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Escherichia coli O104:H4 outbreak in Germany—clarification of the origin of the epidemic

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Background: In 2011, Germany was hit by one of its largest outbreaks of acute gastroenteritis and haemolytic uraemic syndrome caused by a new emerging enterohaemorrhagic *Escherichia coli* O104:H4 strain. The German Haemolytic Uraemic Syndrome/Enterohaemorrhagic *E. coli* (GHUSEC) outbreak had unusual microbiological, infectiological and epidemiological features and its origin is still only partially solved. The aim of this article is to contribute to the clarification of the origin of the epidemic. **Methods:** To retrospectively assess whether the GHUSEC outbreak was natural, accidental or a deliberate one, we analysed it according to three published scoring and differentiation models. Data for application of these models were obtained by literature review in the database Medline for the period 2011–13. **Results:** The analysis of the unusual GHUSEC outbreak shows that the present official assumption of its natural origin is questionable and pointed out to a probability that the pathogen could have also been introduced accidentally or intentionally in the food chain. **Conclusion:** The possibility of an accidental or deliberate epidemic should not be discarded. Further epidemiological, microbiological and forensic analyses are needed to clarify the GHUSEC outbreak.

Introduction

A total of 2987 cases (18 lethal) of diarrhoea without enteropathic haemolitic uraemic syndrome (HUS) and 855 cases (35 lethal) of HUS were attributable to the German Haemolytic Uraemic Syndrome/Enterohaemorrhagic *Escherichia coli* (GHUSEC) outbreak in 2011, making this the largest German enterohaemorrhagic *E. coli* (EHEC) outbreak and one of the world's largest outbreaks of HUS.[1–5] The outbreak started on 1 May, peaked on 21 and 22 May and was declared finished by the Robert Koch Institute (RKI), Berlin, on 26 July.[1–5] Due to the unusual severity of the diseases, the rapid superregional spread of this epidemic and sporadic cases in several countries across Europe, mostly after visiting outbreak areas in Germany, the outbreak was notified first on 22 May 2011 to European Union (EU) by the Early Warning and Outbreak Response System and on 24 May 2011 to World Health Organization under the International Health Regulations as a 'potential public health event of international concern'.[6]

In national and international interdisciplinary cooperation, a new enteroaggregative strain of EHEC O104:H4 (HUSEC 041) was identified.[2–3,5,7]As a result of epidemiological investigations, fenugreek sprouts grown from probably EHEC contaminated seeds imported from Egypt were assumed as the most likely source of the outbreak.[7]

The GHUSEC outbreak showed typical clues of an unusual epidemiological event (UEE). The sudden and unexplainable emerging of a fast increasing number of cases and deaths from bloody diarrhoea and HUS might have been caused naturally, accidentally or intentionally.[8–10] Outbreaks developing after a deliberate contamination of the food chain may mimic natural events, which makes identification of their origin difficult.[4,11]

Assessing the likelihood of criminal or terroristic act behind a UEE is of great public health importance, as it may be helpful in improved response and the resolution of epidemics.[10] Three epidemiological assessment tools are available to differentiate between natural and accidental and deliberate epidemics. Two of them are scoring models[9,12] and the third one[13] is based on potential typical clues to a deliberate epidemic without a numerical ponderation. Grunow and Finke[12] and Dembek *et al.*[13] procedures have been used to retrospectively assess several outbreaks: salmonellosis in Dalles, Oregon, 1984; shigellosis in Dallas, Texas, 1996; anthrax, the USA, 2001; anthrax, Sverdlovsk, Soviet Union, 1979; West Nile Virus, New York City, 1999; and tularaemia, Kosovo, 1999.

The present article is an attempt to further clarify the origin of the GHUSEC outbreak and to assess the likelihood of its accidental or deliberate causes by using three aforementioned procedures. This study reflects the concerns of the EU and its member states regarding chemical, biological or radiological attacks against the food chain and its activities to improve the plant and food biosecurity.[14]

Methods

The GHUSEC outbreak is scored and assessed with three published models for differentiation between accidental, deliberate or natural infectious disease outbreaks.[9,12,13] Necessary data were obtained by a literature review in the database MEDLINE for the period 2011–13 using key words 'outbreak', 'epidemic', '*Escherichia coli*', 'EHEC O104:H4', 'HUS', 'haemolytic uraemic syndrome', 'STEC O104:H4' and 'Germany'.

In Model 1,[9] there are 10 indicators describing an epidemic. Each indicator is scored with 0 or 1 for a low or high probability of an unnatural outbreak, respectively. Total score may point to a natural, accidental or a deliberate epidemic (Table 1).

In Model 2,[12] relevant political, demographical, epidemiological, hygienic, microbiological and clinical data and findings concerning the outbreak are analysed to assess two conclusive and 11 non-conclusive criteria. Conclusive criteria indicating unambiguously biological warfare attacks, bioterrorist or biocriminal acts are excluded because there was no evidence for such events. The scores of non-conclusive criteria are classified into three groups. The first one includes assessment scores from 0 to 3, higher scores

Table1 Scoring of the GHUSEC outbreak according to a model of Radosavljević and Belojević[9] for differentiation between natural, accidental and deliberate outbreaks

No.	Epidemiological/infectiological indicators	Score
1	Unusual/atypical manifestation (fulminant course) of a known disease	1
2	Several unusual/unexplained syndromes coexisting in the same case without any other explanation	0
3	A sudden unexplainable increase in the number of cases or deaths in human populations	1
4	Higher than expected morbidity and/or mortality rates	1
5	Clustering of patients with fever only or with fever and other symptoms	1
6	A disease identified in the region for the first time, again after a long period of time or after its eradication	0
7	A new strain of pathogen identified in the region for the first time, after a long period or after its eradication	1
8	A disease with an unusual/atypical seasonal distribution	1
9	One or more explosive epidemics/outbreaks with indicators of a point-source origin	1
10	A disease with an unusual geographic distribution	1
	Total score	8

1 = High probability of a deliberate or accidental outbreak.
0 = Low probability of a deliberate or accidental outbreak.
Assessment of scores:
1–4 probably natural outbreak.
5–7 possibly deliberate or accidental outbreak.
8–10 probably deliberate or accidental outbreak.

Table 2 Scoring of the GHUSEC outbreak according to the model of Grunow and Finke[12] for differentiating between natural and deliberate epidemics

No.	Non-conclusive criterion	Assessment score[a]	Weighting factor[a]	Calculated/maximum number of points[b]
1	Existence of a biological risk	1	2	2/6
2	Existence of a biological threat	0	3	0/9
3	Special aspects of the biological agent	2	3	6/9
4	Peculiarities of the geographic distribution of the biological agent	1	1	1/3
5	High concentration of the biological agent in the environment	1	2	2/6
6	Peculiarities of the transmission mode of the biological agent	1	1	1/3
7	Peculiarities of the intensity and dynamics of the epidemic	2	2	4/6
8	Peculiarities of the time of the epidemic	1	1	1/3
9	Unusually rapid spread of the epidemic	2	1	2/3
10	Limitation of the epidemic to a specific population	2	1	2/3
11	Peculiarities of the clinical manifestation	2	1	2/3
Total				23/54

a: Assessment of a criterion:
0 = Criterion ruled out or no data available.
1 = Existence of peculiarities or suspicions, but uncertain and indistinct.
2 = Existence of obvious peculiarities or indications, causes yet to be clarified for certain.
3 = Existence of considerable peculiarities or deviations from expected norm, clear indication or proof of an intentional release.
b: Assessment score × weighting factor.

indicating higher probability of an unnatural outbreak. In the second one, there are three weighting factors, scored from 1 to 3, higher scores also indicating a higher probability of an unnatural outbreak. In the third group, an assessment score is multiplied by a corresponding weighting factor. The likelihood of an intentional release of a pathogen is calculated comparing the given number of points of the non-conclusive criteria with an arbitrary scale of probability (Tables 2 and 3).

In Model 3, eleven epidemiological, infectiological and forensic clues are scored as positive, negative, supportive or not applicable.[13] Resulting clues are evaluated separately to distinguish the likely origin of the outbreak (Table 4).

Results

In Model 1, the total score indicates that the GHUSEC outbreak was probably a UEE with the features of accidental or intentional epidemics (Table 1).

Indicator 1. The clinical picture of GHUSEC deviated from the expected one in several clinical features.[1–4,15] There were high percentages of previously healthy adults exhibiting severe courses of EHEC gastroenteritis and enteropathic HUS with increased rates, >50%, of otherwise rare neuro-psychiatric complications.[16,17] Acute gastroenteritis caused by the new strain exhibited an unusually long incubation period of 8 days on average (range 2–18 days) in contrast to 3–4 days incubation period in earlier EHEC O157:H7 epidemics. The median interval between the start of EHEC O104: H4 diarrhoea and start of HUS was 5 days in the outbreak, compared with median 7 days in case of EHEC O157-caused diseases.[1,18] There were higher rates of severe HUS forms requiring hospitalization and intensive care in younger adults and especially women. A significant number of patients with EHEC diseases did not respond to the usual antibiotic therapy.[1,4,15,16]

Indicator 2. There were no coexisting syndromes. Most patients suffered initially watery or bloody diarrhoeal illness.

Indicator 3. The outbreak was characterized by an initially unexplainable rapid rise of the number of patients with acute gastroenteritis and HUS cases since 8 May 2011, which peaked around 22 May 2011. Analysing the epidemic slope, one can estimate that until this day, already ca. 30% of all GHUSEC cases have occurred.[11]

Table 3 Assessing the likelihood of a deliberate use of a biological agent (pathogen, toxin)[12] based on non-conclusive criteria and a comparison of the number of scores with an arbitrary scale of probability

Step	Assumption of a deliberate use of a biological agent	Confidence interval (%)	Limits of calculated points[a]
3	Highly likely	95–100	51–54
2	Likely	67–94	36–50
1	Doubtful	34–66	18–35
0	Unlikely	0–33	0–17

a: Given a maximum number of 54 points.

Table 4 Scoring of the GHUSEC outbreak according to the model of Dembek *et al*.[13] for differentiating between natural and deliberate epidemics

No.	Clue	Score
1	A highly unusual event with large numbers of casualties	Positive
2	Higher morbidity or mortality than is expected	Positive
3	Uncommon pathogen	Positive
4	Point-source outbreak (s)	Positive
5	Multiple epidemics (outbreaks)	Positive
6	Lower attack rates in protected individuals	n. a.[a]
7	Dead animals	Negative
8	Reverse spread	Negative
9	Unusual disease manifestation (fulminant course)	Positive
10	Downwind plume pattern	n. a.
11	Direct evidence	Negative
	Total score: 6 of 11	

a: not applicable.

Indicator 4. According to RKI in the period 2001–10, there were on average 934 cases of EHEC gastroenteritis per year.[19] In the GHUSEC outbreak, this number was about four times higher. The incidence (cases/100 000 inhabitants) of EHEC diseases was particularly increased in Northern Germany:Schleswig Holstein (2010: 1.4; 2011: 32.9), Hamburg (2010: 1.4; 2011: 32.0) and Mecklenburg Western Pomerania (2010: 0.5; 2011: 10.5). The GHUSEC outbreak caused a 13.5-fold rise of the number of HUS cases in comparison with the median number in the years 2001–10 ($n = 65$) and an increase of the incidences from median ca. 0.8 to 10.3 (Hamburg) or 6.0 (Schleswig Holstein). This was accompanied by a high hospitalization rate. During the outbreak, unusually high lethality rates of EHEC haemorrhagic colitis (18 deaths, lethality: 0.6%) and HUS (35 deaths, lethality: 4.1% compared with <1% in recent years) in almost exclusively adults (98%) were notified. The median age of deceased HUS patients was 74 years.[1,7,15]

Indicator 5. The outbreak was first notified to local health departments on 18 May 2011 with a cluster of eight adult patients with bloody enteritis who had lunch in a canteen of a company in Frankfurt/Main. On 19 May 2011, three children with HUS were notified from a Hamburg pediatric hospital.[2,20] From the total of 61 registered clusters, the newly established 'Task Force EHEC' at the German Federal Office of Consumer Protection and Food Safety investigated 41 clusters mainly in restaurants, where >300 EHEC/HUS cases had been exposed to meals containing sprouts from one company in Lower Saxony.[1,5]

Indicator 6. Acute watery or bloody gastroenteritis and HUS are known clinical notifiable diseases endemic in Germany.

Indicator 7. The outbreak was caused by a new pathotype of EHEC O104:H4 (041). It differs essentially from typical EHEC O104:H4 strains because it comprises a hybrid virulence profile that combines typical molecular and phenotypic characteristics of shiga toxin *E. coli* (STEC) and enteroaggregative *E. coli* (EAEC).[21,22] The main virulence factor of STEC with a role in the pathogenesis of HUS is the production of at least one Shiga toxin type.[23] The identification of this new enteroaggregative EHEC O104:H4 was notified immediately on 25 May 2011 to the ECDC in Stockholm via the outbreak information platform EPIS.[24] EHEC strains of the serotype O104:H4 were described already in Germany as HUSEC041 in 2001 by Karch *et al*. (HUSEC041), Korea (2006), Georgia (2009) and Finland (2010).[1] On sequence level, a human EAEC strain endemic in Central Africa shows 93% similarity with the outbreak strain.[5,18] Latter was identified for the first time in Germany in about 99% of travellers suffering from EHEC and/or HUS after visiting Germany during the epidemic and also in an autochthon outbreak in Bègles, France in June 2011.[1,4,25,26] The epidemic strain expresses an extended-spectrum β-lactamase[4,27] in contrast to earlier identified strains of EHEC O124:H4.[1] It was unexpectedly resistant to penicillin and cephalosporin while being still susceptible to carbapenem.[4]

Indicator 8. EHEC-related gastroenteritis and its complication HUS can occur at any time of the year, especially if the pathogen is introduced via the food chain. In contrast to previous years, the highest number of EHEC/HUS cases was notified in the second quarter of year but not in the third quarter, as usual.[1,19]

Indicator 9. There was a rapid exponential rise of the number of cases with a peak around 22 May 2011 and a rapid drop in the following week. That indicates a point-source outbreak, which might have originated from the ingestion of contaminated food distributed to final consumers by one or more producers within a limited time window. That assumption was supported by case-control/restaurant-cohort studies and forward-back-tracing of channels of distribution of food by the EHEC Task Force identifying Egyptian fenugreek seeds as the most probable vehicle.[1,2,4,18] A similar explosive point-source outbreak with 15 cases by an identical EHEC O104:H4 strain occurred in Bègles, France, between 15 and 20 June 2011 after the consumption of contaminated raw home-grown fenugreek sprouts on 8 June 2011.[1,26]

Indicator 10. The outbreak exhibited an unusual geographical distribution with the highest incidences in Northern Germany and smaller clusters and sporadic cases throughout all 16 federal states.[1] See also *Indicators 4* and *9*.

In Model 2, total score is in the range of 18–35 points with a confidence interval of 34–66%. This means that the likelihood of a deliberate release of the pathogen in the food chain is doubtful (Tables 2 and 3).

Criterion 1. The existence of a permanent risk of possible criminal or terrorist acts against the food chain is acknowledged by European and German authorities of public health and consumer protection.[14,30]

Criterion 2. There was no information on threat with biological attack before, during or after the outbreak.

Criterion 3. See *Indicator 7* in Model 1.

Criterion 4. See *Indicator 10* in Model 1.

Criterion 5. The new EHEC O104:H4 strain was detected only in each of the samples of cucumber and sprout mixture found in household garbage of people who suffered from EHEC infection, in salmon and paprika presumably contaminated by an infected employee of a party service.[5] The pathogen was isolated neither from the suspected fenugreek seeds and sprouts at the production facility nor in about 8000 investigated samples of different vegetables.[5] The large number of clusters of disease and high rate of patients with severe EHEC diarrhoea and HUS may indicate an exposure to high doses of the pathogen due to a heavy contamination of sprouts or an ingestion of large volumes of suspected vegetables.

Criterion 6. Ingestion of contaminated food (raw vegetables, esp. sprouts) was postulated as the main incriminated mode of

transmission, especially in the first explosive phase of the outbreak.[1,5] Additional smaller waves starting in the last week of May through the first weeks of June may have been caused by consumption of remaining commercial sprouts.[5] Later, secondary fecal–oral transmission from person to person or indirect transmission by contact with contaminated fomites may have prolonged the epidemic.[1,25,31] However, there exist only little information on asymptomatic shedders and the duration of excretion.[4] The modus of transmission of enteric bacteria like EHEC by contaminated sprouts is known but rare.[1,5]

Criterion 7. See *Indicators* 3, 4 and 9 in Model 1.

Criterion 8. *See Indicator* 8 in Model 1.

Criterion 9. See *Indicators* 4 and 5 in Model 1.

Criterion 10. Compared with the period 2001–10 when 69% of 696 reported cases of HUS were children under 5 years of age, in the GHUSEC outbreak only 2% of reported cases were small children. A higher rate of women developed HUS (68%) during the explosive phase of outbreak in contrast to 56% female patients in the period from 2001 to 2010. Considering EHEC gastroenteritis, the percentages of female patients were similar in 2011 and in the period 2001–10 (58 vs. 61%).[1] The differences in the age and gender distribution of cases could be explained by a higher exposure to the suspected contaminated food. Adults are consuming more raw vegetables than children, and women consume rather raw vegetables and are more likely to be vegetarians than men. Similar differences in age were observed already in 1994 during a food borne outbreak of a haemorrhagic gastroenteritis in Helena, Montana/USA.[32]

Criterion 11. See *Indicator* 1 in Model 1.

In Model 3, six positive out of 11 scores indicate that the GHUSEC outbreak was probably a natural one, but mimicked in some epidemiological and microbiological features accidental or deliberate epidemic events (Table 4).

Clue 1. See indicators 1 and 3 in Model 1.

Clue 2. See indicator 4 in Model 1.

Clue 3: See indicator 7 in Model 1.

Clue 4: See indicator 9 in Model 1.

Clue 5: See indicator 9 in Model 1.

Clue 6: The clue was not applicable because there are no vaccines or pre-exposure chemoprophylaxis against EHEC infections.

Clues 7 and 8: No animal reservoir of the outbreak strain could be found at present. Therefore, no ill or dead animals and transmission from infected animals or their tissues and excretions to humans were observed in this epidemic. The ecologic niche of the infectious agent, natural reservoir and host(s), primary origin and exact modes of distibution are unknown until now.[4,33]

Clue 9: See indicator 1 in Model 1.

Clue 10: See indicator 9 Model 1.

Clue 11: There was no direct evidence of a biological attack (e.g. threat or admission letter, suspect dissemination devices with remains of the outbreak strain) in comparison with the deliberate anthrax, salmonella and shigella outbreaks in the USA.[13]

Discussion

Analysis of the unusual GHUSEC epidemic in 2011 by three models for differentiation between natural and unnatural infectious diseases outbreaks showed that a deliberate or accidental act may not be discarded.

There is a general agreement between the results of the three models. Model 1 is strictly focused on outbreak characteristics, and almost completely overlapping in eight indicators with six criteria/clues from models 2 and 3 (all contributing to deliberate or accidental outbreak nature). Indicators 2 and 6 in Model 1 are of essential importance for determining an outbreak, but not contributing to accidental or deliberate outbreak nature. Criteria 6 and 10 in Model 2 are also strictly focused on outbreak characteristics and they are in epidemiological accordance with the outbreak. Criteria 1 and 2 in Model 1 are predictive and realistic. Six important clues in Model 3 are positive thus indirectly supporting deliberate or accidental outbreak nature.

From the onset of the outbreak, there was confusion about the source and mode of transmission.[34] On 10 June 2011, German authorities announced contaminated sprouts of one particular charge of fenugreek seeds imported from Egypt in 2009 as the most probable culprit source of this outbreak.[1,5] The conclusions of the EHEC Task Force were accepted by the European Food Safety Agency (EFSA) who supported the investigations.[5] However, although it might have been expected, no data or evidence on similar outbreaks in Egypt caused by the new German EHEC O104:H4 strain and on the origin of the suspected seeds were available.

Raw vegetables have shown up in the past years as an important transmission factor of enteric pathogens, which may infect or persist dormant in a 'viable but non-culturable' state in/on plants and their seeds.[5,28] Until the GHUSEC outbreak, sprouts were known as a possible but rare vehicle in some outbreaks caused by enteric pathogens.[1,5,35] The high environmental persistence of *E. coli* O157:H7 on raw nut shells imported from the USA was a likely cause of a multi-provincial *E. coli* O157:H7 outbreak in Canada in April 2011.[36] The EFSA therefore some years ago warned that raw sprouts may be contaminated under poor hygienic conditions and become a health risk.[5] However, neither the fenugreek seeds nor remains of the suspected sprout lots distributed in Germany were positive for EHEC O104:H4.[37]

The GHUSEC demonstrates the high impact of awareness of practitioners and clinicians to detect and notify early even 'small clusters' of a disease as an alerting clue of a developing outbreak requiring immediate microbiological and epidemiological investigations of the possible causes.

In conclusion, after using three published models for the analysis of UEE, a generally accepted assumption that GHUSEC in 2011 was a natural one may not be accepted without reserve. This is the first time ever that an *E. coli* O104:H4 pathotype of a high virulence suddenly emerged, which may indicate an unnatural phenomenon. In the interest of the safety and biosecurity of food chains, further epidemiological, microbiological and forensic analyses are needed for a definite answer on a question concerning GHUSEC: 'What was it, actually?'.

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Keypoints

- This is the first time that GHUSEC epidemic is assessed using three published models for differentiation between unnatural and natural infectious disease outbreaks.
- Possibility that the pathogen could have been introduced accidentally or intentionally in the food chain may not be discarded.
- Alerting clues of a developing outbreak given in these three models may be helpful in adequate and timely response to an epidemic.

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