Bundesinstitut für Risikobewertung

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.

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 horticultural 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 was 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 co-ordinate 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.

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.

3 Rationale

3.1 Risk assessment

3.1.1 Hazard identification

Enterohaemorrhagic and enteroaggregative *Escherichia 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 (*stx*1 or *stx*2) 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 *stx*1 or *stx*2 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.

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 *stx*2 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 (*stx*1): negative
- Shiga toxin 2 (*stx*2a): 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
- Enteroaggr. *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 IS*Ecp1* 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 (IncI1 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.

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.

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.

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 childrens camp from seeds (fenugreek, mustard, rocket salad). Further possible exposures are being investigated within the scope of a cohort study.

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.

3.1.3.1 Identification of the incriminated food vehicle

Epidemiological studies by the Robert Koch Institute on the consumed food and analyses of the distribution routes by the *Laender* and the EHEC Task Force contributed towards narrowing down the 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 prepared 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 blends[1] 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 1). 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 inter-connected via supply relationships of sprouts of the horticultural farm in Lower Saxony (Figure 2). 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.

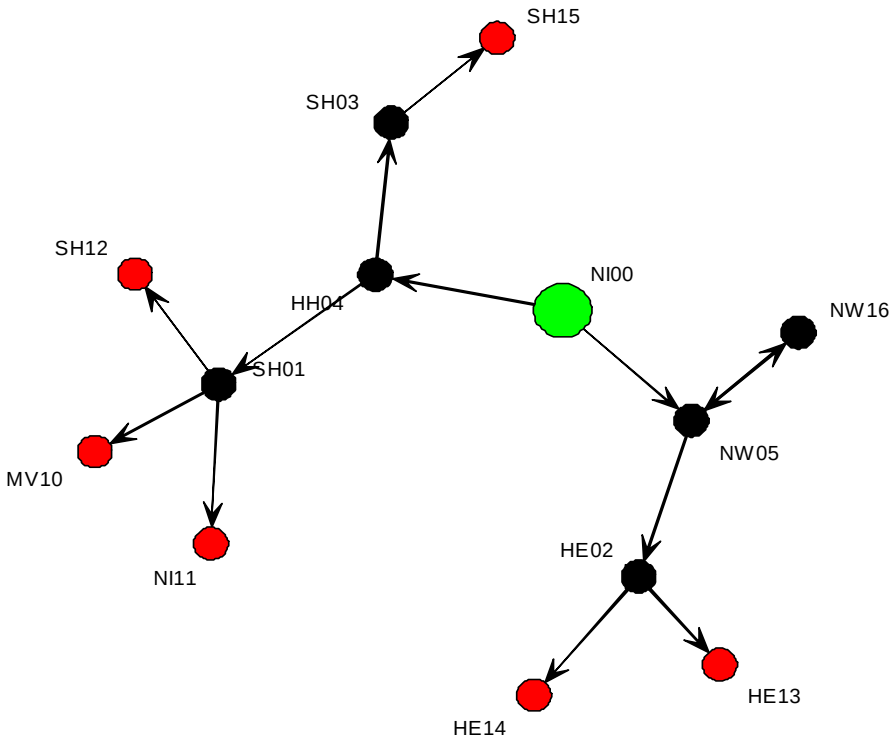


Figure 1: Results of the trace back based on the five outbreak clusters (red) by means of specific batch information (HE13 and HE14 are evaluated as one cluster).

[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)

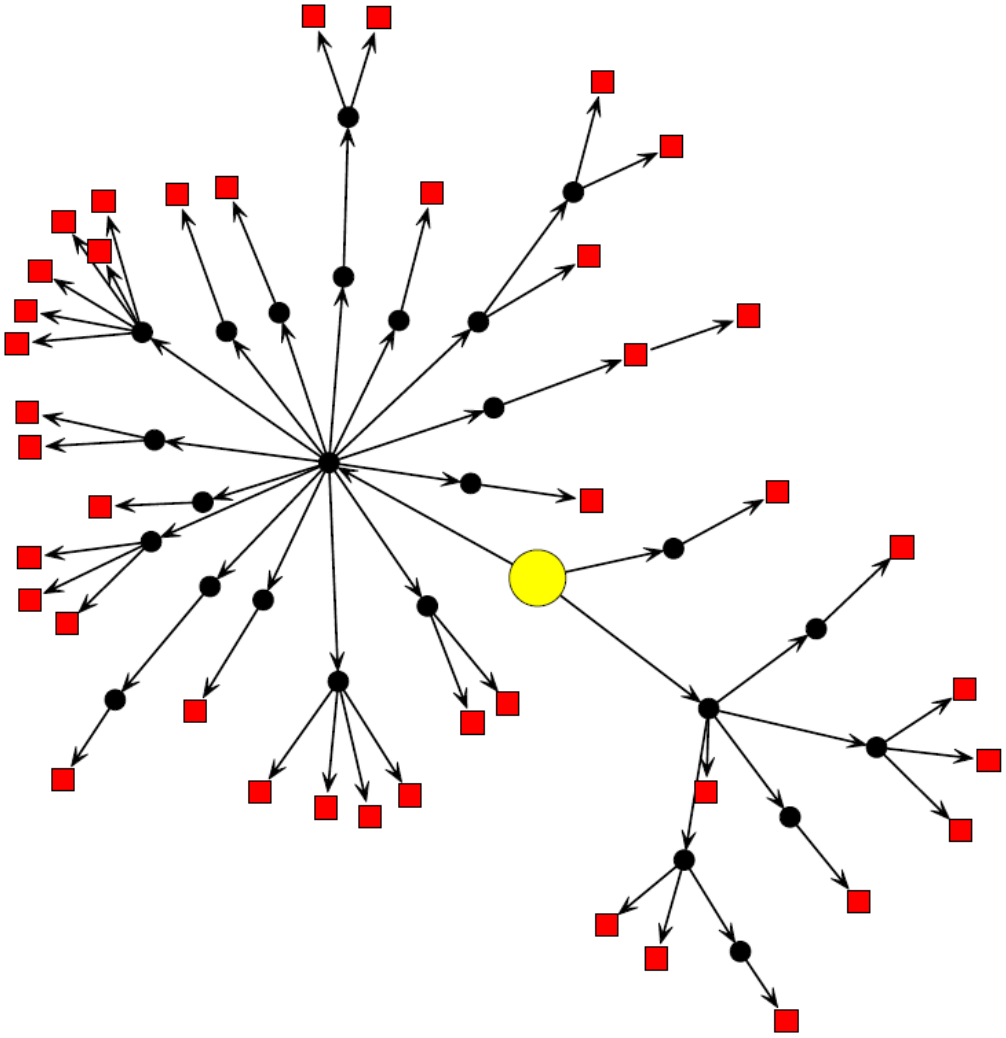


Figure 2: Results of the combined trace forward / trace back strategy. Supply relationships of the horticultural farm in Lower Saxony (yellow) lead to all 41 outbreak clusters associated with sprouts (red). Wholesalers and intermediate dealers (black).

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.

Results of microbiological investigations of samples 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.

Table 1: 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	2	0	0	2
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.

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 5^{th} percentile of 2 g and a 95^{th} percentile of 53 g/portion. The 95^{th} 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 95^{th} and the 5^{th} 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 95^{th} and the 5^{th} 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 95^{th} and the 5^{th} 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 exent 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.

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.

3.1.3.3 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 spreading 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.

3.1.3.4 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 authorities 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.

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 3 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.

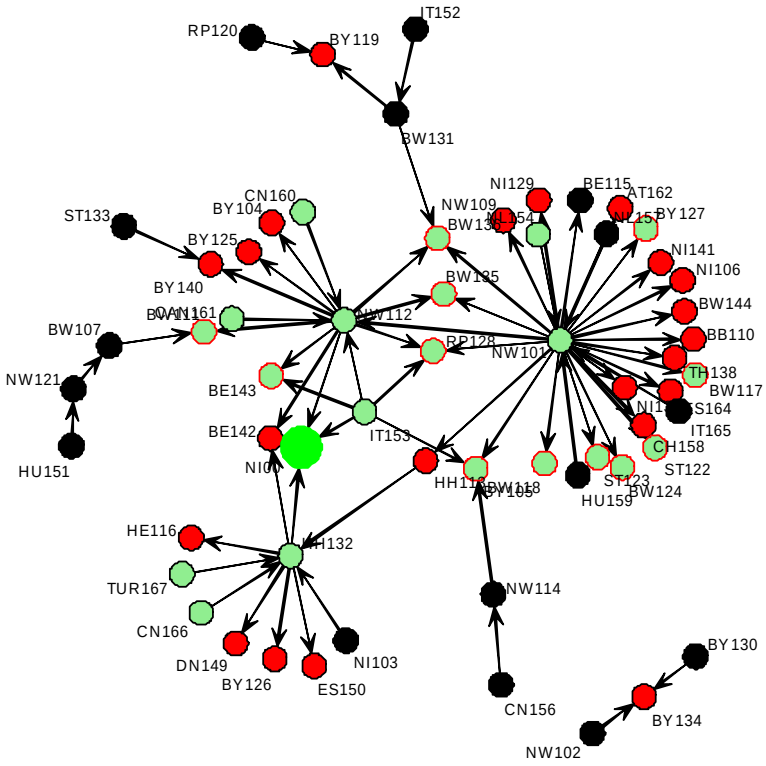
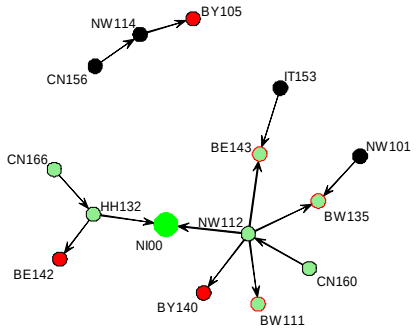
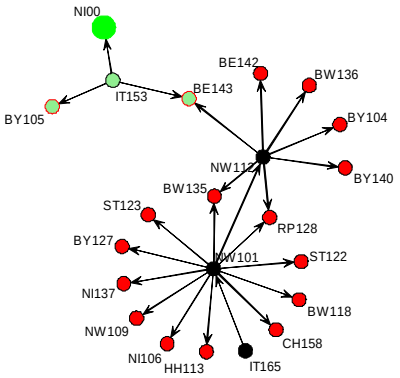


Figure 3: Based on the trace back (combined trace forward / trace back strategy based on specific batch information) of the corresponding seed deliveries to the horticultural farm in Lower Saxony (NI00, large green dot) the determined distribution network (direction of arrow) to German sprout producers (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.

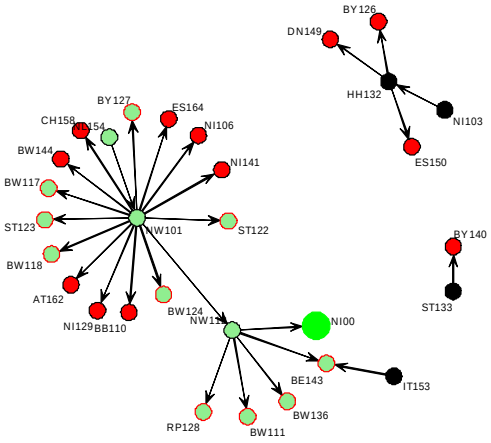
Abbildung 4



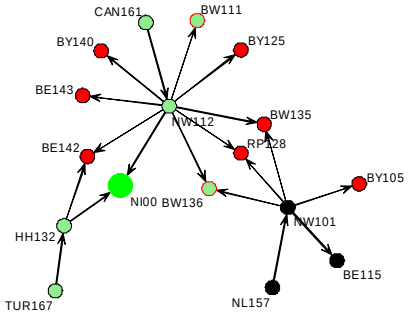
Adzuki



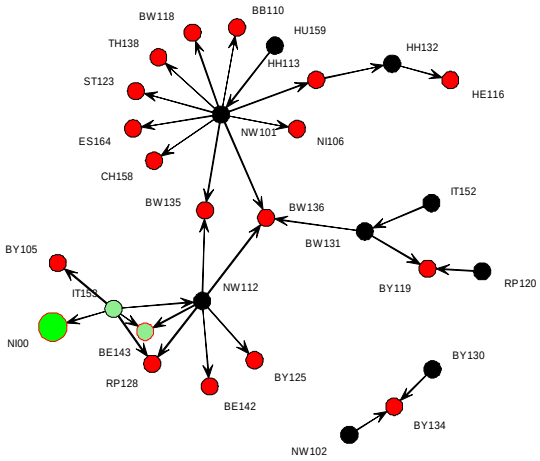
Alfalfa



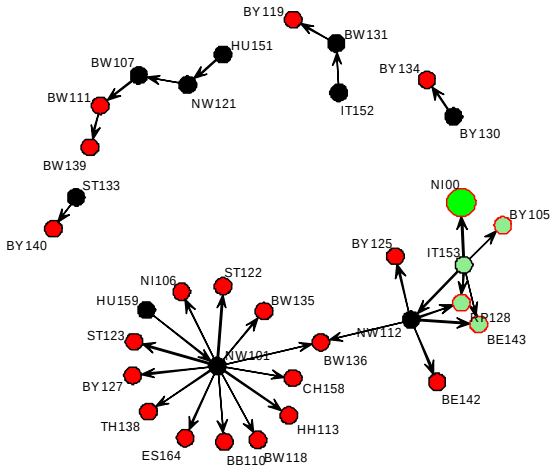
Fenugreek



Lentils

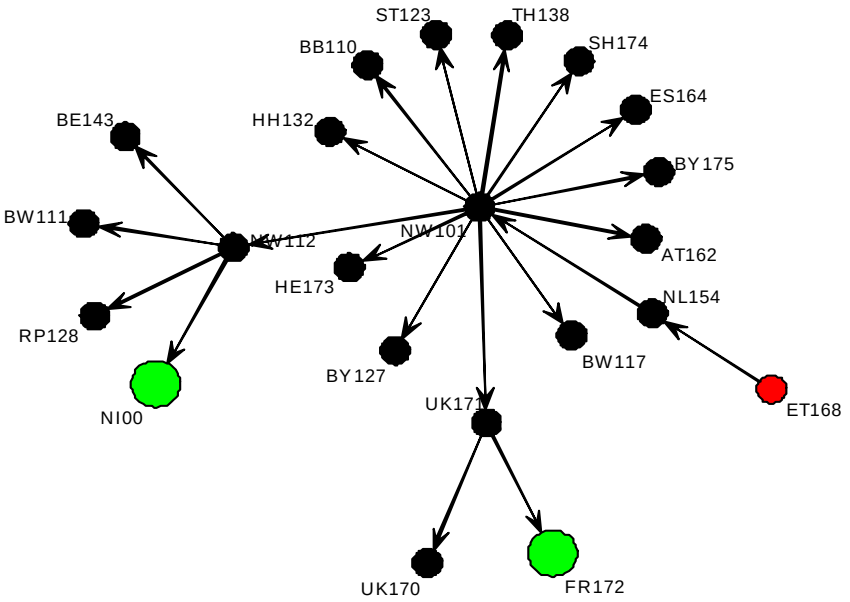


Radish



Daikon

Figure 4: 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 5: 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.

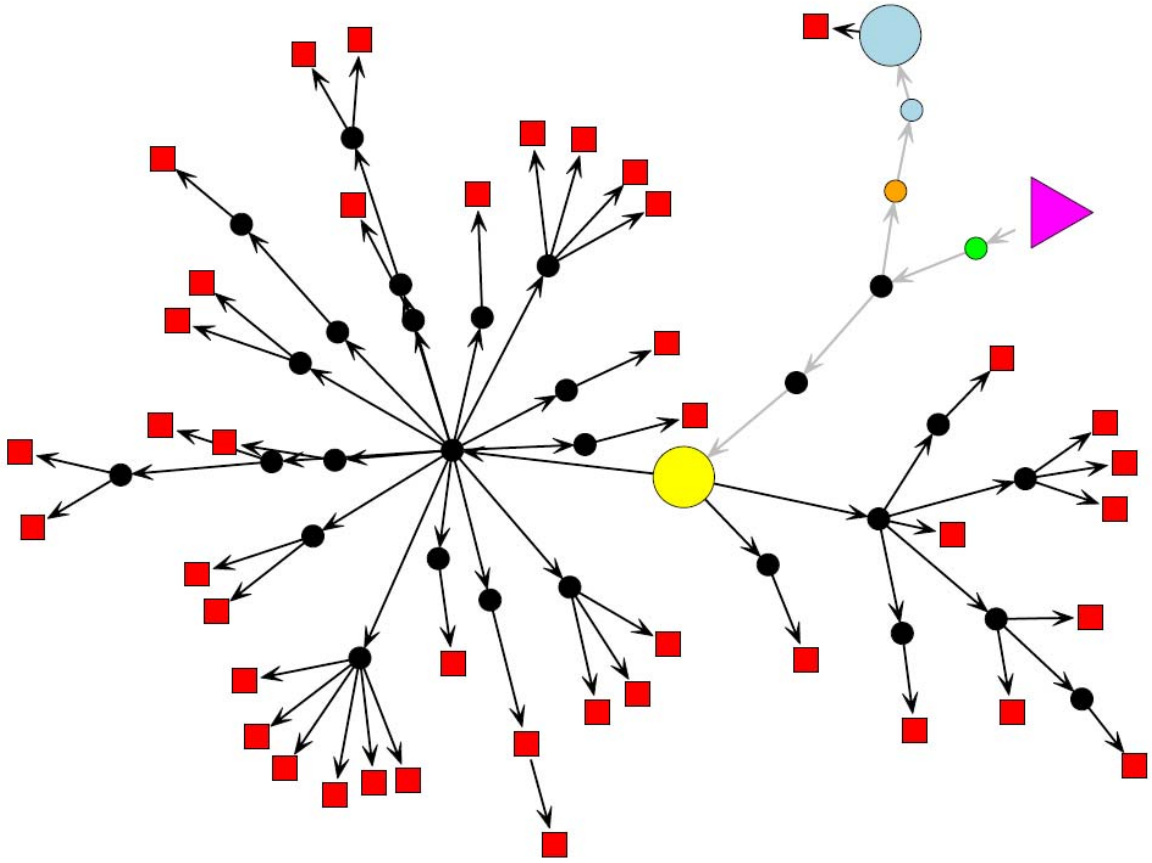


Figure 6: Visualisation of the connection between the German and the French EHEC outbreak with a joint source identified by the EFSA Task Force (magenta-coloured triangle), based on the EFSA Technical Report of 5 July 2011. Furthermore, the horticultural farm in Lower Saxony (yellow), the delivery routes in France (light blue) and the outbreak clusters (red) are shown.

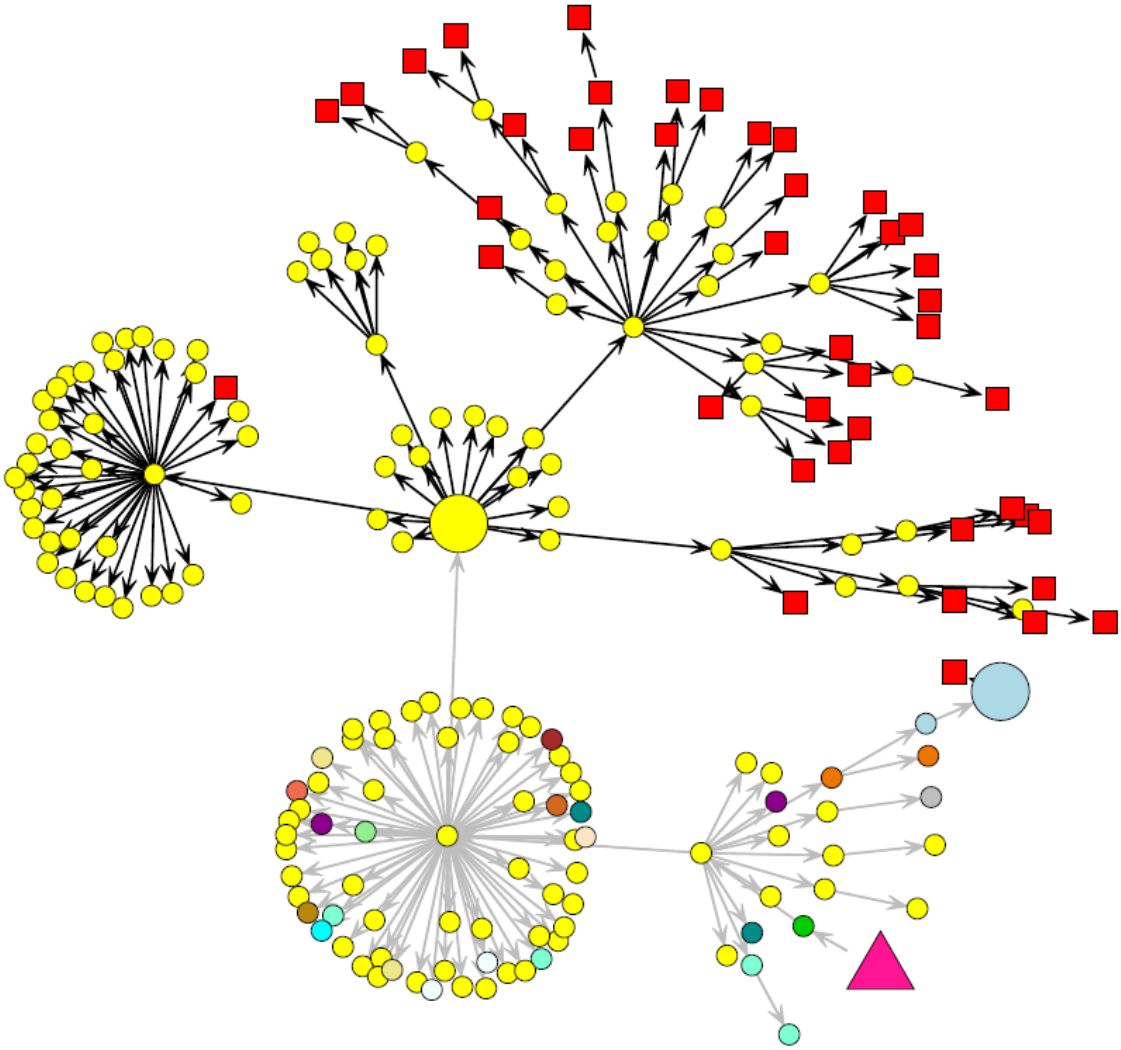


Figure 7: Visualisation of the connection between the EHEC O104:H4 outbreak in Germany and France based on the currently known European distribution network for an identified batch of fenugreek seeds (batch 48088). The distribution network for this seed batch is based on the data compiled by the EFSA 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).

Investigation results of the *Laende*r 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.

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.

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.

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.

3.2 Other aspects

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 Peez), 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.

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.

3.3 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:

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 und 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 hygiene 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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