Relevance of EHEC O104:H4 in fenugreek seeds which are processed into other foods than sprouts and germ buds

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

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

There is a high probability that the cause for the EHEC outbreak event in Germany and France in May and June 2011 was attributable to contaminated fenugreek seeds and sprouts grown from them (see BfR Opinion "Relevance of sprouts and germ buds as well as seeds for sprout production in the current EHEC O104:H4 outbreak event in May and June 2011 " http://www.bfr.bund.de/cm/349/relevance_of_sprouts_and_germ_buds_as_well_as_seeds_for_sprouts_production_in_the_current_ehec_o104_h4_outbreak_event_in_may_and_june_2011.pdf). According to the current state of knowledge, sprouts from fenugreek seeds were involved in the outbreak event. However, the seeds are not only used for sprout production. Therefore, the Federal Institute for Risk Assessment (BfR) has assessed the relevance of EHEC O104:H4 in fenugreek seeds which are used in different food including food supplements. Whether these products can cause an infection if processed with contaminated fenugreek seeds is primarily determined by the preparation and processing methods. Depending on the producer and product, these methods can be very different and are, thus, not known in detail to BfR.

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

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

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

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

Herbal teas containing fenugreek seeds should be infused with boiling water and left to draw for at least 5 minutes like all herbal teas. Water from hot water dispensers is generally not suited for the preparation of herbal teas since it is not hot enough to safely kill bacteria (see also BfR Opinion “Temperierte Heißwasserspender für Kräuterteeaufgüsse nicht geeignet” http://www.bfr.bund.de/cm/343/temperierte_heisswasserspender_fuer_kraeuterteeaufguesse_nicht_geeignet.pdf.

1 Subject of the assessment

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

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

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

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

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

seeds from Egypt until 31 October 2011 was ordered (Commission Implementing Decision of 6 July 2011, 2011/402/EU).

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

Against this backdrop, BfR has made an assessment concerning the processing of products from fenugreek seeds at the production of other foods which should complement the risk assessment of 5 July 2011 on the relevance of sprouts and germ buds[1]. However, BfR is so far not aware of the extent to which fenugreek seeds of the batches withdrawn from the market have been processed into other foods than to sprouts.

2 Result

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

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

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

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

[1] Published under http://www.bfr.bund.de/cm/343/bedeutung_von_sprossen_und_keimlingen_sowie_samen_zur_sprossenherstellung_im_ehec_o104_h4_ausbruchsgeschehen_im_mai_und_juni_2011.pdf

the irradiation of seeds, as allowed for spices according to the Food Irradiation Regulation, to be sufficient for this intended purpose.

Chemical treatment processes, including the use of chlorine solutions or the addition of sulphur dioxide (SO_2) are basically not appropriate to eliminate pathogens which are possibly present in the core of fenugreek seeds.

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

- Recommendations for food companies:

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

- Recommendations for the competent authorities:

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

- Recommendations for consumers:

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

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

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

3 Rationale

3.1 Risk assessment

3.1.1 Enterohaemorrhagic and enteroaggregative *Escherichia coli* as possible hazard

Escherichia coli (*E. coli*) are naturally occurring in the intestines of humans and animals. Certain types of *E. coli*, such as EHEC or EAggEC can cause gastro-intestinal diseases in humans. Since EHEC can also occur in the intestines of ruminants and is excreted with faeces, it can be transmitted directly or indirectly (e.g. through food) from animals to humans and cause diseases. According to the current state of knowledge it has to be assumed that the reservoir for EAggEC is of human origin. A transmission of EAggEC can occur through smear infection from human to human. The pathogen can also get into food during preparation or production and be spread in this way.

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

A characteristic property of EHEC is the production of Shiga toxins (*stx*1 or *stx*2) and to attach through a certain protein (intimin) to the intestines of its hosts. The terms STEC (for Shiga toxin producing *E. coli*) or VTEC (for Verotoxin producing *E. coli*) are therefore used as synonyms for *stx*1 or *stx*2 producing EHEC. However, EAggEC normally does not produce Shiga toxins and attaches through adhesion factors (adhesins) to the human intestinal wall where it can form biofilms. This property of forming biofilms has been described for both EHEC and EAggEC, including for abiotic surfaces.

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

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

The pathogenic role and the transmission pathway of *E. coli* strains which possess both EHEC and EAggEC-specific virulence factors (*stx* production and enteroaggregative adhe-

sion) is at present almost unexplored. Morabito et al. already assumed in 1998 that such recombined strains can be just as pathogenic for humans as classical EHEC strains.

Characteristics of EAggEC EHEC O104:H4 (outbreak strain)

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

By means of DNA sequence analysis it was determined that the outbreak strain had essentially more commonalities with EAggEC than with the conventional EHEC. The outbreak strain is on the sequence level 93 % similar to a human EAggEC strain from Central Africa which has been already characterised. The EHEC-specific feature of the outbreak strain is the bacteriophage-encoded *stx2*- gene. The outbreak strain is obviously a recombination of two *E. coli* pathotypes (EAggEC and EHEC) which lacks the typical *eae* (attaching and effacing) gene for EHEC.

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

Occurrence of EAggEC EHEC O104:H4

Occurrence in humans

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

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

Occurrence in foods

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

However, STEC/VTEC of other serotypes have already been detected in food for many years. In Germany STEC/VTEC are monitored within the scope of food-business operators own checks, controls of the official athorities as well as in the course of zoonoses monitoring

programs. In the course of the controls of the official authorities, STEC/VTEC were detected particularly in fresh meat as well as in raw meat preparations and also game meat.

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

Occurrence in animals and in the environment

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

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

Tenacity of enterohaemorrhagic and enteroaggregative *Escherichia coli*

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

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

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

Since the persistence of the pathogen in food depends on the matrix and the applied food technology, the assessment of the effect of the individual processes requires not only know-

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

Tenacity to heat, high pressure and irradiation

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

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

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

Tenacity to chemical treatment processes

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

Sulphur dioxide used for the preservation of foods can cause a germ reduction. Sulphur dioxide (SO_2) is authorised as a preservative and antioxidant agent for different foods (E220). SO_2 is used, inter alia, for dried fruit, but also for instance for dried potato products or dried or deep-frozen white vegetables with product-specific maximum amounts being authorised. In wine production the use of sulphur dioxide is common. For instance, a germ reduction of EHEC O157:H7 by up to 5 logs was achieved in different sour apple juice products through the use of 50ppm SO_2 (Basaran-Akgul et al., 2009).

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

Tests for the decontamination of foods contaminated with EHEC O157:H7 with 0.5, 1.0 and 1.5% organic acids proved to be ineffective and underline the acid tolerance of this pathogen

(Brackett et al., 1994). In the laboratory it can be shown that cultures with 3 x 10^4 CFU/ml EHEC O157:H7 are stable after 24 hour incubation both at 4 °C and at 24 °C at pH 3.4 and pH 11. At pH 2 there is only a slight reduction (0.5 – 1 log) of the germ count (Miller and Kaspar, 1994). Tests with artificial gastric juice suggest that not only EHEC O157:H7 survive at pH 1.5 but also other pathotypes such as enteropathogenic *E. coli* are extremely acid tolerant (Arnold and Kaspar, 1995). Given this data situation and the capacity of the outbreak strain to form biofilms it should generally be assumed that there is an increased insensitivity to chemical treatments.

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

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

3.1.2 The hazard potential in the EHEC O104:H4 outbreak event

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

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

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

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

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

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

3.1.3 Exposure assessment

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

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

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

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

Processing of fenugreek seeds in the food production

Cheese

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

Spice blends and condiments

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

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

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

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

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

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

Tea

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

Food supplements

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

ever, frequently thermal processing of the seeds is deliberately waived at the production of these products. Especially for these products it must be assumed that existing EHEC bacteria can survive.

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

3.1.4 Risk characterisation

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

Situation 1:

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

It is possible that material from the stock of at least one contaminated fenugreek seed batch was used in a manufacturing plant for foods including food supplements. If contaminated fenugreek seeds have been processed without having been sufficiently treated in advance, depending on further technological effects, it has to be expected that the pathogen survives in cheese, mustard, teabags and food supplements.
The risk of an infection with enteroaggregative EHEC O104:H4 would exist, more particularly, for the direct consumption of these cheeses and mustard types as well as food supplements with fenugreek seeds and seed powders. Other disease outbreaks caused by enteroaggregative EHEC O104:H4 would be possible.

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

Situation 2:

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

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

Assessment of the severity of the health impairment

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

Assessment of the quality of the data

Processing methods

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

Tenacity of the pathogen

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

4 Conclusion and recommended measures

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

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

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

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

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

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

To this end, heating processes can be applied as they are standard for other foods, too. For reasons of taste and technological reasons fenugreek seeds are usually heated prior to their addition to foods. Heating to at least 72 °C for 2 minutes in the core or a comparable temperature time combination with a similar effect is appropriate to eliminate the pathogen in the seeds as well as in most of the other foods. This remains valid for the elimination of the pathogen in seeds in a moist environment (e.g. by hot water steam treatment). When using dry heat only, temperatures around 70 °C require a heat treatment of several hours. Moreover, BfR considers that the irradiation of the seeds, as allowed for spices according to the Food Irradiation Regulation, is sufficient for this intended purpose.

Chemical treatment processes, including the use of chlorine solutions or the addition of sulphur dioxide (SO_2) are basically not appropriate to eliminate pathogens which are possibly present in the core of fenugreek seeds.

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

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

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

- Food companies which process fenugreek seeds should review the risk analysis to be conducted within the framework of the HACCP (Hazard Critical and Control Points) concept in view of the EHEC O104:H4 outbreak and possibly adjust it. Since pathogenic

germs can possibly penetrate the inside of plants through the roots, seeds should be treated anyway before further processing in such a way that possibly existing pathogens in the seed core can be safely killed.

- Consumers should infuse herb teas including those with fenugreek seeds with boiling water and leave to draw them for at least 5 minutes. Since herb teas can be contaminated with pathogens, water from hot water dispensers is generally not appropiate for the preparation of herb teas. This has already been pointed out by BfR in an Opinion back in 2005.

- BfR advises consumers to thoroughly heat fenugreek seeds before their further processing in the private household e.g. by roasting in a pan, in order to kill possibly existing pathogens on or in the seeds. Any seed meal and spice blends made thereof, derived from untreated fenugreek seeds should as a matter of precaution not be consumed but discarded with the household refuse.

- As a matter of principle BfR advises to continue to research the survival capacity and the growth behaviour of enteroaggregative EHEC O104:H4 within the framework of scientific tenacity studies.

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