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

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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.

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 outbreak[1] already published by the BfR in July 2011. For enhanced readability, sprouts and seedlings are summarised as “sprouts” henceforth in this document.

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

[1] 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

http://www.bfr.bund.de/cm/343/bedeutung_von_ehec_o104_h4_in_bockshornkleesamen_die_zu_anderen_lebensmitteln_als_sprossen_und_keimlingen_weiterverarbeitet_werden.pdf

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.

3 Rationale

3.1 Risk Assessment

3.1.1 Enterohaemorrhagic and Enteroaggregative *Escherichia 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 EAggEC 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 EAggEC 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 EAggEC-specific virulence factors (Stx production and enteroaggregative adhesion) are currently virtually unresearched. Morabito et al. surmised as early as 1998 that such recombined strains may be as pathogenic to humans as conventional EHEC strains.

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 EAggEC than with conventional EHEC. Thus at the sequence level, the strain responsible for the outbreak shows a 93% similarity to a human EAggEC 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 EAggEC 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, nalidixin 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.

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 serotypisation 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.

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, *stx*2, *eae*-, *hly*A-, 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.

Tenacity of Enterohaemorrhagic And Enteroaggregative *Escherichia 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 EaggEC 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.

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.

3.1.2 The Hazard Potential in the EHEC O104:H4 Outbreak

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.

3.1.3 Exposure Assessment

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.

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 1 and 2). 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.

Table 1: 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

Table 2: 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.

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 propable 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.

Figure 1: Distribution in percentages of the probable uses of seed Batch 48088

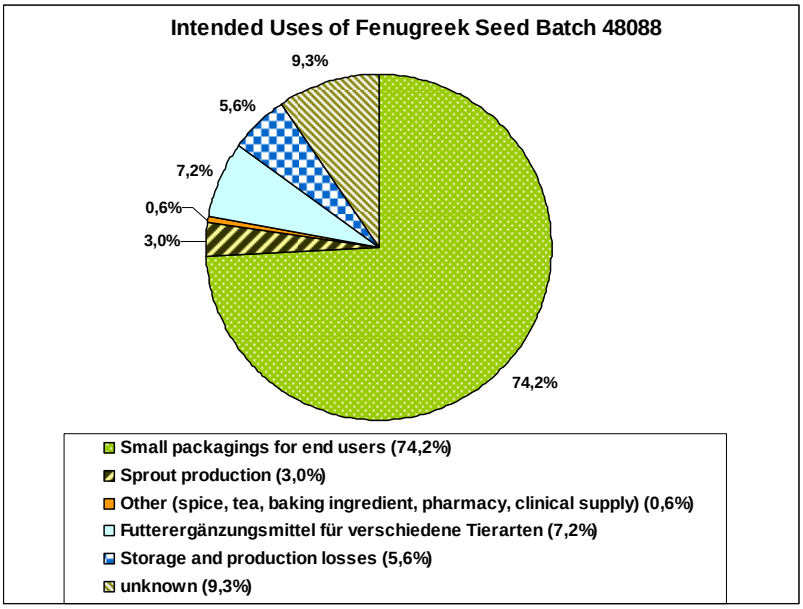
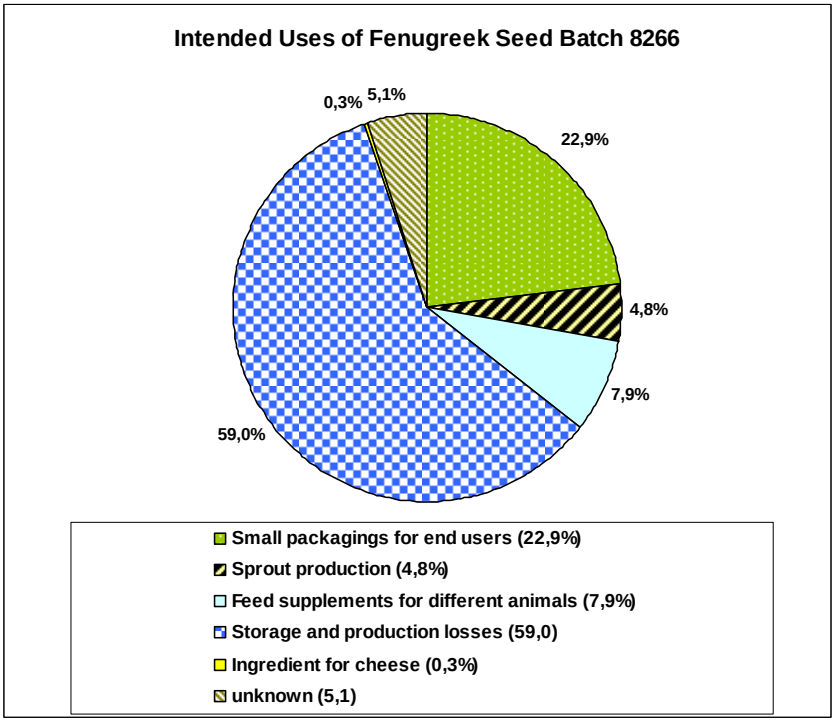


Fig. 2: Distribution in percentages of the probable uses of seed Batch 8266



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.

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.

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.

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.

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.

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.

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.

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.

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