an EHEC O104:H4 (ESBL-negative) outbreak among French tourists travelling to Turkey. They were travelling through Turkey as part of an organised bus tour in September 2011.

The EHEC isolate of a HUS case was characterised as *E. coli* O104:H4, *stx2*, *eae*-, *hlyA*-, ESBL- and is therefore not identical with the outbreak strain.

Enteroaggregative *E. coli* of the O104:H4 type without Shiga toxin genes are known from at least one major English case control study with patients suffering from infectious intestinal diseases (Wilson et al. 2001).

Occurrence in Foods

The occurrence of the serotype O104:H4 in foods had not been described in Germany and the EU prior to the outbreak.

However, the methods to detect STEC/VTEC of other serotypes in foods have been in existence for several years. In Germany, STEC/VTEC are monitored as part of food business operators' own checks, controls by the government authorities and in the course of zoonosis monitoring programmes. As part of controls by government authorities, STEC/VTEC have notably been detected in fresh meat, raw meat preparations and in game meat.

Within the EU, individual cases of detection of STEC/VTEC in plant-based food (vegetables, fruit) have also been reported, though they invariably concerned non-O104:H4 strains.

Occurrence in Animals and in the Environment

The outbreak strain EHEC O104:H4 had not been observed in animal stocks or in samples from the environment within the EU prior to the onset of the outbreak. None of the isolates differentiated at the National Reference Laboratory for *E. coli* (NRL *E. coli*) at the BfR belonged to this serovar. Even as part of the notifications on zoonosis reporting, the serovar has not been reported to date.

Based on the current state of knowledge, it must generally be assumed that the outbreak strain with its genetic features described in detail has its reservoir in humans, since this *E. coli* type has so far not been found in animals. At present there are no indications to suggest that the outbreak strain has overcome the human/animal species barrier. However, it cannot be ruled out that the outbreak strain could colonise animals secondarily, for instance through the uptake of contaminated water or feed. At this point in time it would appear that the pathogen multiplies in humans and reaches the environment, e.g. waste water, after excretion through faeces. It is to be assumed that for effective multiplication of the pathogen, it must colonise the human digestive system again.

6.3.3.1.1.4 Tenacity of Enterohaemorrhagic and Enteroaggregative *E. coli*

Very little is currently known about the resistance of the outbreak strain in the environment. However, it cannot be excluded at present that the enteroaggregative EHEC O104:H4 strain can survive in the environment, e.g. in water, for long periods of time. Similarly little is known about its survival capability in food. In 2011, Scheutz et al. investigated the outbreak strain's capacity to form biofilms and concluded that, as is typical for EggEC strains, it is a moderate to good biofilm producer.

Intensive research has been done on EHEC bacteria, including serovar O157. The current assessment including its recommendations is therefore largely based on knowledge concerning the behaviour of EHEC O157:H7, the underlying assumption being that enteroaggregative EHEC O104:H4 exhibit comparable tenacity. EHEC are resistant to dehydration, freezing and acidification, meaning that they can survive in the environment (soil, water, faeces) for weeks or even months.

EHEC bacteria of the O157:H7 serovar are capable of colonising both abiotic (Saldaña et al., 2009) and biotic surfaces such as lettuce with biofilms (Takeuchi et al., 2000). In biofilms, these bacteria are more resistant to cleaning agents than in free life forms (Stopforth et al., 2003). The increase in tenacity of the EHEC bacteria to a large extent depends on the food matrix and the accompanying biotic and abiotic factors. For example, tenacity is increased if the biofilm, in addition to the EHEC bacteria, consists of other bacterial groups and if the biofilm remains undisturbed during the first 48 hours (Stopforth et al., 2003). EHEC can also form biofilms on the surface of iceberg lettuce and cos lettuce within a few hours (Patel et al., 2011). For that reason, salad mixtures to which contaminated fenugreek sprouts have been added can remain contaminated with the pathogen even after the removal of these sprouts.

Since the persistence of the pathogen in food depends on the matrix and the applied food technology, the assessment of the effect of the individual processes requires not only knowledge of the processes themselves but also detailed information on the matrix. Notably for oleiferous products it is known that the tenacity of pathogens is significantly higher. Equally, evidence shows longer survival in biofilms. The pathogen is not sufficiently inactivated by processes such as maturing, drying and acidification (Mathusa et al., 2010). EHEC germs may also be resistant to salt. Many EHEC strains can multiply despite salt concentrations of 4 to 5 % at ambient temperature (25 °C), while some strains even survive 15 % salt concentrations for at least 24 hours, also at ambient temperature (Olesen und Jespersen, 2010; Cheville et al., 1996).

Since EHEC O104:H4 is a new and highly pathogenic germ, it should be characterised in more detail in terms of its properties including its survival capacity and its growth behaviour in different matrices.

6.3.3.1.1.5 Treatment Procedures for Seeds Intended for Sprout Production

With a view to preventing sprout-related disease outbreaks, a number of studies on the effectiveness of decontamination procedures for sprout seeds have been conducted in recent years. The Panel on Biological Hazards of the EFSA has drawn up a list of available studies and their results in its opinion “Scientific Opinion on the Risk Posed by Shiga Toxin Producing *Escherichia Coli* (STEC) And Other Pathogenic Bacteria in Seeds And Sprouted Seeds”. These are almost exclusively chemical and physical procedures such as the use of chlorine solutions and acids, the application of dry or humid heat, high pressure and irradiation. In its assessment, the Panel of the EFSA concludes that suitable treatment methods are not available for all seed types and that the procedures described in the literature must, before application, be optimised for the seed types to be used. Over and above this, the panel recommends that the safety and effectiveness of treatment methods for seeds be evaluated and harmonized at EU level, since the treatment methods known so far cannot guarantee complete elimination of pathogenic germs for all seed types without impairing their germination capacity and yield.

The procedures tested so far enable differing degrees of germ count reduction. The goal of the seed treatment is a germ count reduction by at least 5 log when germination capacity is reached, even if in this matrix only low concentrations of pathogenic germs are expected. This is necessary for seeds intended for sprout production, because, due to the mesothermal and humid conditions during sprout cultivation, germs undergo growth amounting to several log. Whether EHEC O104:H4 too can grow in these conditions is not currently known.

The possibility must certainly be considered that the pathogen may also exist inside the seeds. Experimental studies have shown that some EHEC strains can enter the inside of plants via the roots. In the case of alfalfa, the absorption of both pathogenic and apathogenic bacteria into the inside of the seeds has been observed. It is assumed that bacteria enter the

plant through cracks in the lateral roots (Dong et al., 2003). Even if this is not known to be the case for fenugreek seeds yet, the seeds should be treated in such a way that any pathogens that may exist in the seed core are killed.

However, chemical treatment procedures are generally not suitable for eliminating pathogens that may exist in the inside of fenugreek seeds. Even treatments of sprout seeds with chlorine solutions which, for example, contain 2 % chlorine from calcium hypochlorite, are said not to achieve a complete elimination of EHEC germs (Fett et al., 2005). One of the reasons for this observation may be that the germs in biofilms exhibit increased chlorine tolerance. Thus a 100-fold chlorine tolerance is to be assumed for bacteria in biofilms (Prof. Exner, University of Bonn, personal communication from 21 June 2011).

For seeds used for sprout production, the principle of maximising the overall effect with a number of individual inhibitory factors is usually adopted in the form of combinations of several mild reduction procedures for bacteria in order to ensure that the germination capacity of the seeds is not impaired. Studies on the inactivation of EHEC O157:H7 through thermal treatment of sprout seeds have shown that a 5 log germ reduction was achieved by the application of dry heat only at 70 °C for 24 hours or 70 °C for 6 hours followed by high pressure treatment (600 MPa) for 2 min at 35 °C (Neetoo und Chen, 2011). The current state of knowledge also suggests that heat treatment of 50 °C for one hour followed by evenly distributed gamma irradiation (2 to 2.5 kGy) is suitable for achieving an EHEC O157:H7 reduction by 4 to 5 log for different sprout seeds.

6.3.3.1.2 The Hazard Potential in the EHEC O104:H4 Outbreak Event

The EHEC O157 infection dose is very low and amounts to less than 100 germs. No data are available on the infective dose of EHEC O104:H4.

At present it is to be assumed that the pathogen does not need to multiply in the environment or in the products in order to infect humans. Efficient multiplication of the pathogen notably appears to occur in the gastrointestinal tract of humans which can then cause a severe case of the disease.

The period from May to July 2011 saw an accumulation of HUS and bloody diarrhoea cases in connection with infections caused by EHEC O104:H4. In most cases, the disease caused by the outbreak pathogen took the form of non-bloody, mostly watery diarrhoea. Some patients developed haemorrhagic colitis with spasmodic stomach pains, bloody stool and, in some cases, fever. However, EHEC infections may take an unapparent and hence unnoticed course. The RKI attributed a total of 855 HUS cases to the outbreak. The full clinical picture of HUS is characterised by acute renal failure in some cases including anuria, haemolytic anaemia and thrombocytopenia (low level of blood platelets). HUS is typically preceded by often bloody diarrhoea. This severe HUS complication occurs in approximately 5 to 10 % of symptomatic EHEC infections. It frequently leads to short-term dialysis dependency and more rarely to an irreversible loss of renal function with chronic dialysis dependency. During the acute phase, the fatality rate of HUS is approximately 2 %.

During the outbreak caused by serotype O104:H4, neurological symptoms were frequently observed in clinically affected persons. The reason for these symptoms could be that more toxin is released into the organism due to the heavy colonisation, leading to increased incidences of a severe progression of the disease (Bielaszewska 2011).

The incubation period for EHEC infections is usually 2 to 10 days (3 to 4 days on average), this data being based for the most part on investigations into EHEC of serogroup O157. For the outbreak caused by enteroaggregative EHEC O104:H4, a median incubation time of 8

days is assumed. This means that the incubation time for infections with EHEC O104:H4 is significantly longer compared to the median incubation period for EHEC O157.

During this outbreak, the symptoms of EHEC-associated HUS cases on average commenced five days after the onset of the diarrhoea. The median time period between the onset of diarrhoea and the onset of HUS therefore seems to be shorter for the outbreak strain than for infections caused by EHEC O157 (seven days).

For further information reference is made to the concluding summary and assessment of the epidemiological insights into the EHEC O104:H4 outbreak of the Robert Koch Institute.

6.3.3.1.3 Exposure Assessment

6.3.3.1.3.1 Microbiological Testing of Food and Environmental Samples

In Germany, enteroaggregative EHEC O104:H4 were first detected in or on food as part of the current investigation into the outbreak. More than 8000 food and environmental samples had been tested for the pathogen causing the outbreak in Germany by 30 August 2011. Detection was successful in a cucumber sample and a sprout sample which had been collected at different places from kitchen scraps by persons infected with the outbreak pathogen. In addition, EHEC O104:H4 was found in three food samples (salmon raw and cooked, sweet peppers) which evidently had been contaminated by an employee of a party service.

However, it was not possible to detect the outbreak pathogen in the tested seed batches, but that is not unusual. No EHEC outbreaks associated with sprouts are known to the BfR in which the pathogen causing the outbreak was found in the implicated seed batches. Microbiological detection of outbreak pathogens in involved seed batches was successful only for a few sprout-associated salmonella outbreaks. Nevertheless, it can be concluded from the results of the epidemiological investigations that in most cases seeds are the source of sprout-associated outbreaks (Puohiniemi et al., 1991; Mahon et al., 1997, FDA 1999).

On the occasion of the FVO inspection in August 2011, the competent authorities in Egypt advised that between 1 January 2009 and 15 July 2011, a total of 180 samples of fenugreek seeds had been tested for *E. coli* by the CPHL (Central Public Health Laboratory). In one sample, *E. coli* O114:K90 were detected without the ability to form Shiga toxin. According to the CPHL, *E. coli* were not found in the other samples. In addition, 554 fenugreek samples from the trade, 5 samples of fenugreek seeds from exporters and 10 environmental samples (water, soil and fertiliser) from the producer of the fenugreek seed batch (Batch 48088) which was implicated in both outbreaks were tested for *E. coli* O104 in Egypt as part of the investigation into the outbreak before 21 August 2011. According to the Egyptian authorities, *E. coli* O104 was not found in these samples taken in connection with the outbreak either. No samples were taken at the implicated packing plant in Egypt, even though control samples of the three recalled seed batches were still stored there.

6.3.3.1.3.2 Production and Distribution Channels of the Suspected Seed Batches

The recall issued by the European Commission on 6 July 2011 concerns three fenugreek seed batches which were imported from Egypt by the same German-based importer between December 2009 and February 2011. Two of the three batches (Batches 48088 and 8266) were used for sprout production by a horticultural farm in Lower Saxony in the spring of 2011. Both batches were obtained by the horticultural farm in Lower Saxony from the same wholesaler (Wholesaler A). The third recalled batch (Batch 2660002) was produced in Egypt under the same conditions and during the same time period as Batch 8266. The three batches were produced by three different farms. None of these three farms cultivates seeds for the purpose of sprout production, nor did the type of seed cultivation even of the fenugreek seeds comply with the standards of the Codex Alimentarius for sprout seeds (Annex 2 of the CODEX Code of Hygienic Practice for Fresh Fruits and Vegetables CAC/RCP 53/2003). As part of the FVO inspection, hygiene deficiencies were discovered which may, starting from humans and animals as well as via the sprinkling water, have led to contamination of the fenugreek seeds. The path of contamination into the seeds was not identified within the scope of the investigations in Egypt, however.

The recalled fenugreek seed batches were temporarily stored at the same packing plant, cleaned with sieves and put into paper bags. The packing plant provides the farms with the implements necessary for harvesting. All three farms and the packing plant are owned by the same extended family.

Batch 48088 (15 tons) is the connection between the EHEC O104:H4 outbreak in France and the outbreak in Germany and was produced on a small farm (Farm A) in Egypt in the 2008/2009 season.

Batches 8266 (10 tons) and 2660002 (12 tons) were cultivated on two other small farms, located approximately 120 km away (Farms B and C) in the winter of 2009/2010. These two adjacent farms use the same irrigation water and the same animal-based fertiliser for seed cultivation.

To provide a good overview, the BfR has summarised in a table the available information about production and distribution channels of the two Batches 48088 and 8266 used in the horticultural farm in Lower Saxony in the spring of 2011 (Table 8 and 9). Since Batch 2660002 was only exported to Germany in January 2011 and most of it was still stored in the warehouse of the importer at the point in time of the EHEC outbreak, the BfR has dispensed with a portrayal of the production and distribution channels for this batch.

The documentation of the packing plant showed that in the year 2009, a further fenugreek seed batch was exported to the EU which, according to the FVO report, originated from a different farm. According to statements made by the affected packing plant, seeds from there are, on the basis of a contract with an importer, only delivered to Germany. Based on the available data, the BfR acts on the assumption that this batch mentioned in the inspection report concerned roughly 8.5 tons of fenugreek seed (Batch 2044) which the importer distributed in 2009. Batch 2044 was not subject to the investigation into the outbreak, because by the time the EHEC outbreak started, it had already exceeded its stated expiry date (February 2011). In consequence, it is assumed that at the point of time of the outbreak this batch had already been processed and used up.

Tab. 8: Production and Delivery Path of Fenugreek Seed Batch 48088

Level	Quantity	Process step	Comments
1.) Harvest in Egypt 2008/2009 (Farm A)	15 t	Transportation in 50 kg units to the packing plant	
2.) Packing plant in Egypt	15 t	Temporary storage, cleaning with sieves and packing into 25 kg paper bags	
3.) Shipment, December 2009	15 t	from Damietta, Egypt, in a closed container	
4.) Arrival in Europe, December 2009	15 t	Unloading in Rotterdam, Netherlands	
5.) German importer, December 2009	15 t	Storage for resale in 25 kg bags	including 75 kg from the warehouse (outgoing goods 15,075 kg)
6 a.) most important buyer, Wholesaler A	10.50 t	Distribution of 25 kg bags and weighing for packing into small mixed and unmixed packagings for end consumers, to over 60 customers including the horticultural farm in Lower Saxony (associated with 41 outbreak clusters in Germany)	For details on intended uses, see also Fig. 1
6 b.) 12 additional buyers/distributors	4.58 t	Distribution of 25 kg bags and weighing for packing into small mixed and unmixed packagings for end consumers, including sprout producers among the many customers, via an English distributor distribution to France (outbreak cluster)	For details on intended uses, see also Fig. 1

Tab. 9: Production and Delivery Path of Fenugreek Seed Batch 8266

Level	Quantity	Process step	Comments
1.) Harvest in Egypt 2009/2010 (Farm B)	10 t		No information provided in the FVO report
2) Packing plant in Egypt	10 t	Temporary storage, cleaning with sieves and packing into 25 kg paper bags	
3.) Shipment, October 2010	10 t	from Alexandria, Egypt, in a closed container	
4.) Arrival in Europe, October 2010	10 t	Unloading in Rotterdam, Netherlands	
5.) German importer, October 2010	10 t	Storage for resale in 25 kg bags	
6 a.) most important buyer, Wholesaler A	4.50 t	Distribution of 25 kg bags and weighing for packing into small mixed and unmixed packagings for end consumers, to over 40 customers including the horticultural farm in Lower Saxony and other sprout producers	For details on intended uses, see also Fig. 2
6 b.) 5 additional buyers/distributors	1.15 t	Distribution of 25 kg bags and weighing for packing into small mixed and unmixed packagings for end consumers, including sprout producers among the many customers	For details on intended uses, see also Fig. 2

From the information available at the BfR, a complete delivery path down to the level of the first distributor can be demonstrated for both batches. However, the BfR does not know where the documented additional fenugreek seeds (75 kg, mathematically corresponds to 3 bags) came from which the importer distributed together with the imported 15 tons of fenugreek seeds of Batch 48088.

The BfR is unable to completely reconstruct and outline the traded quantities from the third trading level after importation of the goods. Processing steps such as mixing and putting the seeds into small packagings of various weight shares make forward-tracking of the two batches to the end consumer difficult.

The seeds were distributed not only within Germany but, via different distributors, in a total of 22 member states and 2 other countries. For Batch 48088, these deliveries amount to a quantity of approximately 1611 kg and for Batch 8266 a quantity of about 445 kg. As far as can be told from the information on further delivery relations available to the BfR, no re-importation into Germany took place. However, giving the patchy nature of the data, this possibility cannot be excluded with certainty.

6.3.3.1.3.3 Intended Uses of the Suspected Seed Batches

The BfR has extensive information on the delivery relations of the two Batches 48088 and 8266 which were used by the horticultural farm in Lower Saxony in the spring of 2011. The seeds were assigned to various intended uses based on the information available, predominantly from the Internet, on the individual buyers of these two batches. Figures 1 and 2 show the established probable intended uses of the seeds for the appropriate batch.

The category "Small packagings for end users" comprises packaging units of 30, 40, 50, 60 and 125 grams, both mixed and unmixed. The BfR has based its calculations of the intended uses on the assumption that seed mixtures put into small packagings consisted of one part fenugreek seeds and two parts other seed types. The packagings are largely intended for sprout cultivation in private households. How the seeds were eventually treated in households and what they were used for cannot be reconstructed, however. Nor is it known whether consumers used fenugreek seeds distributed in small packagings by large do-it-yourself markets and garden centres for sowing in order to produce fenugreek plants.

In the "sprout production" category, five companies are found for Batch 48088 and four companies for Batch 8266. One of these sprout producers explicitly stated that they only used the fenugreek seeds as an ingredient in baked goods.

The "storage and production losses" category contains the quantities known to the BfR which did not go into production and were not resold.

For some delivery addresses, it was not possible to obtain any information regarding intended use. The quantity distributed to those addresses was therefore assigned to the "unknown" category.

It cannot be established what proportion of Batch 48088 has already been eaten or used up and how much has been recalled and destroyed. The time span of the flow of goods covers 1.5 years, from the importation to the recall from the trade.

Since Batch 8266 was only imported in October 2010, a large quantity of it was still stored in warehouses at the time the outbreak began in early summer 2011. Almost 6 tons of Batch 8266 were successfully recalled and secured.

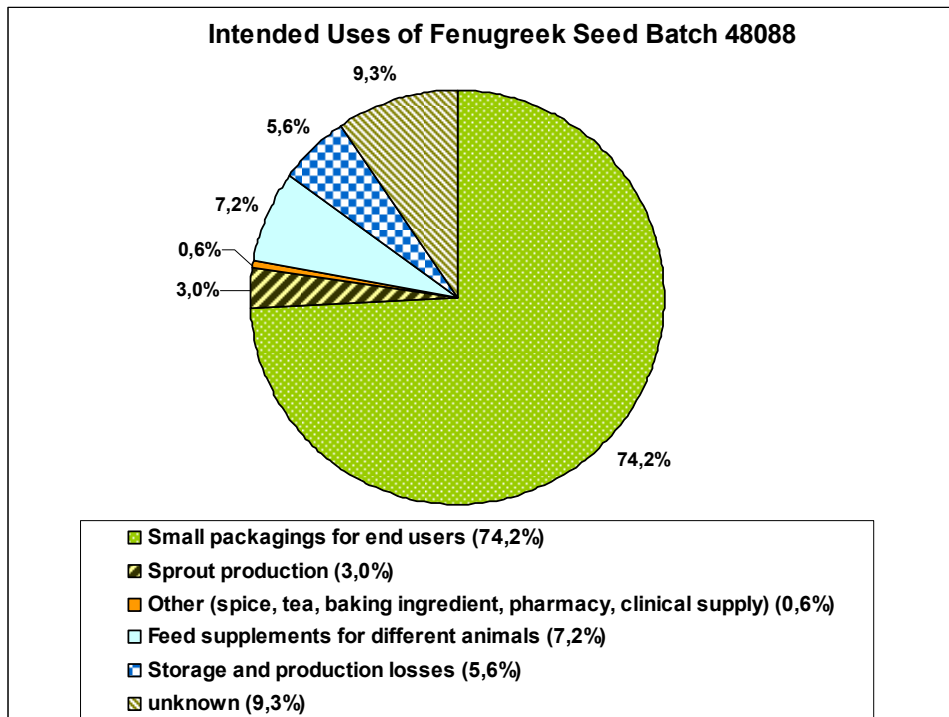


Fig. 18: Distribution in percentages of the probable uses of seed Batch 48088

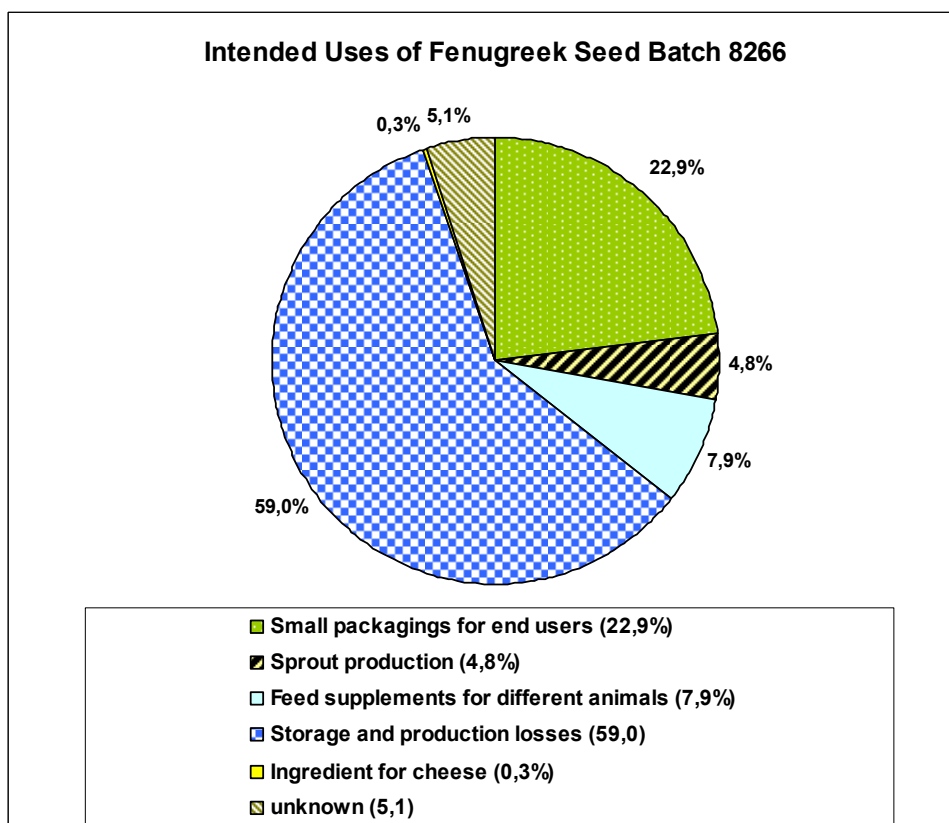


Fig. 19: Distribution in percentages of the probable uses of seed Batch 8266

6.3.3.1.3.4 Influence of Eating Habits

As part of the outbreak of early summer 2011, healthy individuals and persons of all age groups (especially adults) came down with EHEC O104:H4 symptoms. It is conceivable that the health-conscious eating habits of women in particular led to higher exposure to the contaminated seeds. According to the data of the National Nutrition Survey II (NVSII), women and men have the same exposure risk, however. In addition, sprouts are eaten unknowingly as well, as the first case control studies of the RKI impressively demonstrated. It follows that the risk cannot be confined to certain sections of the population.

6.3.3.1.4 Risk Characterisation

The consumer risk arising from fenugreek seeds produced in Egypt, if they are used for cultivating sprouts, is characterised below. The consumer risk which emanates from fenugreek seeds from Egypt that are processed further into foods other than sprouts was already assessed by the BfR in July 2011. This assessment continues to apply. The risk of sporadic transmissions of the outbreak pathogen through human excretion or via contaminated and inhabited surfaces of implements into other food supply chains is not taken into account.

According to the RKI, the disease outbreak resulting from EHEC O104:H4 in Germany ended on 26 July 2011. Most cases of illness can be attributed to exposure in May. Since September 2011, infections with EHEC O104 have been reported to the RKI only infrequently. In the course of the investigation into the outbreak along the food supply chain, fenugreek seeds from Egypt were identified as the most likely cause of the disease outbreak. Seed batch 48088 which was used for sprout production both in France and in the horticultural farm in Lower Saxony is especially suspect. However, it is to be assumed that only one part of this batch was contaminated with the outbreak pathogen or at any rate that the contamination was very uneven (heterogeneous). It is the case that the seeds of the suspected batches were distributed largely within Germany. However, given the wide distribution of the seeds, for the most part in small packagings for private cultivation by end consumers, more cases of illness would have been reported from other parts of Germany and the EU, if the pathogen had been homogeneously distributed in the seed batch.

Since the pathogen's pathway into the fenugreek seeds has not been identified, the continued risk arising from fenugreek seeds from Egypt, if any, cannot be estimated. In order to reduce the risk, the European Commission ruled that three fenugreek seed batches imported from Egypt in the period 2009-2011 which had been identified as part of the reconstructive investigation at EU level must be recalled and destroyed in a non-harmful way. To complement this measure, the Commission also imposed an import ban on certain seeds and beans from Egypt which has been extended to 31 March 2012.

Since the recall measures of the member states have been completed according to the European Commission (see press release of the EFSA of 3 October 2011), it is unlikely that fenugreek seeds of the three affected batches will continue to be used for commercial sprout production in Germany. For this reason, the risk of contracting an EHEC O104:H4 infection from the raw consumption of commercially produced fenugreek seeds is significantly lower than it was before completion of the recall measures. The risk emanating from the fourth batch (Batch 2044) from Egypt which was not affected by the Commission Implementing Decision must be seen as very low in any case, since it had already exceeded its stated expiry date (February 2011) by the time the EHEC outbreak began.

However, if fenugreek seeds of the batches used by the horticultural farm in Lower Saxony in the spring of 2011 were to be used again for commercial sprout production in Germany, a new and similarly severe outbreak could develop, if the sprouts were to be eaten raw. The

risk of falling ill with EHEC is probably greatest when seeds of Batch 48088 are allowed to germinate, because this batch is the only known epidemiological connection between the outbreak clusters in Germany and France.

Because the recall from the trade also included small packagings for end consumers containing fenugreek seeds of the three batches, the risk of contracting an EHEC O104:H4 infection from the raw consumption of privately produced fenugreek sprouts is also clearly lower. It is not likely that fenugreek seeds currently being traded originated from the recalled batches. However, it is possible that fenugreek seeds from Egypt still exist in private households and that they are used for sprout production there, because consumers are not sufficiently informed on the potential danger and the recall of the seed batches. This could result in new EHEC cases. Due to the probably uneven distribution of the contamination in the batch, it can be assumed that not all end user packagings are contaminated with the outbreak pathogen, however.

6.3.3.1.4.1 Assessment of the Severity of Health Impairments

The health impairments are to be seen as severe. The disease pattern can include everything from bloody diarrhoea, renal failure with dialysis dependency to severe neurological symptoms and death. How long the damage to health continues, whether it leads to chronic illness (for example in the form of irreversible kidney damage) or whether the damage is reversible and what long-term complications can occur, cannot currently be assessed.

6.3.3.1.4.2 Assessment of Data Quality

Microbiological Test Results

The results of the microbiological testing of fenugreek seeds from Egypt for the presence of EHEC O104:H4 are marked by great uncertainty and cannot be conclusively assessed. The BfR does not have any information on how many samples and what quantities of the three batches were tested, on the basis of what sampling plan the samples were taken, and what diagnostic procedures were employed. These data are necessary for the assessment, not least because it is to be assumed that the contaminated seed particles within the batches are not homogeneously distributed but instead form "nests". In addition, the available method was developed for detecting STEC in fresh plant-based foods (e.g. pre-cut mixed salads and sprouts) and has not been validated for the testing of seeds. Moreover, it would appear that the pathogen can be in a state of dormancy which complicates cultivation. For these reasons alone, erroneously negative test results are conceivable.

Furthermore, an absolute absence of pathogen germs from a matrix is not possible in microbiological tests anyway. When applying sampling plans, it is possible, however, to draw conclusions regarding the probability of the percentage proportion of the contamination of tested batches. Larger samples sizes allow more accurate statistical results with regard to possible contamination.

6.3.3.1.4.3 Production and Distribution Channels of the Suspected Seed Batches

Due to missing and contradictory statements, an inspection of the documents presented at the three affected farms in Egypt in August 2011 raised doubts among the FVO inspectors as to the integrity of the recalled batches. This assessment is therefore largely based on data which were made available to the BfR by the German authorities.

Although the quality of the data for the delivery relations for seeds is batch-dependent, it can, overall, be regarded as good. On the basis of delivery notes, the data was entered by the trained employees of an EHEC Task Force set up at the BVL. Once the work of the EHEC Task Force had been completed, the BVL further processed the data sets pertaining to the various countries to the extent where their traceability, from the importer to the main distributors including delivery quantities, could be almost completely represented in the complex database format of the EFSA. According to the State Authority responsible for food inspection for Wholesaler A, difference quantities between incoming and outgoing goods incurred by Wholesaler A in connection with Batches 48088 and 8266 are attributable to cleaning and production losses.

However, the information received on the subsequent distribution stages and on possible intended uses of the recalled fenugreek seeds is incomplete. Processed investigation results of the involved authorities in the member states which had been made available by the European Commission in the form of 91 follow-up messages on the RASFF communication 2011.0842 turned out to be of little use for the purpose of risk assessment, because the batch reference was frequently missing and more generally because the data was not detailed enough. In addition, returns by recipients of the relevant fenugreek seeds were not recorded systematically and quantitatively at federal level. The BfR is therefore unable to assess what quantities of the three seed batches were returned, destroyed, sold or eaten. For this reason, any assessment of the continued exposure of users through residues of the contaminated batches even after completion of the recall measures must be seen as uncertain. However, within the scope of this assessment, allowance is made for this uncertainty with regard to distribution and use in that the various possible scenarios are analysed.

6.3.3.1.4.4 Tenacity of the Pathogen

The quality of the data relating to the outbreak pathogen can be said to be highly incomplete. Therefore, available information on enterohaemorrhagic and enteroaggregative *E. coli* were used to assess the possible risks. But even for these bacteria species, the data situation must be regarded as incomplete. This uncertainty was considered accordingly during the assessment.

6.3.4 Conclusion and Recommended Measures

The EHEC O104:H4 outbreak of the early summer 2011 in Germany is now over. According to the RKI, it was the largest outbreak by EHEC infection in Germany so far and, with regard to the number of reported HUS cases, the largest outbreak of its kind reported anywhere in the world. Fenugreek seeds imported from Egypt which were used for sprout production both by a horticultural farm in Lower Saxony and by private individuals are, upon completion of the investigations, seen as the cause of the EHEC outbreak. This conclusion is in agreement with the results of other epidemiological studies which indicate that in most cases seeds are the source of sprout-related outbreaks. Where and how the seeds came into contact with the pathogen leading to the outbreak could not be determined. Nor was the pathogen causing the outbreak detected in the tested fenugreek seeds. However, for methodological reasons, a negative test result does not mean that EHEC O104:H4 was not present in the seeds.

Fenugreek seed Batch 48088 is the most likely batch to have caused the outbreak, since this batch is the only known epidemiological connection between the outbreak clusters in Germany and France. This batch was recalled together with two additional fenugreek seed batches which in the subsequent years were cultivated on the farms of the same extended family and treated at the same packing plant. This recall has significantly reduced the risk of

consumers of contracting an EHEC infection following the consumption of raw sprouts made from these fenugreek seeds.

Irrespective of the EHEC outbreak which is now over, the consumption of raw sprouts generally involves a non-quantifiable risk of contracting a food-borne infection. In its expert opinion published on 15 November 2011, the Panel on Biological Hazards of the EFSA too concludes that sprouts pose a microbiological risk from a food safety viewpoint. The reasons for this are that seeds used may be contaminated with pathogens and that the cultivation conditions for sprouts facilitate the multiplication of existing pathogens. In addition, sprouts are not at all or only lightly heated before consumption, meaning that pathogens may survive.

Based on the insights gained in the course of the investigation into the outbreak and on the current state of knowledge generally, the BfR therefore makes the following preventive recommendation to ensure the protection of consumers from food-borne infections:

1. When cultivating, storing, treating, transporting and analysing seeds used in the production of sprouts, at least the standards of the Codex Alimentarius (Annex 2 of the CODEX Code of Hygienic Practice for Fresh Fruits and Vegetables CAC/RCP 53/2003) should be observed in order to reduce the risk of contamination with pathogens.
2. Producers of sprouts are advised to use only seeds that have been cultivated specifically for this purpose and that comply with the above-mentioned standards of the Codex Alimentarius. This risk management measure aims to reduce the probability that pathogens are imported, via the seeds, into sprout production where they can then settle and multiply. For the same reason, it is additionally recommended to sprout producers to treat or have treated seeds with suitable germ-reducing procedures before cultivation, especially if the sprouts may be intended for raw consumption. In its expert opinion published on 15 November 2011, the Panel on Biological Hazards of the EFSA concludes, however, that suitable treatment methods are not available for all seed types and that the procedures described in the literature must, before application, be optimised for the seed types to be used. The panel of the EFSA recommends that the safety and effectiveness of treatment methods for seeds be evaluated and harmonized at EU level.
3. In addition, sprout producers are advised to monitor critical points in the production process by means of microbiological checks at regular intervals. Since no reliable method for detecting STEC in seeds is currently available, intermediate products must instead be tested (e.g. germinated seeds 48 hours after germination) for the presence of pathogens. Whether the probability of detection of EHEC O104:H4 in seeds can thereby be increased as well is not known yet, however. As a complementary measure, the taking and microbiological testing of swab samples from the production environment as well as regular personnel testing can be helpful in identifying contamination sources.
4. However, consumers who cultivate sprouts from seeds themselves for the purpose of raw consumption have no way of making the production process safer nor of verifying its safety through microbiological tests. For this reason, it is especially important that the used seeds do not contain any pathogens. Food business operators who circulate seeds for the purpose of producing sprouts in private households should therefore only use seeds which were cultivated for this purpose and which comply with the above-mentioned standards of the Codex Alimentarius. In addition, as part of incoming goods inspection, seed batches should be microbiologically tested for the presence of pathogenic germs. Due to methodological uncertainties, it is furthermore recommended that those circulating such sprout seeds additionally treat or have treated the seeds using suitable germ-reducing procedures before the seeds are put into end user packagings.
5. Since fenugreek seeds of the recalled batches may still be found in private households, consumers are, as a precautionary measure, advised not to allow fenugreek seeds purchased before October 2011 to germinate. The seeds should be processed into meals, for example by thorough roasting in a pan or by cooking, or else disposed of as household rubbish.

6. Moreover, the BfR advises consumers producing their own sprouts only to use seeds which are marketed for sprout production by the producer.
7. By thoroughly heating sprouts, any pathogens that may exist are killed. For this reason, the BfR recommends that persons with a not fully developed or weak immune system (infants, pregnant women, the elderly, and sick people) should, as a precaution, only ever eat sprouts after they have been sufficiently heated.
8. In order to reduce contamination by germs, sprouts should be thoroughly washed before they are eaten and consumed as quickly as possible. However, pathogens cannot be safely eliminated by washing the sprouts.
9. In addition, general rules of body and kitchen hygiene should be observed in order to avoid human-to-human transmission (smear infection) and contamination of foods with pathogens.

With a view to preventing food-borne infection, the growth and survival of enteroaggregative STEC in various food matrices including seeds and sprouts should be researched. This research should also probe the question what influence accompanying flora existing on sprouts has on the growth and survival of pathogens. Furthermore, research is needed on the detection of enteroaggregative STEC in the “sprout production” food supply chain.

6.3.5 References

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7 Occurrence and Distribution of Enterohaemorrhagic *E. coli* in the Agricultural Production

Long before the EHEC outbreak in May 2011, it was known that Shiga toxin-forming *E. coli* (STEC), which also include enterohaemorrhagic *E. coli* (EHEC), occur naturally in the intestines of ruminants without causing any illness, and are excreted with the animal faeces. Of the approx. 13 million cattle in Germany, mainly dairy cows (approx. 4.1 m) are kept on pasture land during the main vegetation period from May to October. When farm animals are kept on pastures, their droppings are not distributed evenly over the entire pasture area, but they tend to concentrate around certain neuralgic points, such as watering places, shady areas where the animals can lie down, and droving routes. This concentration and the use of water ditches as a natural boundary between pastures fundamentally enable the introduction of coli bacteria to surface water.

In addition to this, roe deer as wild ruminants should be regarded as another EHEC reservoir. It should be taken into account here that roe deer are not only to be found on pasture land but also in other areas, such as arable land, where farm animals are not kept.

7.1 Livestock Drinking Water

Whereas drinking water for humans must fundamentally comply with the requirements of the Drinking Water Regulation in Germany (regulation on the quality of water for human consumption of 21 May 2001), there are currently no similarly detailed legal requirements for livestock drinking water.

Certain stipulations are formulated, however, in the “Feed and Water” section of Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 with regulations on feed hygiene. Accordingly, drinking water must be “suitable” for the animals in question. As the lawmakers have restricted themselves to general formulations, the BMELV published an orientation framework for the assessment of the hygienic quality of livestock drinking water from a feed law point of view in May 2007. It is based on a study from the same year. The BfR agrees with the recommendations of the BMELV and sees no need for further action at the moment.

Compliance with these requirements by means of the appropriate control, cleaning and maintenance of watering technology can guarantee an adequate hygiene status of the “acquired” water. It must be remarked, however, that increased germ counts can occur very quickly under certain circumstances if contamination, such as cowshed dust, feed residue and excrement enters the water or if the water is left in the watering equipment for long periods of time at high temperatures.

7.2 Farm Fertilisers

According to the Fertiliser Regulation (regulation on the marketing of fertilisers, soil additives, culture substrates and plant additives [German Fertiliser Regulation – DüMV] of 16 Dec. 2008), farm fertilisers are categorised as organic fertilisers and comprise all organic fertilisers which occur in agricultural farms, e.g. solid dung, liquid manure, slurry. As these are substances of animal origin, it must be assumed that they could contain pathogens which are also of relevance for humans and animals. However, fertilisers may only be approved or put into circulation, if they do not pose a hazard to human or animal health if used properly. For this reason, the Fertiliser Regulation (DüMV) contains requirements for the epidemic hygiene of fertilisers. These requirements are regarded as not complied with if salmonella are found

in 50 grams of sample material. However, the detection of other animal and human pathogens has not been prescribed up to now.

As there are no binding requirements concerning the hygiene of farm fertilisers, it must be assumed that the human pathogenic germs which may possibly exist in animal excrement may not be completely eliminated even after being stored on the premises – usually for several months – as solid dung, liquid manure or slurry. With the current level of available knowledge, however, it is not possible to make a reliable estimate of the extent to which farm fertilisers are contaminated with pathogens relevant to humans and animals.

7.3 Animal By-products

Animal by-products are also listed as base materials for organic fertilisers in the Fertiliser Regulation (regulation on the marketing of fertilisers, soil additives, culture substrates and plant additives [German Fertiliser Regulation – DüMV] of 16 Dec. 2008) under the designation “other organic substances”. When using untreated animal by-products, such as slaughter waste, stomach and rumen content etc, it has to be assumed that these by-products could possibly contain pathogens relevant to humans and animals. Accordingly, in order to guarantee epidemic and hygienic innocuousness in accordance with the provisions of Regulation (EU) No. 142/2011 and the requirements of Regulation (EC) No. 1069/2009, animal by-products are to be dealt with under the hygiene regulations for certain animal by-products not intended for human consumption. The standard processing methods with heat treatment listed here are regarded as sufficient for a reduction of pathogens so that no epidemic and hygienic risk is to be expected from a veterinary point of view from products treated accordingly.

7.4 Digestates from Organic Waste Treatment

Digestates from biogas plants are regarded as farm fertiliser if they were produced from the fermentation of plant materials that occur in agricultural, forestry or horticultural businesses, even when mixed with animal excrements. No binding requirements for hygienisation currently apply to these digestates, as explained in Item 7.2.

If other substances, such as organic waste which also includes animal by-products, are fermented in biogas plants the end product is organic fertiliser and not farm fertiliser in accordance with the Fertiliser Regulation. These are subject to the hygiene requirements of the Organic Waste Ordinance (BioAbfV).

As outlined above, digestates can result from many different base substrates which are subject to different decomposition rates during the fermentation process, depending on the retention period, temperature and mixing ratio. The possible contamination of fermentation residues with pathogens also relevant to humans and animals depends essentially on the base materials used and on the treatment method.

In general it must be assumed that the requirements for the hygienisation of organic waste in fermentation plants (biogas plants) laid down in the Organic Waste Ordinance (BioAbfV) are sufficient to eliminate the vegetative forms of bacteria that occur in fermentation residues.

7.5 Conclusion

In summary, it must be stated that the possibility exists that zoonotic pathogens and other pathogenic germs can exist in organic fertilisers, especially if farm fertilisers (e.g. solid dung, liquid manure and slurry) and other organic substances are used as base material, thus constituting a health hazard for humans and animals. Even when ruminants are kept on pastures, the introduction of pathogens is still possible through animal excrement. In addition to this, pathogens can also be spread via contaminated livestock drinking water as well as surface water adjacent to pasture land.

During the processing of animal by-products, on the other hand, the standard heating methods used are sufficient to kill pathogenic microorganisms. With digestates from the treatment of organic waste the hygienisation requirements are suitable for the reduction of vegetative bacteria cells, but not for the elimination of heat-resistant spore formers.

Wherever the possibility of introducing microorganisms into organic fertilisers exists and subsequent treatment does not result in the elimination of the pathogens, a hygienic risk cannot be excluded. This applies to the same extent to the incidence of salmonella and other pathogenic microorganisms as it does to enterohaemorrhagic *E. coli*.

However, the EHEC strain O104:H4 which triggered the outbreak in May and June 2011 possesses a special characteristic. It is a recombinant of an enteroaggregative and an enterohaemorrhagic *E. coli* which has not been possible to isolate in animals or from foods up to now and which has only been detected previously in humans. According to the latest level of available knowledge, it is not to be assumed that EHEC O104:H4 is of any substantial significance for the contamination of agricultural matrices.

7.6 References

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- Regulation on the quality of water for human consumption – TrinkwV 2001 of 21 May 2001
- Organic Waste Ordinance – BioAbfV of 21 September 1998

8 Risk Communication



Fig. 20: 05 July 2011: Press conference by the RKI, BfR and BVL (l to r: Prof. R. Burger, Prof. A. Hensel, Dr. H. Tschiersky-Schöneburg)

An important legal task of the Federal Institute for Risk Assessment (BfR) is risk communication. It is defined as a continuous and interactive process and characterised by a participative dialogue with various target groups. Risk communication is therefore much more than the general conveyance of risk assessment results. Timely information of the general public in regard to possible health risks, acquired knowledge and work results forms the basis of this dialogue.

One of the necessary prerequisites for adequate risk communication is the knowledge of how risks are perceived and which factors have an influence on risk percep-

tion. Differences in the perception of what is described as risk become apparent in that “on the one hand relatively insignificant risks take up some considerable space in public perception while on the other hand some severe risks are underestimated or even ignored” (Risk Commission, 2003). Factors which influence risk perception can be allocated to one of the three categories *Properties of the risk*, *Properties of the perceiver and his situation* and *Properties of risk communication*. This means that the analysis of individual risk perception involves establishing what kind of risk is perceived by whom on the basis of which information (Kurzenhäuser & Epp, 2009).

The type and extent of the communication of risks also have a considerable influence on individual risk perception. This applies just as much on the level of mass communication through newspapers, TV and internet as on the level of individual communication. In this way, selective or unilateral information on risks can lead to false estimations, such as when the media focus mainly on the disaster potential of new risks. Only on the basis of knowledge of the factors which have the greatest influence on risk perception can effective risk communication be practiced (Kurzenhäuser & Epp, 2009).

In general, risk communication pursues the following objectives (Wiedemann & Schütz, 2006): on the one hand, the communication of risks should improve the general level of knowledge concerning risks and their scientific foundations (e.g. risk estimation). This involves primarily information on and the explanation and/or clarification of risks. The improvement of the level of knowledge is a necessary but not sufficient condition for the initiation of behavioural changes and the promotion of preventive measures, which is a fundamental objective of risk communication as well. A further goal, which should be placed more in the area of crisis communication, is effective information in emergencies and disasters.

Risk communication therefore involves prevention and preparation (through clarification and sensitisation) and relates to unknown occurrences in the future. In this way, an attempt is made to prevent damage. Risk communication sets its sights on longer timeframes, whereas crisis communication relates to reactions after an actual incident and has the aim of restoring the affected infrastructure (cf. Federal Office of Civil Protection and Disaster Assistance [publisher], 2007, P. 320).

The communication measures of the authorities played an important and relevant role during the EHEC outbreak. They provided information (directly or indirectly through the media) to

the general public who always have an increased demand for information in times of crisis. Matter-of-fact, easily understandable and unequivocal communication is required in crisis communication in order to minimise fears among the general public. The communication of political institutions to the outside must be open and transparent. This also implies that any uncertainties which can arise in various contexts should be explained (Günther et al., 2011).

8.1 Press and Public Relations Work

The open information policy during the EHEC outbreak was based on close cooperation with all institutions involved, continuous press work and an established network of regional and suprarregional media and journalists.

In 2011, ten press releases were published on the subject of EHEC, three of them jointly with the Robert Koch Institute (RKI) and Federal Office of Consumer Protection and Food Safety (BVL) and one jointly with the RKI (see appendix, Table 10). The consumption recommendations in particular were coordinated and communicated jointly with the RKI. With over 200,000 visits per week – far more than the average of 30–40,000 times a week – a BfR website dedicated to the subject of EHEC was set up to support external communication.

The BfR also published eleven scientific statements, including one jointly with the RKI (see appendix, Table 11). These statements usually included the so-called “grey box” in which the results of risk assessment are summarized in a generally comprehensible manner, thus constituting an important instrument for the BfR to enhance the level of understanding, transparency and usefulness of scientific appraisals. Frequently asked questions (FAQ) and answers to four theme blocks were used to provide consumers with comprehensive information (see appendix, Table 13).

The BfR answered more than 300 press inquiries and gave more than 50 TV interviews. Five press conferences were held on the subject of EHEC in which the BfR was actively involved. Two of the press conferences were held at the BfR, two at the RKI and one at the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV). The numerous inquiries from members of the general public were answered with the help of the hotlines set up by the ministries and cooperation with *aid*.

A representative media response analysis was prepared during the crisis to provide reliable results on the basis of which strategic decisions for communication could be made. The following newspapers and magazines were included in the media response analysis: *Berliner Morgenpost*, *Berliner Zeitung*, *Bild*, *Bild am Sonntag*, *Die Welt*, *Die Zeit*, *FAZ*, *FAZ-Sonntag*, *Financial Times Deutschland*, *FOCUS*, *Frankfurter Rundschau*, *Hamburger Abendblatt*, *Handelsblatt*, *Lebensmittelzeitung*, *Rheinische Post*, *Spiegel*, *Stern*, *Stuttgarter Zeitung*, *Süddeutsche Zeitung*, *Tagesspiegel*, *TAZ*, *Wirtschaftswoche*. Within this selected media sample, all of the editorial articles that mentioned the topic of EHEC were recorded and analysed in regard to how often reports appeared on EHEC within a specific period. It was also determined how often reports were run on the BfR in connection with EHEC overall and which of the analysed media reported most frequently on the subject.

A total of 1,598 articles that mentioned EHEC were published in print media in the analysis period from 16 May 2011 to 15 June 2011. As time progressed, reporting on EHEC and/or the BfR in connection with EHEC reached its peak between 03 and 11 June 2011 with a total of 855 articles, 79 of which referred to the BfR.

It becomes clear in Fig. 21 that there were an awful lot of reports on the subject on 27 May 2011, one day after the Hamburg Institute for Hygiene and the Environment identified cucumbers from Spain as the EHEC carrier. There was another peak in media reporting on 03 and

04 June 2011 because shortly beforehand, on 02 June 2011, scientists at the University Clinic Hamburg-Eppendorf had decoded the genetic makeup of the pathogen and detected a previously unknown version through the combination of certain genetic characteristics. Overall, the media named above contained the most reports on EHEC on 08 June 2011. German crisis management was also one of the topics of these reports.

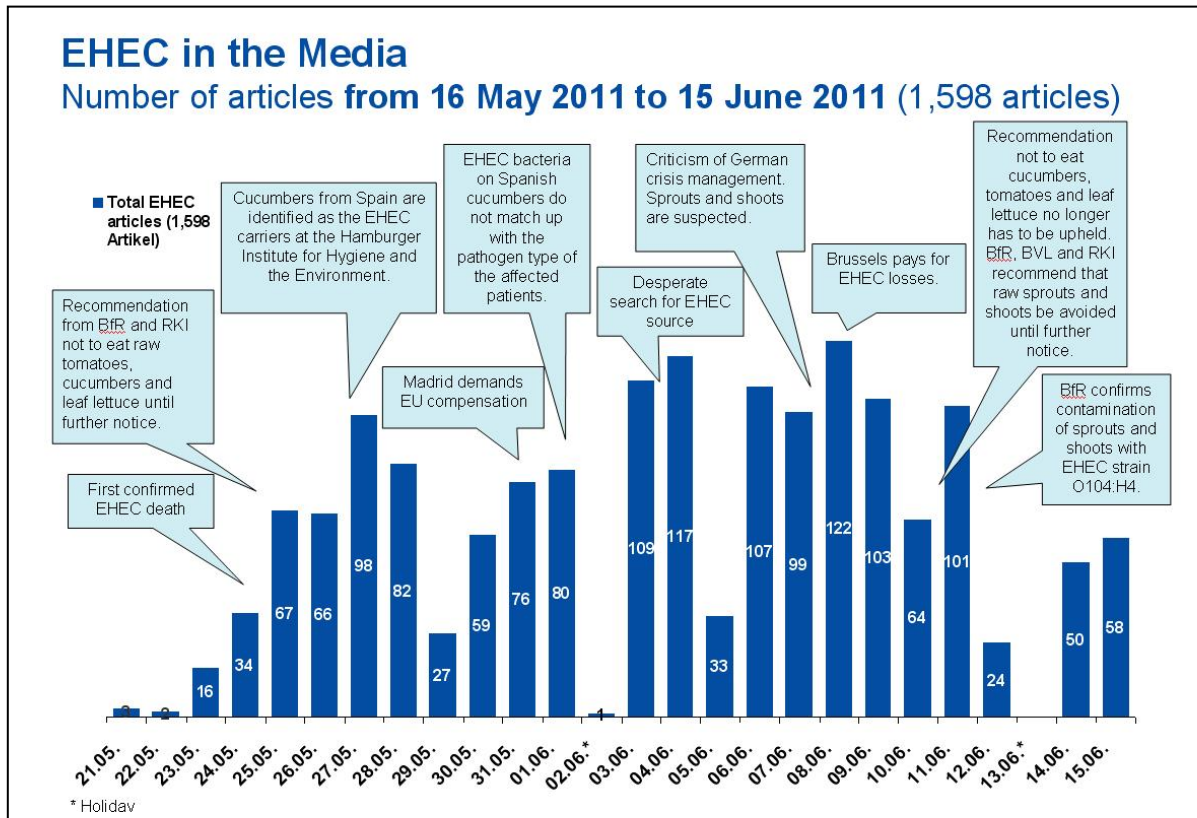


Fig. 21: EHEC in the Media

The BfR was mentioned in connection with EHEC in 130 articles (see Fig. 22). It was mentioned mainly in connection with consumer tips within the scope of the Federal Government's crisis management. The BfR appeared most frequently in the media in connection with EHEC on 07 June 2011. On this day, a BfR statement announced "that the pathogen could have been introduced to the affected foods in the current outbreak by humans or by humans via the environment" (statement available through: www.bfr.bund.de/cm/343/enterohaemorrhagische_escherichia_coli_o104_h4.pdf). The BfR also appeared very frequently in connection with EHEC in the analysed media set on 11 June 2011. On 10 June 2011, the BfR, BVL and RKI identified sprouts from a horticultural farm in Lower Saxony as the source of the EHEC outbreak and revoked the recommendation not to consume cucumbers, tomatoes and lettuce. The BfR also appeared relatively often in connection with EHEC on 14 June 2011. This was preceded on 12 June 2011 by the BfR's recommendation not to consume home-grown and raw sprouts.

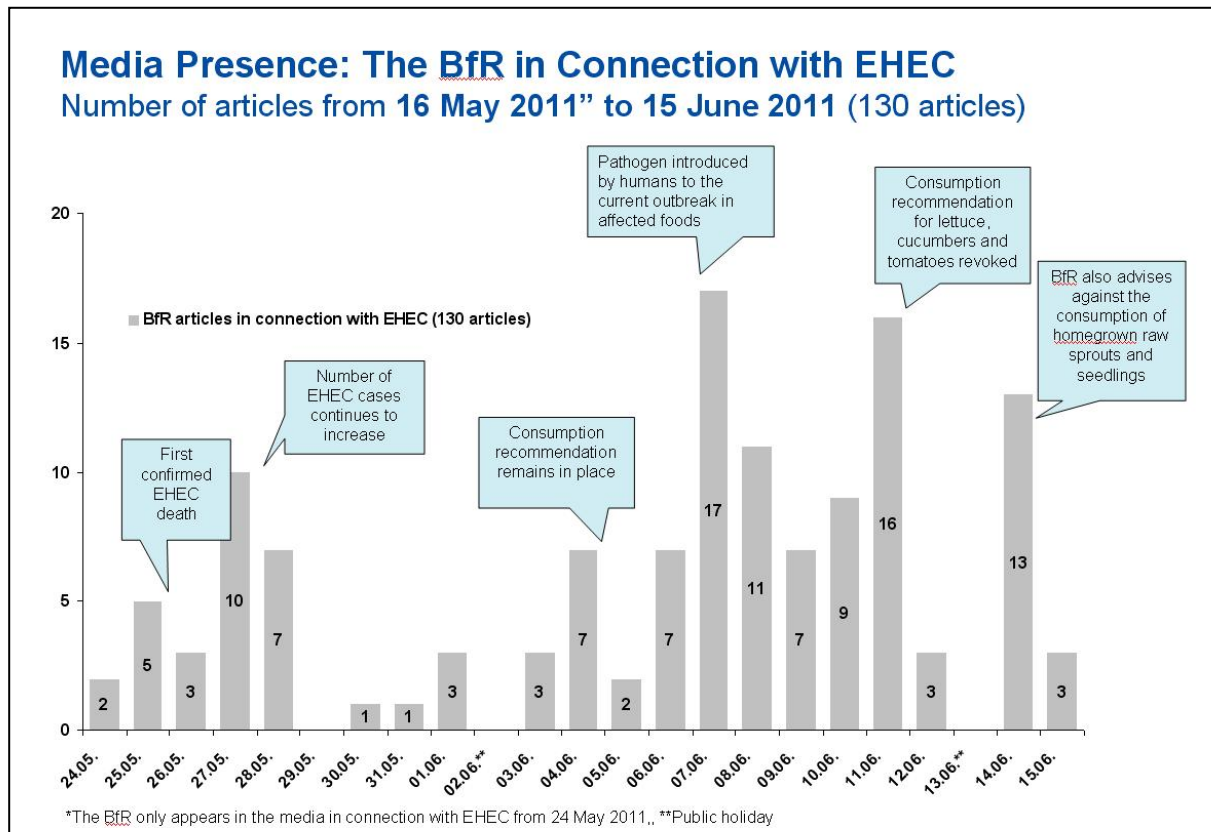


Fig. 22: Media Presence: The BfR in Connection with EHEC

The term EHEC was used most frequently in the reports of the *Süddeutsche Zeitung* (207 times), *Bild* (161 times) and *Hamburger Abendblatt* (144 times).

8.2 BfR Risk Communication on European Level

From 27 May 2011, shortly after the news broke in Germany of the first deaths in connection with the EHEC outbreak, the BfR in its capacity as the German EFSA contact point kept its national sister authorities in Europe and the EFSA informed of the latest status of the outbreak in Germany with 17 memos.

In addition to the 26 remaining EU member states, six other European countries were kept up-to-date with the occurrences so that they could act responsibly towards the citizens of their countries.

Even in the first communication on European level on 25 May 2011, the BfR made reference to the various information sources in Germany regarding the EHEC outbreak. In this way, the responsible authorities in Europe had the necessary information available to them to inform the general public in the appropriate manner, including information on inner-European transport, and prepare protective measures in the countries affected by the EHEC outbreak.

To intensify contacts with the responsible authorities on European level, the BfR conducted a telephone conference on 09 June 2011 to which representatives of all of the other 26 member states were invited along with EFSA representatives. During the telephone conference, information was exchanged with a view towards identifying the source of the EHEC outbreak and coordinating scientific activities.

BfR scientists were also members of the EFSA Task Force, thus contributing towards the investigation of the outbreak in Europe.

Through the special technical information it provided from Germany, the BfR created the basis for consumption recommendations on a European level. It also supported the investigation of the French outbreak through a transfer of know-how on the process for detecting the pathogen strain and participated in other international exchanges of scientific information, such as WHO telephone conferences.

8.3 Analysis of Risk Perception by the General Public

When the EHEC outbreak began to subside, a representative population survey was conducted on knowledge of EHEC, risk perception, changed behaviour since news of the first EHEC cases broke and appraisal of EHEC-related communication (especially by the authorities). The survey was conducted from 08 to 20 August 2011 by means of computer-supported telephone interviews. A total of 1,002 persons aged 14 and over were questioned. The random sample of interviewees is representative of all German-speaking persons living in private households with a telephone connection in the Federal Republic of Germany⁴.

8.3.1 Knowledge of EHEC

Of the 1,002 persons questioned, 931 (= 93 %) stated that they had heard or read about the EHEC outbreak. 7 % were not aware of it, however. There were distinct regional differences regarding awareness of the EHEC outbreak. All of the persons questioned had heard or read about it in several *Laender* (Hamburg and Saxony-Anhalt). By way of comparison, the fewest number of people had heard or read about the EHEC outbreak in Saxony (84 %) and Saarland (82 %). The state-specific results (about which more is reported below) should in general be interpreted with a degree of caution because although the random sample of the population questioned is representative of all of the Federal Republic of Germany, it is not representative of each individual federal state. There were no or only marginal differences regarding this question between men and women and with regard to the age of the persons questioned.

When presenting the results below, each instance relates to the 931 persons who had heard or read about the EHEC outbreak.

⁴ The appendix includes tables of detailed information on the age, sex, highest level of school and/or university education attained and number of persons questioned per federal state (Tables 15 to 18 in the annex).

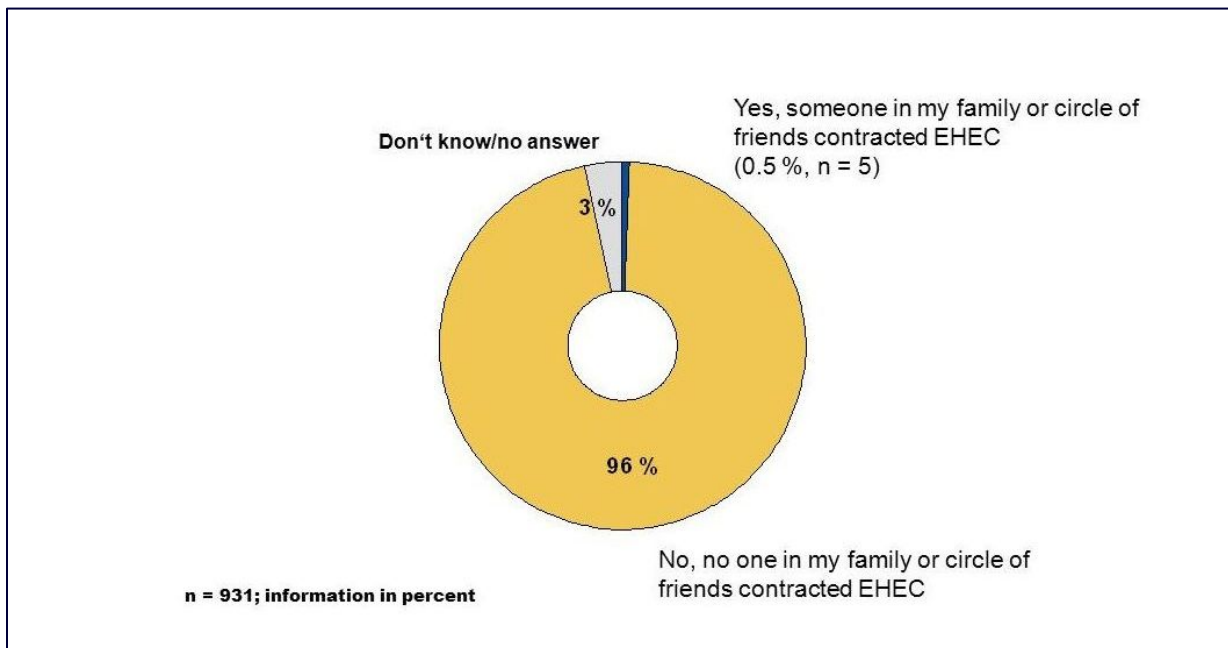


Fig. 23: Did you, a member of your family or anyone in your circle of friends or acquaintances contract an EHEC infection?

Almost all of the persons questioned (96 %) stated that they were not personally affected by EHEC, i.e. that neither they themselves nor anyone in their family or circle of friends and acquaintances contracted an EHEC infection (see Fig. 23). 3 % of those questioned either did not answer the question or were not sure whether someone in their family or circle of friends and acquaintances had contracted EHEC. Only five interviewees (= 0.5 %) reported that a member of their family or circle of friends and acquaintances had contracted EHEC. None of the persons questioned had themselves been infected with the EHEC pathogen.

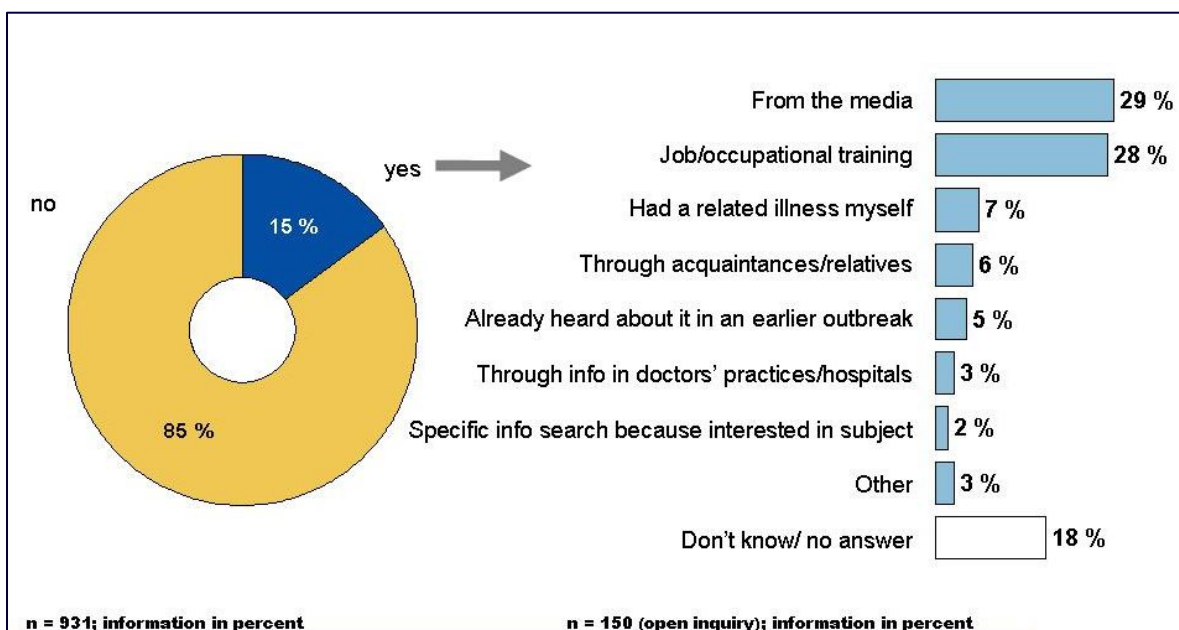


Fig. 24: Had you heard or read about EHEC prior to the EHEC outbreak? In which context did you hear about it?

A clear majority (85 %) of the persons questioned had never heard or read about EHEC prior to the outbreak in May, June and July 2011. The 15 % (n = 150) who had heard or read about the subject before the outbreak were confronted by it mainly through the media (29 %) or in the course of their jobs or occupational training (28 %) (see Fig. 24).

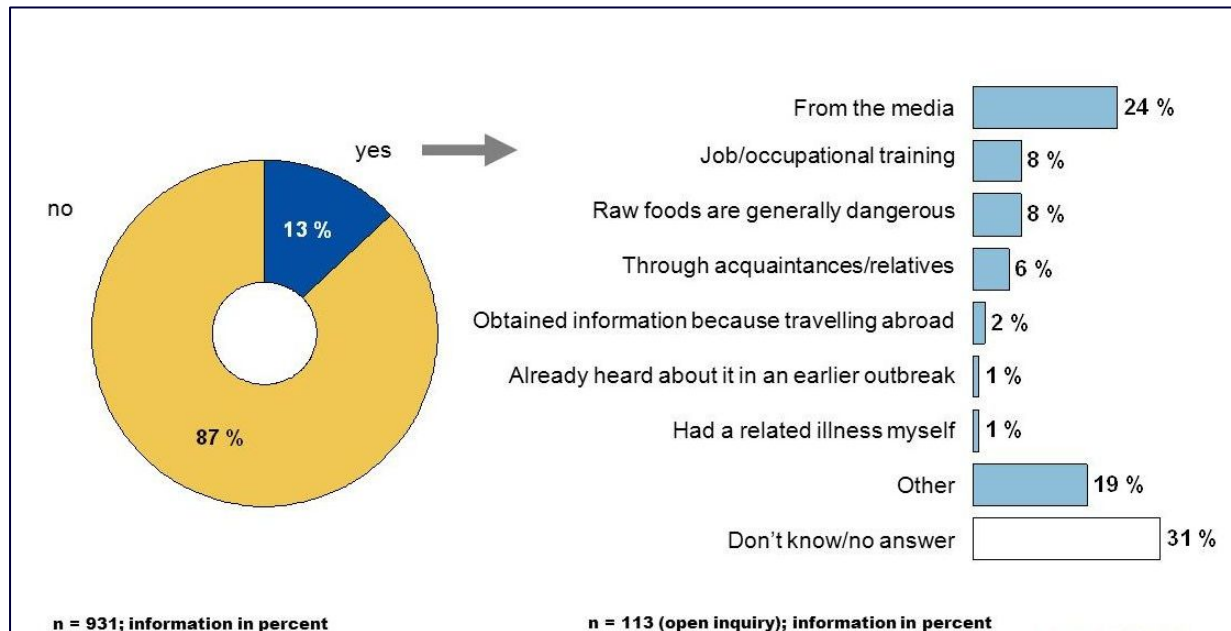


Fig. 25: Had you heard or read before the EHEC outbreak that the consumption of raw sprouts could trigger a food infection? In which context did you hear about it?

The interviewees were also asked whether they had heard or read prior to the EHEC outbreak in 2011 that the consumption of raw sprouts could trigger a food infection. 13 % answered this question with yes. When subsequently asked in which context this was, almost a third (31 %) gave no answer or stated that they did not know in which context it had been. Roughly a quarter of those questioned (24 %) stated that they had heard or read in the media that the consumption of raw sprouts could trigger a food infection (see Fig. 25).

When asked if they knew of any other foods in which EHEC bacteria can occur, half of the persons questioned (49 %, n = 446) answered with yes. A further 50 % answered with no and 1 % gave no answer or stated that they did not know.

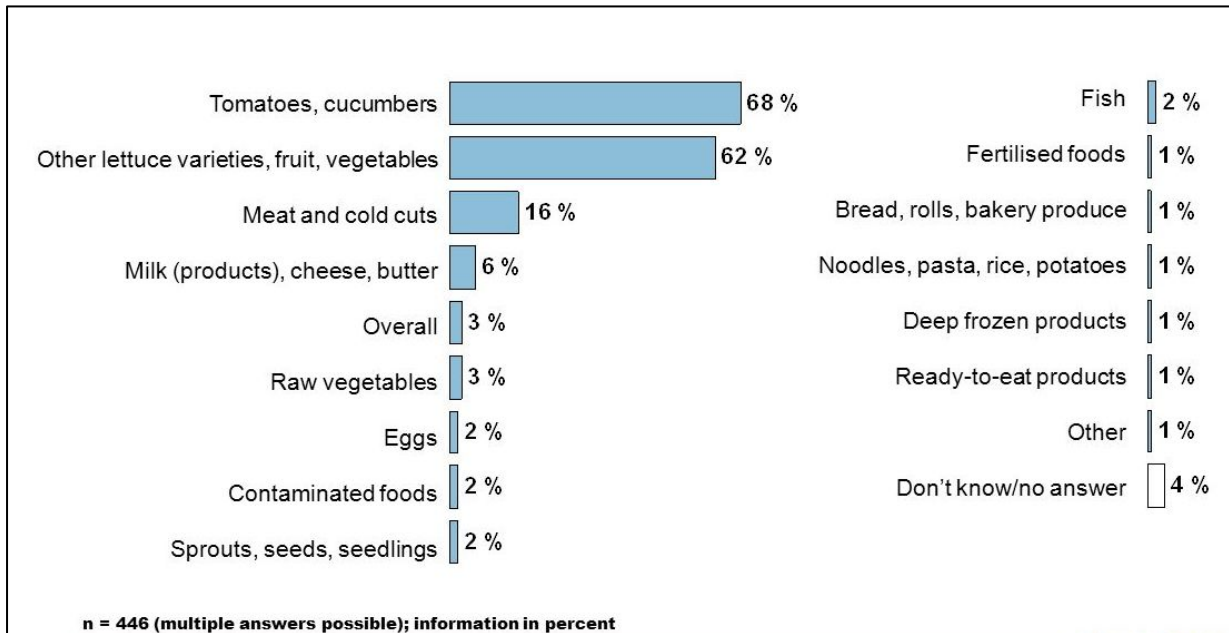


Fig. 26: In which foods can EHEC bacteria occur in your opinion?

Of the persons who stated that they knew in which other foods EHEC bacteria can occur, tomatoes and cucumbers were named most often (by 68 % of those questioned) and lettuce, vegetables and fruit (by 62 %). It was only mentioned comparatively seldom that EHEC bacteria can occur in meat and cold cuts (by 16 % of those questioned) and in milk (products), cheese and butter (by 6 %) (see Fig. 26).

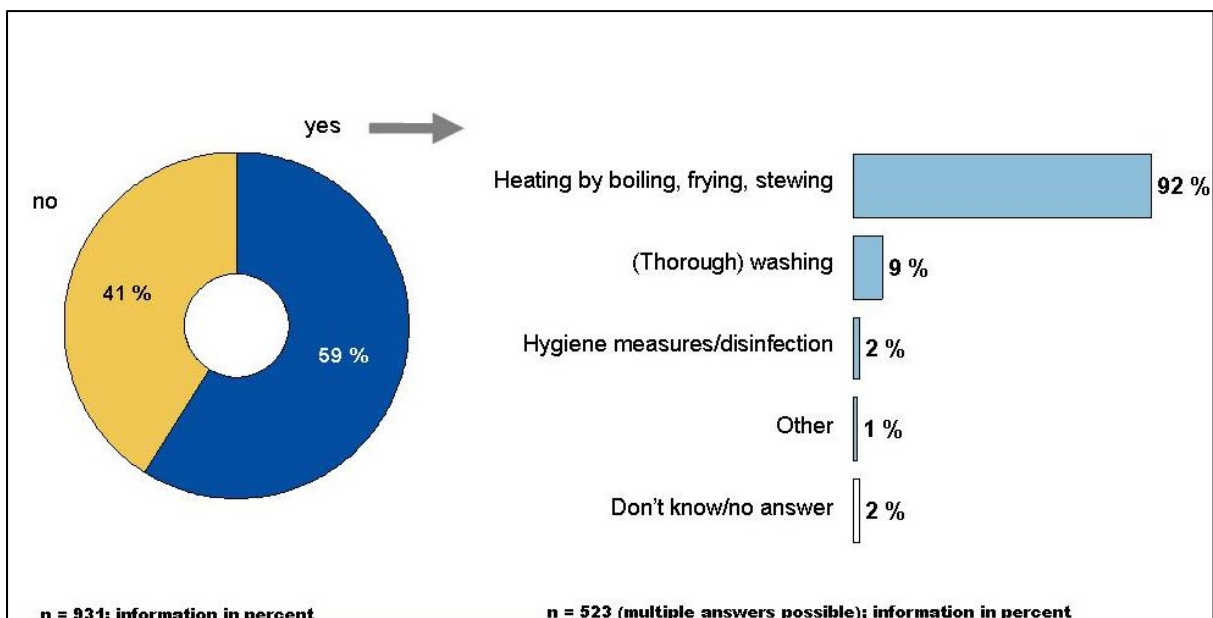


Fig. 27: Do you know how EHEC bacteria in food can be killed off? By what means can EHEC bacteria in food be eliminated?

Asked if they knew how to kill off EHEC bacteria in food, the majority (59 % of the people questioned) answered with yes. Despite this, 41 % stated explicitly that they did not know

how EHEC bacteria in food can be killed. Almost all (92 %) of the people who answered the question with yes stated that it was possible to kill EHEC bacteria through heating the food (boiling, frying, stewing). A small percentage (9 %, n = 51) stated that it was possible through (thorough) washing and/or other hygiene measures such as disinfection (2 %, n = 9) (see Fig. 27).

8.3.2 Risk Perception and Changed Behaviour

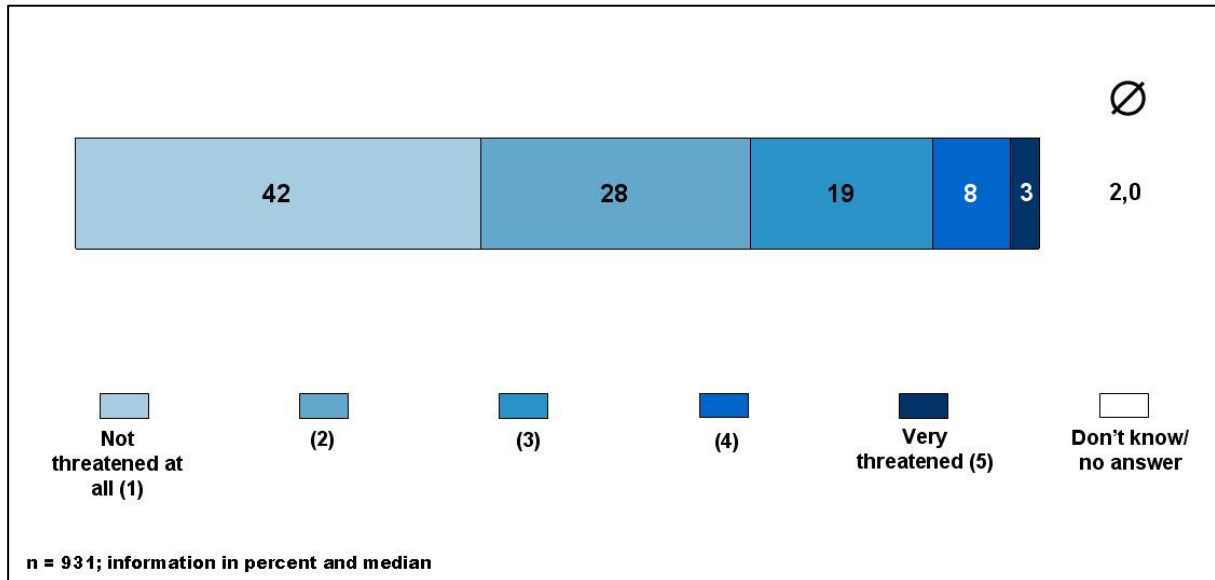


Fig. 28: Have you or your family felt threatened by EHEC?

In reply to the question as to whether people themselves or their families had felt threatened by EHEC, 70 % of those questioned stated that they had not felt threatened (at all) by EHEC (see Fig. 28). Roughly a fifth (19 %) of the persons questioned stated that they or their families had felt a little threatened. Only a comparatively small percentage (11 %) of the people questioned felt (very) threatened.

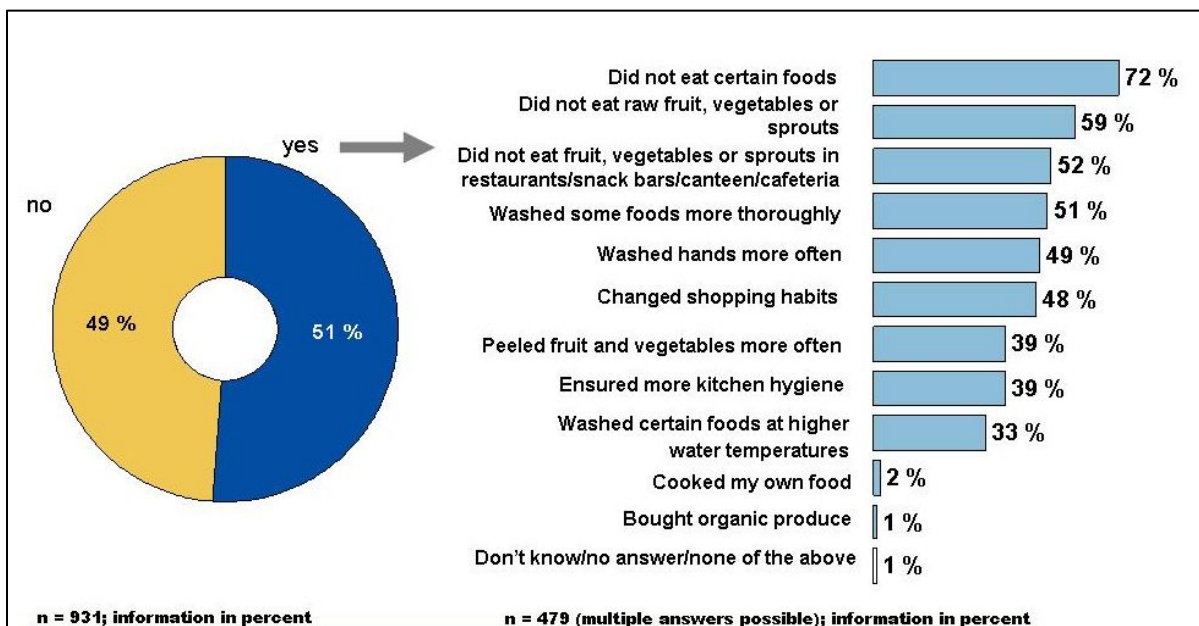


Fig. 29: Did you change your behaviour during the EHEC outbreak to protect yourself from the bacteria? What did you do to protect yourself against EHEC?

Roughly half of the persons questioned (51 %) stated that they had changed their behaviour during the EHEC outbreak to protect themselves from the bacteria (see Fig. 29). There were clear difference regarding this question between persons who felt threatened by EHEC and persons who did not. Almost all (92 %) of the persons who felt threatened by the EHEC pathogen changed their behaviour. On the other hand, only about half of the persons who felt hardly, slightly or not at all threatened changed their behaviour.

It was reported most frequently that people had avoided certain foods (72 %) or had not eaten raw fruit, vegetables or sprouts (59 %). People also stated very often that they had not eaten fruit, vegetables or sprouts in public gastronomy businesses (52 %), had washed certain foods more thoroughly (51 %), had washed their hands more often (49 %) and had changed their shopping habits (48 %) (see Fig. 29).

8.3.3 Appropriateness and Understandability of Consumption Recommendations

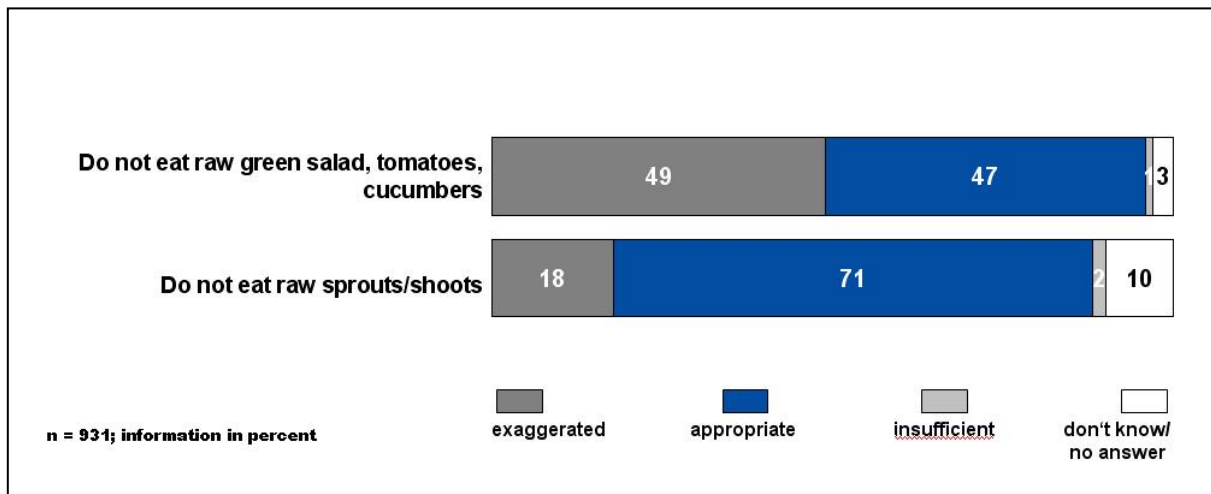


Fig. 30: Did you find the following recommendations issued by public authorities appropriate?

Consumption recommendations were issued by public authorities during the EHEC outbreak. Among other things, from 25 May 2011 the RKI and BfR jointly advised against the consumption of raw lettuce, tomatoes and cucumber. In retrospect, 47 % of the persons questioned found this recommendation appropriate in August 2011, but just as many (49 %) regarded it as exaggerated in retrospect. A very small percentage of those questioned (1 %) regarded the recommendation as insufficient or gave no answer to the question (3 %) (see Fig. 30). This consumption recommendation was revoked on 10 June 2011.

The advice not to consume raw sprouts (joint recommendation of the BfR, Federal Office of Consumer Protection and Food Safety [BVL] and RKI of 10 June 2011) was regarded as appropriate in retrospect by 71 % of those questioned. Almost one fifth of the persons questioned (18 %), however, evaluated this recommendation as exaggerated in retrospect (see Fig. 30).

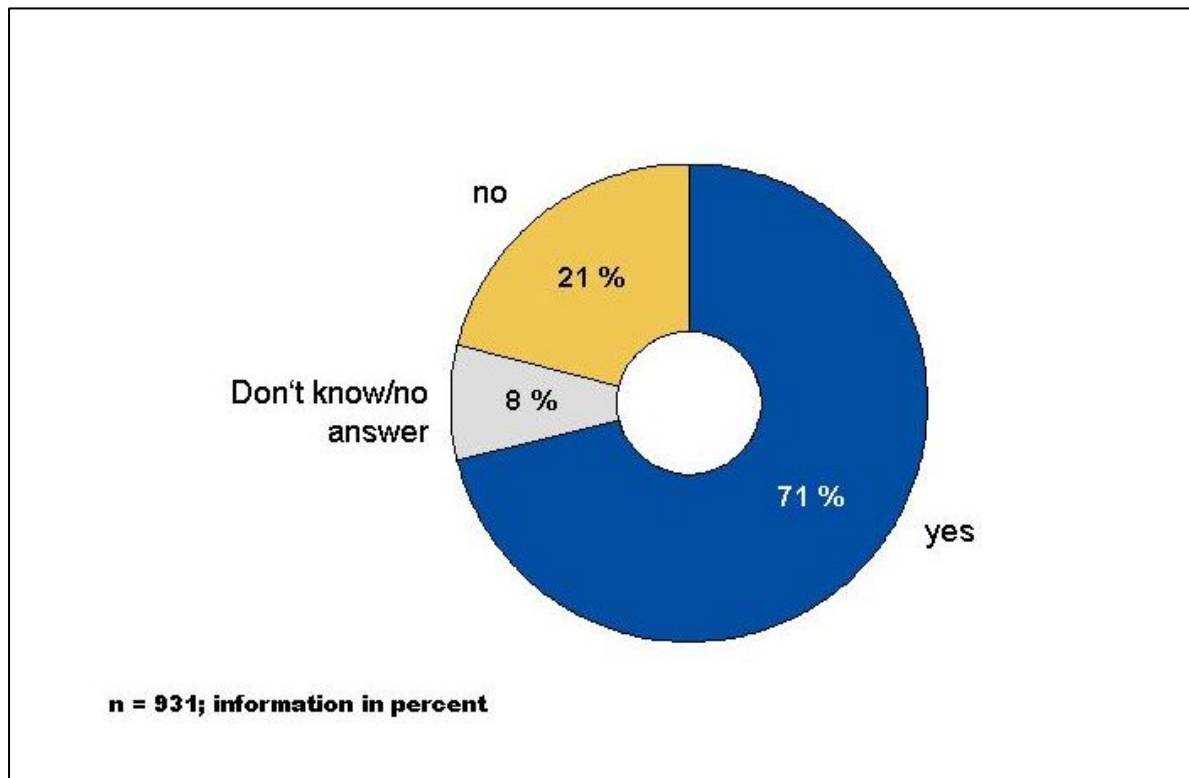


Fig. 31: Did you understand why the initial recommendation not to eat raw lettuce, tomatoes and cucumbers was revoked in the light of new information?

Roughly three quarters of those questioned (74 %) understood that there was initially a recommendation not to consume raw lettuce, tomatoes and cucumbers and that this recommendation was then revoked in the light of fresh information. Almost a quarter of the persons questioned (23 %), however, did not understand why the consumption recommendation was revoked (see Fig. 31).

8.3.4 The Players in Consumer Protection

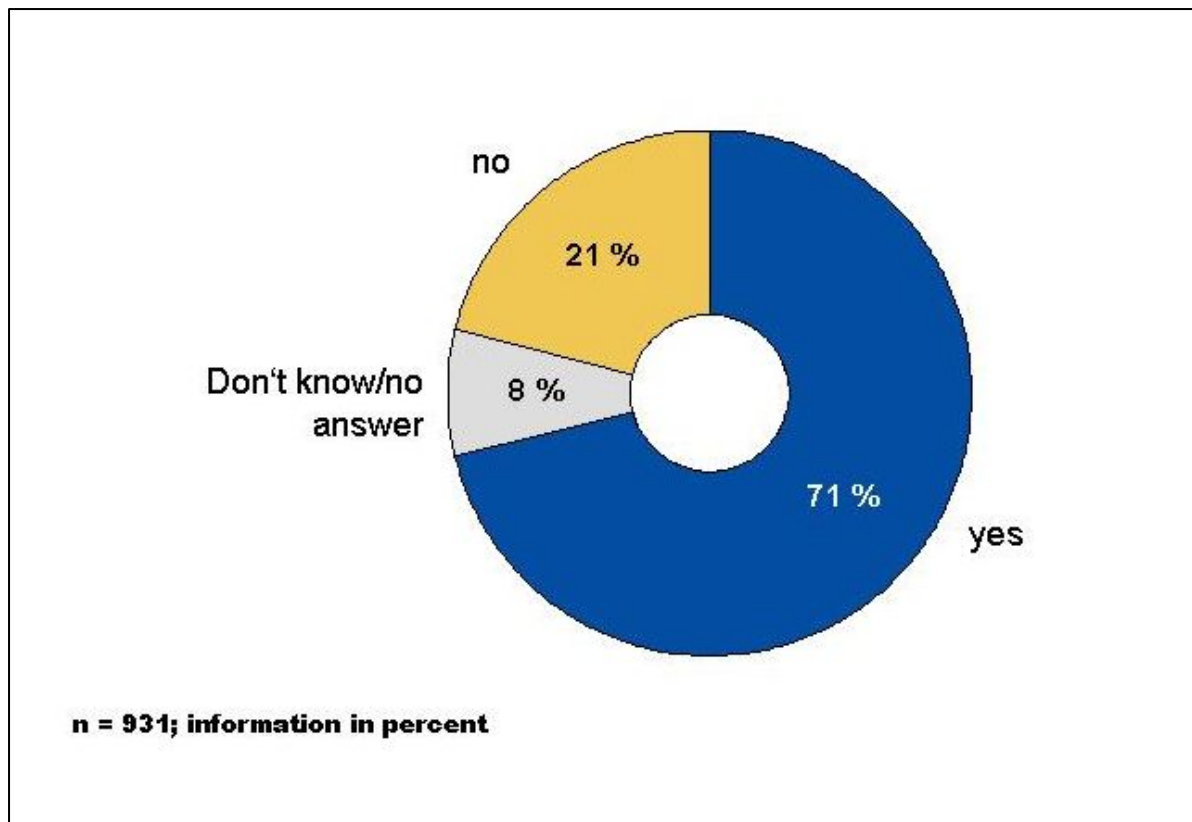


Fig. 32: Did the responsible authorities in Germany make enough efforts to protect the population from the EHEC pathogen in your opinion?

In reply to the question as to whether the responsible authorities in Germany made enough efforts to protect the population from the EHEC pathogen, 71 % of those questioned answered with “yes”. The same question was also answered with “no”, however, by 21 %. A further 8 % of the persons questioned gave no answer to the question (see Fig. 32). There are slight regional differences where this question is concerned. There were particularly critical appraisals in the *Laender* of Berlin and Mecklenburg-Western Pomerania where almost 30 % of the persons questioned stated that the responsible authorities had not done enough in their view to protect the general public from the EHEC pathogen.

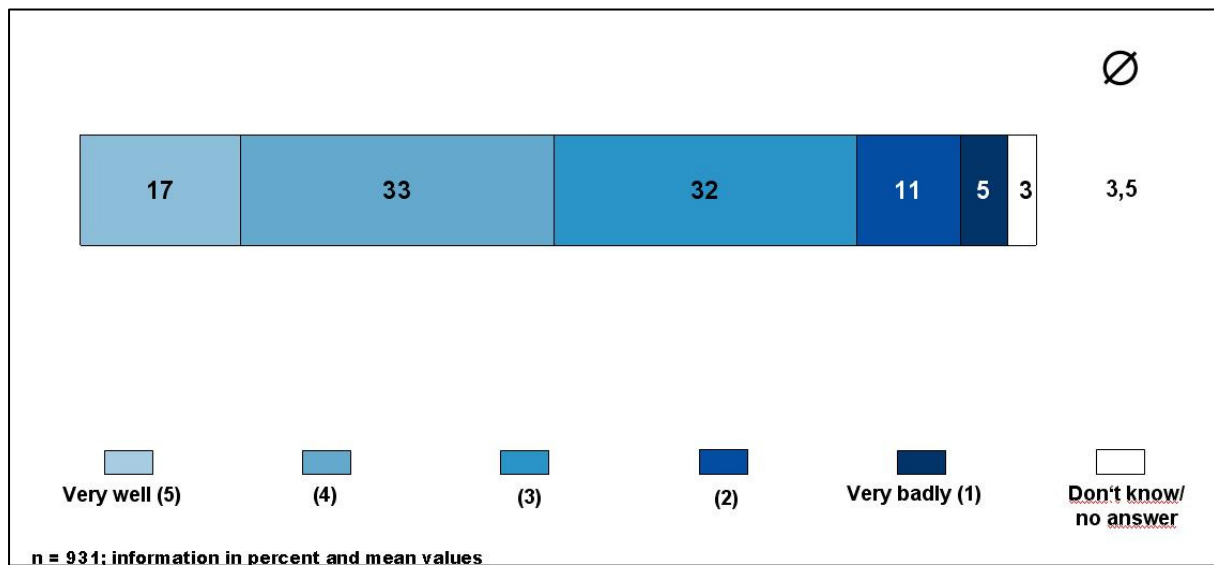


Fig. 33: Did you feel sufficiently well informed about EHEC by the national authorities?

The question as to whether people had felt themselves sufficiently well informed by the national authorities in regard to EHEC was evaluated as good to average by the majority of those questioned (see Fig. 33). Half of those questioned answered with “very good” or “good”. Almost a third, however, (32 %) answered this question with “partly partly” and a further 16 % did not consider themselves sufficiently well informed (11 % poorly informed and 5 % very poorly informed).

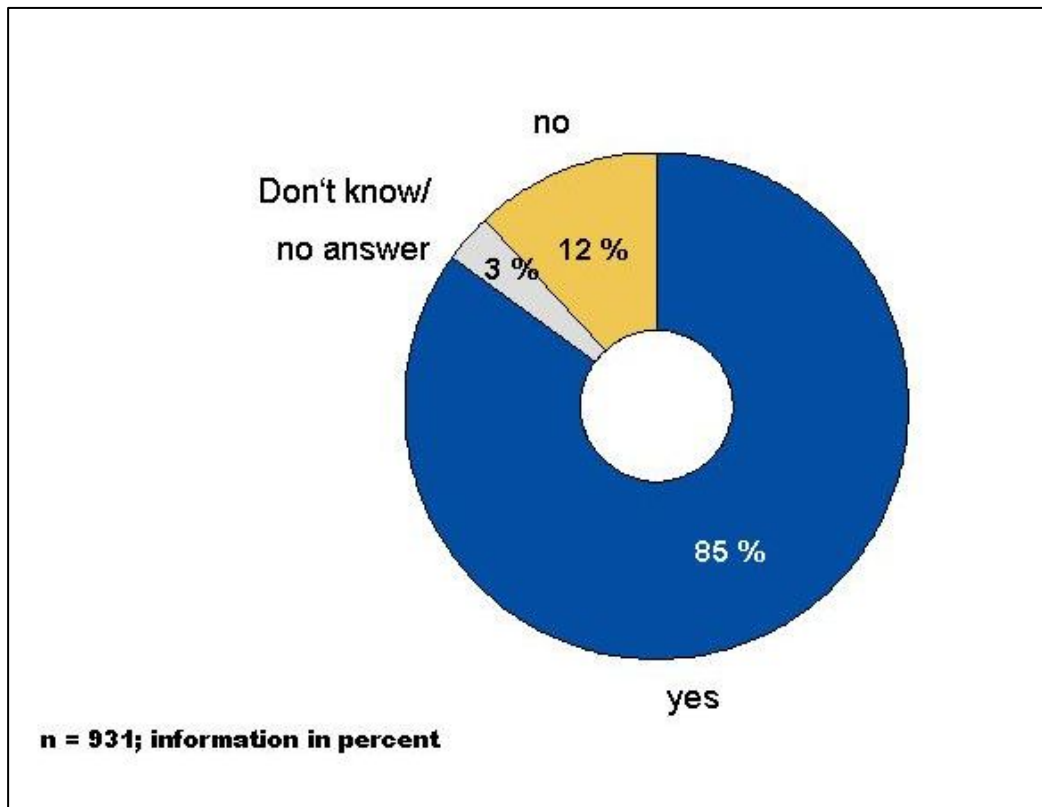


Fig. 34: Did you find the information on EHEC easy to understand?

The information conveyed on the topic of EHEC was evaluated as understandable by the vast majority (85 %), as opposed to 12 % who regarded the conveyed information as incomprehensible. A further 3 % did not answer this question (see Fig. 34).

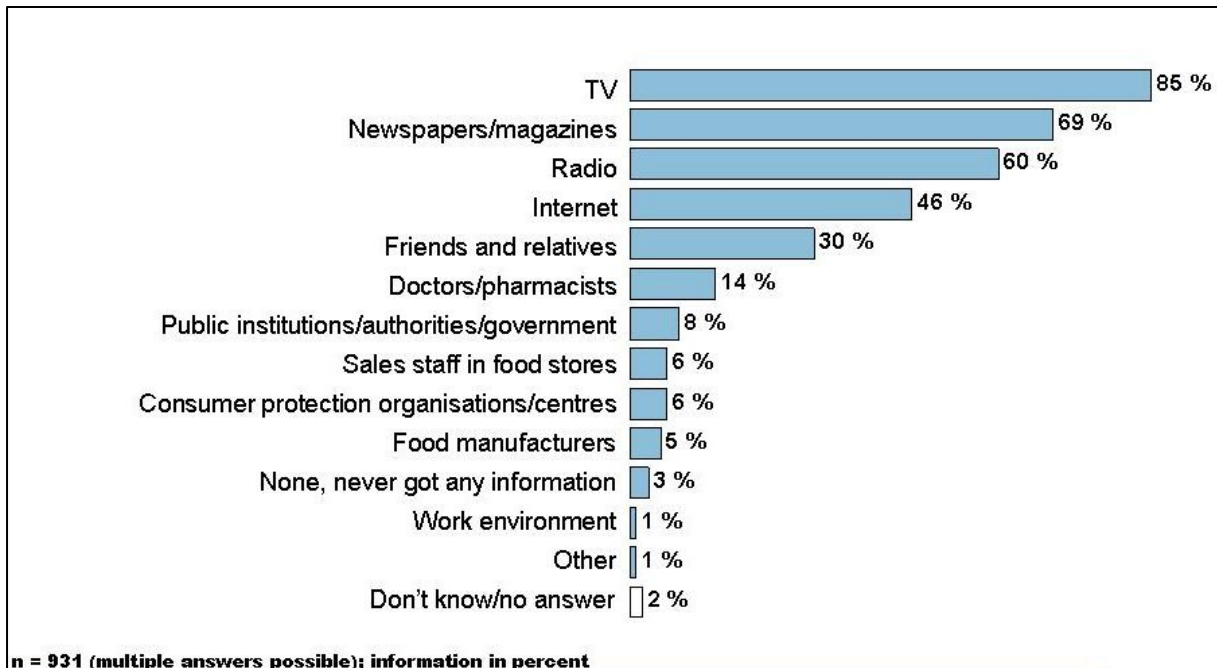


Fig. 35: Which information sources did you use personally to find out about EHEC?

Most of the persons questioned (85 %) used television to find out about EHEC. Newspapers and magazines (69 %), radio (60 %), the internet (46 %) and friends and relatives (30 %) were also used as information sources (see Fig. 35).

The information sources used were assessed in the main as trustworthy. Television was appraised as trustworthy by 75 % of the persons who used it as an information source. Information from newspapers and magazines was appraised as trustworthy by 78 % of users, from the radio by a further 78 %, from the internet by 80 % and from friends and relatives by 69 % of users.

8.3.5 Comparative Risk Assessment

In conclusion, the persons questioned were asked to give comparative estimations in regard to various food-related risks, six of which were named:

1. Infection with EHEC
2. New technologies, such as the cloning of animals, genetic technology or nanotechnology
3. Animal infections or diseases which can be transferred to humans, such as mad cow disease/BSE
4. An unhealthy diet due to too much fat, for example
5. Chemical substances in foods, such as pesticide residues, preservatives or artificial aromas and
6. Bacteria in foods, e.g. salmonella in eggs⁵.

For each of these risks, a scale from 1 to 5 was to be used to ascertain whether no risk or a very high risk of health damage was presumed.

⁵ The risk of infection with EHEC is actually a bacterial risk. Because the survey dealt mainly with the subject of EHEC, however, "Infection with EHEC" was deliberately listed separately. The food-related risks in the items 2. to 6. are based on the Special Eurobarometer 354 (2010).

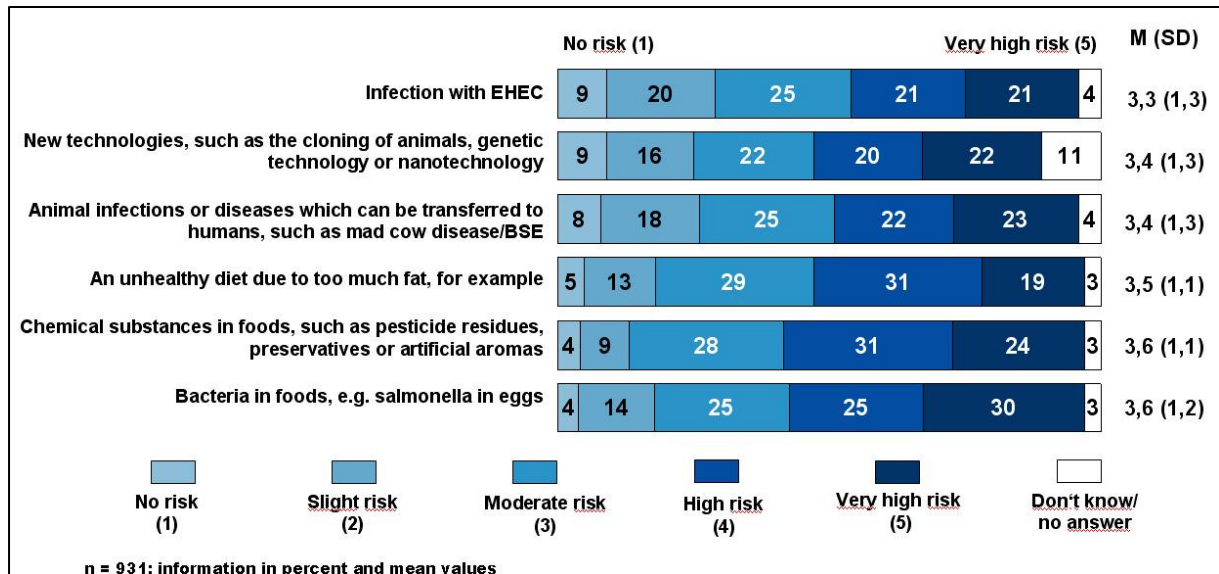


Fig. 36: How high do you estimate the risk of suffering health damage through one of the following food-related risks?

The mean estimations of the persons questioned regarding the listed risks lie close together (see Fig. 36). The risk of suffering health damage through an infection with EHEC is regarded as the lowest ($M_{\text{(EHEC)}} = 3.3$). The risk of suffering health damage through chemical substances in foods (e.g. pesticide residues, preservatives or artificial aromas) or through bacteria in foods (e.g. salmonella in eggs) is regarded as the highest in comparison ($M_{\text{(chemical substances)}} = 3.6$; $M_{\text{(bacteria in foods)}} = 3.6$). Although these mean value differences are significant from a statistical point of view, they are hardly important at all from a practical point of view.⁶ In synopsis it can therefore be stated that there are hardly any differences in the estimation of mean risks. On average, a medium to high risk of suffering health damage is recognised with the listed risks.

Reference was then made to the subject of dioxin in foods and feeds, which was highly topical at the beginning of 2011, as an additional comparative risk. To this end, the interviewees were asked initially if they had heard or read that increased dioxin concentrations had been found in feedstuffs at the beginning of the year as a result of which some foodstuffs, such as eggs and meat, had also contained higher dioxin levels. The vast majority of those questioned (85 %) stated that they had heard or read about it. An additional 14 % had heard or read nothing about it and 1 % gave no answer or were not sure if they had heard or read about it. The persons who knew about the dioxin ($n = 803$) were asked to compare the two food-related risks of dioxin in foods and infection with EHEC (see Fig. 37).

⁶ The significance of a mean value difference is determined by the effect size (d) which gives the mean value difference in units of standard deviation (SD) (Bortz, 1993). According to Cohen (1988), the following guidelines can be used to evaluate effect sizes: $d \geq .20$ indicates a small effect, $d \geq .50$ a medium effect and $d \geq .80$ a great effect. In this examination, the standard deviations range from $SD = 1.1$ (for chemical substances in foods) to $SD = 1.3$ (for new technologies). For this reason, when placed in relation to the standard deviation, the greatest mean value difference of .3 is so slight that one can only speak of a small effect.

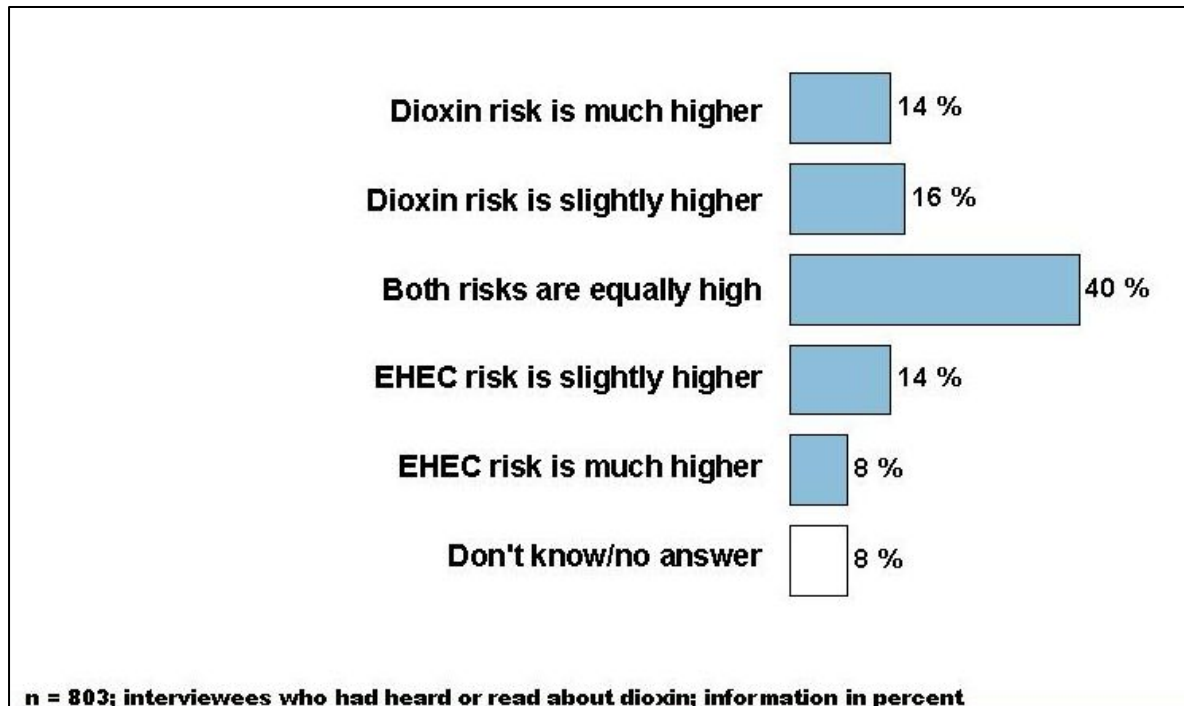


Fig. 37: How would you estimate your own personal risk of suffering health damage when comparing the two possibilities of dioxin in food and EHEC?

Two fifths (40 %) of the persons questioned stated that in their view, the risk of suffering health damage is just about equally great in both instances. A further 30 % reported that in their opinion, the risk of suffering health damage through dioxin is very much (14 %) or slightly (16 %) higher than through EHEC. Slightly fewer than a quarter of the persons questioned (22 %) estimated the risk of EHEC in comparison to dioxin as very much (8 %) or slightly (14 %) higher. Eight percent of those questioned gave no answer to this question. Overall therefore, the two scenarios – dioxin in foods and EHEC – were estimated to be just about equally threatening.

8.3.6 Summary and Discussion of the Results of the Population Survey

8.3.6.1 Knowledge of EHEC

Almost all of the persons questioned (93 %) had heard or read about the EHEC outbreak in May, June and July 2011. In light of the fact that the EHEC outbreak constituted the biggest outbreak of EHEC infections in Germany, however, with over 3,800 people contracting HUS or acute gastroenteritis and more than 50 fatalities (RKI, 2011), it must be emphasised that not all of the persons questioned stated that they had heard or read about the outbreak. Prior to this year's outbreak, however, only a few people (15 %) were aware of the subject of EHEC.

It must also be noted that around 40 % of the persons questioned did not know how EHEC bacteria in food can be killed off. The persons questioned stated mainly that they had dispensed with certain foods, but the option of heating foods for at least two minutes at temperatures of at least 70 °C (at the core of the food) was not known to the majority of them. This may also have had something to do with the foods suspected of having caused the EHEC outbreak: lettuce, tomatoes, cucumbers and sprouts, which are often eaten raw. This may have had the result that people tended to dispense with these foods altogether. Had other foods been involved which are not normally consumed raw so much (e.g. meat prod-

ucts), the possibility of killing off EHEC bacteria through heating might have been perceived more strongly. Add to this the fact that some of the persons questioned had erroneous information on how EHEC bacteria in foods can be eliminated. Accordingly, 60 of the persons questioned stated that EHEC bacteria in foods can be killed off by washing them thoroughly or by means of certain hygiene measures or disinfection. This also underscores the fact that the general public was given insufficient or wrong information here.

8.3.6.2 Risk Perception and Changed Behaviour

On average, the threat posed by the EHEC outbreak was regarded as relatively low. Only about one in ten of the persons questioned felt threatened. Overall, roughly half of those questioned reported that they had changed their behaviour. Virtually everyone who felt threatened changed their behaviour, but so did roughly half of all of the persons who only felt slightly threatened or not at all.

At the same time, however, it also means that almost half of the persons questioned did not change their behaviour. This can be explained by clear differences between the *Laender*. The EHEC outbreak had a strong regional component. The five northern federal states (Hamburg, Schleswig-Holstein, Bremen, Mecklenburg-Western Pomerania and Lower Saxony) were affected in particular. Accordingly, there were big differences from state to state as to how many persons changed their behaviour. Roughly two thirds of the persons questioned in Schleswig-Holstein and Lower Saxony reported that they had changed their behaviour, whereas the percentage of persons who changed their behaviour in Saarland, Rhineland-Palatinate, Baden-Württemberg, Brandenburg and Bavaria was relatively low (between 20 % and 45 %).

8.3.6.3 Appropriateness and Understandability of Consumption Recommendations

Roughly half of the persons questioned found the recommendation not to consume lettuce, tomatoes and cucumbers appropriate in retrospect, while just as many regarded it as exaggerated. The recommendation not to eat sprouts was considered appropriate by the majority of those questioned (71 %). It might be possible to explain these differences by the findings made within the framework of the investigation of the EHEC outbreak. The recommendation not to consume lettuce, tomatoes and cucumbers was revoked as far back as early June because the results of additional epidemiological studies had indicated that the outbreak of EHEC was due to the consumption of contaminated sprouts. It was then confirmed in July 2011 that in all probability, contaminated fenugreek seeds and the sprouts cultivated from them were the cause of the EHEC outbreak. On the basis of these occurrences, it is conceivable that in retrospect, a majority of the persons questioned in August 2011 regarded the recommendation not to consume lettuce, tomatoes and cucumbers as exaggerated.

The decision of the authorities, on the other hand, to revoke the recommendation not to consume lettuce, tomatoes and cucumbers initially, then sprouts, was not understood by about a quarter of all of the persons questioned. Here too, there are differences between the individual federal states, whereby considerably more people in Rhineland-Palatinate (39 %) and Saarland (44 %) in particular regarded the revocation of the consumption recommendation as incomprehensible. In the other federal states this figure varied between 15 and 27 %.

8.3.6.4 Players in Consumer Protection

Only roughly a fifth of the persons questioned stated that the responsible authorities in Germany had not done enough in their view to protect the general public from the EHEC patho-

gen. Half of them, on the other hand, felt that enough information had been provided by the national authorities.

The majority of the persons questioned obtained their information on EHEC through classical media (TV, newspapers/magazines, radio). Most of them regard the information sources they used as trustworthy.

8.3.6.5 Comparative Risk Estimation

When asked about the extent to which they believed they could suffer impaired health through various food-related risks (infection with EHEC, new technologies, animal infections or diseases, unhealthy diet, chemical substances in foods, bacteria in foods), the persons questioned assessed them all as being more or less equally problematic. This is a slightly surprising result, as it could have been assumed that infection with EHEC would have been regarded as much more of a health risk such a short time after the EHEC outbreak compared with topics and risks which were far less the focus of attention at that point in time. In addition to this, the EHEC outbreak was accompanied by characteristics which normally sharpen risk perception, such as the fact that a large number of people took ill and even died within a very short space of time. Despite this, there were hardly any differences in the estimation of the listed risks.

A possible explanation for this could be that even in August 2011, the risk perception and/or threat experience of the persons questioned in regard to an EHEC infection was at a "normal" level. This probably had to do with the fact that as early as the end of May the number of new infections had declined and that in July 2011 it was announced by the authorities that the EHEC outbreak had in all probability been caused by the consumption of contaminated sprouts.

8.4 Conclusion

The measures taken within the scope of the risk communication of the EHEC outbreak can be summarised as follows:

Which tasks were assumed within the scope of press and public relations work in connection with the EHEC outbreak?

- Communication was by means of interviews, scientific statements, press releases and FAQ on the BfR homepage.
- Joint press conferences were held by the BfR, RKI and BVL.
- Concrete, easily understandable instructions were given to the general public in the form of consumption recommendations based on scientific data.
- Questions on EHEC from the press and general public were answered competently and in a timely manner by various means including support from the BMELV hotline and cooperation with the aid.

Which risk communication measures were taken on a European level?

- The EFSA Focal Point at the BfR kept the EFSA and all European member states continuously and actively informed about EHEC.
- The EFSA Focal Point at the BfR set up a trans-European telephone conference to enable an exchange of information among all members of the EFSA Advisory Forum of the European member states.

How threatened did consumers feel by the EHEC outbreak? Did they change their behaviour?

- On average, only a slight threat was experienced by the general public due to the EHEC outbreak.
- Overall, roughly half of the persons questioned changed their behaviour during the outbreak. It was most common for people to stop buying certain foods and/or stop eating them raw. There were differences regarding changes of behaviour between persons who felt threatened and those who felt less threatened. Almost all of the persons who felt threatened changed their behaviour whereas fewer than half of the persons who only felt a little bit threatened changed their behaviour.
- Looking back at the EHEC outbreak, consumers viewed the risk of contracting an EHEC infection compared to other food-related risks as more or less equal.

How did consumers acquire information on the EHEC outbreak? How well informed was the consumer? How did consumers assess official communication?

- Most people acquired information on EHEC through classical media (TV, radio, newspapers/magazines). These sources of information were regarded by the majority as trustworthy.
- Half of the persons questioned felt sufficiently well informed by the public authorities in regard to EHEC.
- 80 % of those questioned stated that the responsible authorities had done enough to protect the general public from the EHEC pathogen.
- Many of them did not know, however, or had obtained erroneous information on how or by what means EHEC bacteria in foods can be eliminated.
- In addition to this, the revocation of the consumption recommendation was incomprehensible to roughly a quarter of those questioned.

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8.6 Annex

Tab. 10: Published documents on EHEC – Press releases

Date	Title
21.07.2011 23/2011	EHEC: BfR, BVL and RKI issue specified consumption recommendations for uncooked sprouts and shoots (germ buds)
05.07.2011 21/2011	EHEC O104:H4 outbreak event in Germany clarified: sprouts of fenugreek seeds imported from Egypt as underlying cause
12.06.2011 018/2011	EHEC outbreak: BfR also advises against the consumption of home-grown raw sprouts and germ buds
11.06.2011 17/2011	EHEC outbreak: BfR confirms contamination of sprouts with O104:H4
10.06.2011 16/2011	EHEC: Current State of Knowledge Concerning Illnesses in Humans
09.06.2011 15/2011	Consumption Recommendations to protect consumers from EHEC
03.06.2011 14/2011	New epidemiological data corroborate existing recommendation on consumption by BfR
01.06.2011 13/2011	EHEC germs on Spanish cucumbers do not correspond to the pathogen type of the patients concerned
31.05.2011 12/2011	BfR and ANSES develop test system for the identification of EHEC contaminations in foods
14.01.2011 02/2011	EHEC infections can have serious consequences for children

Tab. 11: Published documents on EHEC – BfR-Opinions

Date	Title
23.11.2011 BfR Opinion No. 049/2011	EHEC Outbreak 2011: Updated Analysis as a Basis for Recommended Measures
26.07.2011 Updated BfR Opinion No. 031/2011 des BfR	Relevance of EHEC O104:H4 in fenugreek seeds which are processed into other foods than sprouts and germ buds
05.07.2011 BfR Opinion No. 023/2011	Relevance of sprouts and germ buds as well as seeds for sprouts production in the current EHEC O104:H4 outbreak event in May and June 2011
30.06.2011 BfR Opinion No. 022/2011	High probability of responsibility of fenugreek seeds for EHEC O104:H4 outbreak
18.06.2011 BfR Opinion No. 021/2011	EHEC: Observance of general hygiene rules is particularly important for the protection against infections
07.06.2011 BfR Opinion No. 019/2011	Enterohaemorrhagic Escherichia coli (EHEC) O104:H4: a brief bacteriological intro-ductory profile
06.06.2011 BfR Opinion No. 020/2011	EHEC: What is the role of BfR in the current EHEC outbreak event?
06.06.2011 BfR Opinion No. 018/2011	Sprouts and germ buds as possible cause for the EHEC infections: BfR supports Lower Saxony at the clarification
31.05.2011 BfR Opinion No. 016/2011	EHEC pathogen not yet typed: tomatoes, cucumbers and salads should nonetheless continue not to be consumed raw
26.05.2011 BfR Opinion No. 015/2011	EHEC: Consumers to continue to refrain from eating tomatoes, cucumbers and green salads raw
25.05.2011 Joint Opinion No. 014/2011 of BfR and RKI	Preliminary results of the EHEC/HUS Study

Tab. 12: Published documents on EHEC – Publikationen: Consumer Leaflets

Datum	Titel
14.01.2011 Consumer Leaflet	“Verbrauchertipps: Schutz vor Infektionen mit enterohämorrhagischen <i>E. coli</i> (EHEC)“ (in German only)“

Tab. 13: Published documents on the subject EHEC – Questions and answers

Date	Title
22 Jul. 2011 Updated FAQ from the BfR	Questions and answers on the use of fenugreek seeds in foods
06 Jul. 2011 Updated FAQ from the BfR	Questions and answers on the origin of enterohaemorrhagic <i>E. coli</i> O104:H4
15 Jun. 2011 Updated FAQ from the BfR	Questions and answers on EHEC infections through plant-based foods
31 Aug. 2007 FAQ from the BfR	Questions and answers on EHEC

Tab. 14: Published documents on the subject EHEC – Miscellaneous

Date	Title
21 Jun. 2011 Status	BfR recommendation for the examination of seeds and production of sprouts
20 Jun. 2011 Status	Protocol of the enrichment and isolation of STEC/EHEC from plant-based foods

Tab. 15: Age of the persons questioned (absolute and relative⁷ numbers)

Age	N	Percent
14–19 years	70	7 %
20–29 years	136	14 %
30–39 years	137	14 %
40–49 years	191	19 %
50–59 years	158	16 %
60–69 years	126	13 %
70 and older	165	16 %
Don't know/no answer	19	2 %
Total	1,002	100 %

Tab. 16: Sex of the persons questioned (absolute and relative numbers)

Sex	N	Percent
Female	515	51 %
Male	487	49 %
Total	1,002	100 %

⁷ Percentages are rounded off. The statistical error tolerance is ± 3 percentage points.

Tab. 17: Highest level of school or university education achieved by the persons questioned (absolute and relative numbers)

Highest level of school/university education	N	Percent
(Without) junior high school leaving certificate (8 th grade)/no apprenticeship	38	4 %
(Without) junior high school leaving certificate (8 th grade)/with apprenticeship	163	16 %
Intermediate high school leaving certificate	347	35 %
General or technical university entrance qualification	227	23 %
Completed (technical) university degree (incl. study of Engineering)	185	18 %
No final certificate because still at school	17	2 %
Total	1,002	100 %

Tab. 18: Persons questioned per federal state (absolute and relative numbers)

Federal state	N	Percent
Baden-Württemberg	121	12 %
Bavaria	174	17 %
Berlin	27	3 %
Brandenburg	37	4 %
Bremen	10	1 %
Hamburg	10	1 %
Hesse	76	8 %
Mecklenburg-Western Pomerania	20	2 %
Lower Saxony	116	12 %
North Rhine-Westphalia	189	19 %
Rhineland-Palatinate	63	6 %
Saarland	13	1 %
Saxony	46	5 %
Saxony-Anhalt	27	3 %
Schleswig-Holstein	33	3 %
Thuringia	32	3 %
No answer	8	1 %
Total	1,002	100 %

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