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Public health hazards in Poland posed by foodstuffs contaminated with *E.Coli* O104:H4 bacterium from the recent European outbreak

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Abstract

Shiga toxin producing *Escherichia coli* (STEC) are the most virulent diarrhoeagenic *E. coli* known to date. They can spread with alarming ease via the food chain, as recently demonstrated by the large outbreak of STEC O104:H4 borne by sprouted seeds in 2011, clustered in northern Germany, and subsequently affecting other countries. Indeed, a significant number of infections to verocytotoxin producing *Escherichia coli* O104:H4 have been reported from the WHO European Region resulting in many cases of bloody diarrhoea and haemolytic uraemic syndrome in Germany, 15 other European countries and North America. Eventually, the European Food Standards Agency, (EFSA), identified the likely source to a single consignment of fenugreek seeds from an Egyptian exporter as being linked to the two outbreaks in Germany and France. The situation was closely monitored by the Chief Sanitary Inspectorate public health authority in Poland where actions undertaken ensured that the public was well informed about the dangers of STEC contamination of food, how to avoid infection, and what to do if infected. Tracing the fenugreek distributors also enabled the identification of suspected batches and their isolation. As a result, there were very few reported cases of STEC infection in Poland. Effective control over such outbreaks is therefore a vital public health task. This should include early detection and rapid identification of the contagion mode, followed by removing the foodstuff(s) from the market, providing consumer advice, and preventing secondary spreading. As a mitigation measure, screening/monitoring those involved in food handling is also warranted to exclude carriers who can be asymptomatic.

Key words

E. coli O104:H4, infections, contamination of food, public health

CHARACTERISTICS OF ENTEROHAEMORRHAGIC *ESCHERICHIA COLI* (EHEC) STRAINS

E. coli bacteria are found normally in gut flora of the lower intestines in animals or humans and are usually transmitted via the faecal/oral route. Most strains are harmless commensals, but some are pathogenic to humans and these can be divided into different groups: enteropathogenic *E. coli* (EPEC) – associated with infantile diarrhoea, enteroinvasive *E. coli* (EIEC) – cause dysentery-like illnesses, enterotoxigenic *E. coli* (ETEC) – produce enterotoxins causing diarrhea, enteroaggregative *E. coli* (EAEC) – express aggregative adherence, diffusely adherent E. coli (DAEC) – adhere to the surface of epithelial cells, enterohaemorrhagic *E. coli* (EHEC) – produce Verocytotoxin or Shiga-like toxin, (Vtx, Stx), which is frequently identified as a causative agent of life-threatening diseases in humans such as haemorrhagic colitis (HC) and haemolytic-uraemic syndrome (HUS) [1, 2].

The primary sources of pathogenic *E. coli* contaminating foods are human shedders which constitute the primary reservoir for pathogenic *E. coli* belonging to the first of the aforementioned groups. The EHEC strains, however, can cause severe food-borne disease where the primary reservoirs are domestic ruminants, especially cattle, sheep and goats; these farmed ruminants being healthy EHEC carriers [2]. As a zoonotic pathogen, EHEC can be transmitted from animals to humans through direct contact with animals, but more often through consumption of undercooked meat, unpasteurized dairy products and vegetables, or water contaminated by the faeces of carriers. Person-to-person transmission has also been documented [1, 3, 4]. EHEC is known to produce characteristic toxins similar to those produced by *Shigella dysenteriae* and are known

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as Vtx or Stx, as defined earlier. Typical EHEC strains produce Stx but also encode a LEE, (locus of enterocyte effacement), pathogenicity island which is important for adherence in the colon, (i.e. through the presence of the *eae* gene expressing the intimine protein). However, those E. coli strains that encode a Shiga toxin but do not contain the LEE pathogenicity island are designated as STEC [2-3]. Two types of these toxins have been described: Shiga toxin 1 (Stx1), which differs from true Shiga toxin by one to seven amino acids, and Shiga toxin 2, (Stx2), which shares about 60% sequence homology with Stx1. These bacteria are therefore often called Shiga toxin-producing *E. coli* (STEC). Functionally-active Shiga toxins may be detected using the Vero cell toxicity test which is why bacteria producing these are also called verotoxin or verocytotoxin-producing *E. coli* (VTEC). These Shiga toxins may cause symptoms of uncomplicated diarrhoea through to HC that may progress into HUS, consisting of a micro-angiopathic haemolytic anaemia, thrombocytopenia, and severe acute renal failure requiring intensive care [5]. Shiga-toxin genes are coded in lambda-type bacteriophages which are incorporated into the *E. coli* genome as lysogenes. These phages have the ability to infect pathogenic and non-pathogenic *E. coli* strains from the human intestine and are capable of producing Shiga-toxins and infectious particles of bacteriophages, which may enable the toxin genes to be spread among other *E. coli* strains (lytic cycle). This, therefore, is the reason why the best way to identify EHEC strains are genetic tests based on the detection of Shiga-toxines genes [6].

EPIDEMIOLOGY

STEC have been recognised since the early 1980s as important food-borne pathogens [7] and the serogroup STEC/VTEC O157 was the first of its kind to be identified as a source of human disease; being responsible for a large number of reported outbreaks during recent decades [6]. STEC infection began to be a public health problem when it was first recognized in 1982 following an outbreak in the USA associated with undercooked hamburgers [2]. From 1982–2002, a total of 350 *E. coli* O157 outbreaks were reported from 49 states in the USA, accounting for 8,598 cases of *E. coli* O157 infection consisting of 1,493 (17.4%) hospitalisations, 354 (4.1%) cases of HUS, and 40 (0.5%) deaths. The number of reported outbreaks began rising in 1993, and peaked in 2000 at 46, where food remained the predominant transmission route [8]. According to data from EFSA, the serogroup O157 has been the most commonly detected serogroup in recent years in Europe, representing about 52% of the confirmed cases with known serotypes. Altogether, 16,263 confirmed human VTEC cases have been notified in EU Member States in the period 2005–2009, including two to six cases of death. STEC/VTEC O157 accounted for almost two-thirds (63.2%) of the 242 HUS cases [5]. O157 is also the most important EHEC serotype with respect to public health in North America, the United Kingdom, and Japan [2]. However, it has been observed recently that the disease in humans is also caused by other serotypes; the so called non-O157 STEC, where the full spectrum of pathogenic non-O157 serogroups and the illnesses they cause remain poorly defined [9]. Evaluation of non-O157 STEC isolates from subjects in the United States found that 6 of the 61 serogroups identified, (i.e. O26, O111, O103, O121, O45, and O145), accounted for 71% of the isolates recovered from 1983–2002, whereas three of these serogroups, (O26, O111, and O103), accounted for 50% of the isolates. The non-O157 STEC demonstrated peaks in summertime similar to that for the STEC O157:H7, and were isolated more frequently from children [10]. Apart from classical EHEC, the newly emerging enteroaggregative EHEC O104:H4 strain was identified as the causative agent in a large outbreak of HC and HUS in Germany and other European countries which occurred in May and July of 2011 [9, 11].

Sporadic cases of Shiga-toxin gene *E. coli* serotype O104:H4 carriers have been reported before, and nine cases of STEC O104 infection were reported in the EU Member States during 2004–2009, with single cases occurring in France (2004), Belgium and Denmark (2008), and in Austria, Finland and Sweden (2010), as well as Norway having one case in 2006 and 3 in 2009. 56% of these cases were male, with ages ranging from < 1 year to 76 years. One of the cases (11%) developed HUS and four (44%) were travel-related; the countries of origin being Afghanistan (2008), Turkey (2009), Egypt and Tunisia (2010). Only two of the STEC O104 cases were of serotype STEC O104:H4 – in France (2004) and Finland (2010). The latter case was travel-related with infection acquired in Egypt [5, 12, 13]. STEC O104:H4 has also been isolated twice in Germany (2001) [14] and once in Korea (2005) [15].

As previously mentioned, in May and July 2011, a major outbreak of gastroenteritis with bloody diarrhea and HUS related to infections with STEC O104:H4 was reported [12]. Most of these were among residents in Germany (96.5%) and travellers to northern Germany from other countries. Additional cases were also reported from Switzerland, USA and Canada [16]. In Poland, two confirmed cases of STEC O104:H4 occurred in a household outbreak, (in Gizycko). Two clinical samples collected from a 7-year-old boy with HUS and his nanny were observed to have the same unique virulence properties as the STEC O104:H4 strain from the international outbreak. Retrospective serological investigations proved person-to-person transmission of the STEC O104:H4 strain originating from the boy's father who had previously visited Dortmund in Germany [17]. This outbreak was the first and largest of its kind in which 25% of all laboratory-confirmed STEC O104:H4 serotype-infected cases evolved into the HUS, while the others presented with STEC related gastroenteritis. The mortality rate was 3.3% in the former and 0.5% in the latter group [16]. The outbreak demonstrated three unusual features. Firstly, a large proportion of the patients suffered from HUS, (> 800 cases) [18, 19], and secondly, it was largely confined to adults (89%) of whom the majority were women (68%) [19], (although it usually affects children); and finally, frequent development of neurologic symptoms was observed in patients when clinical and laboratory indicators of HUS were improving [18]. It has been tentatively suggested that differences in antibiotic treatment regimens between adults and children may explain why the incidence of HUS was observed to be higher in the former, although further studies are ongoing to confirm this [20].

The likely source of contamination for this outbreak strain was found to be fenugreek sprouts and seeds; however, neither detection nor isolation of the *E. coli* O104:H4 has thus far been possible. This may be due to the known ability of bacteria, including the *E. coli* strain, to exist in a dormant state, (e.g. the viable but non-culturable VBNC state), where

detection by culture-based methods may be possible only under very specific conditions [21].

More recently, in the autumn of 2011, a diarrheal illness cluster was detected in elderly female tourists in France (8/22 subjects), returning from Turkey. Two cases were of HUS caused by *E. coli* O104:H4, genetically similar to the German outbreak strain. The timing of the symptoms appearing together with the known incubation period make it highly likely that exposure occurred in Turkey, adding evidence that this *E. coli* serogroup circulates in this region of the world [22].

EHEC O104:H4 STRAIN CHARACTERISTICS AND VIRULENCE

Some differences have been discovered in genetic construction between previously isolated EHEC strains and this last outbreak strain [1, 19]. Comparisons of genome sequences between two *E. coli* O104:H4 strains derived from the 2011 German EHEC outbreak in patients with other pathogenic *E. coli* strains suggests that the O104:H4 strain represents a new *E. coli* pathotype, which has been named Entero-Aggregative-Haemorrhagic Escherichia coli (EAHEC). The analyses indicate that a number of horizontal gene transfer events took place to create the genome of the German outbreak strain [2].

The O104:H4 epidemic strain was found to have a genetic marker characteristic of enteroaggregative *E. coli* (EAEC) [18]; however, unusually, it lacked the attaching/effacing pathogenicity island of virulent STEC strains. In this strain, scientist have also identified the presence of the receptor for iron-chelating aerobactin, known to be a virulence factor associated with the extra-intestinal *E.coli* pathotype (ExPEC) [23]. Because of the merged virulence profiles it has been postulated that the outbreak strain is a typical EAEC strain that acquired the bacteriophage encoding stx2 (Shiga toxin 2) [16, 24], with the capacity to colonise the human GI tract effectively in combination with the production of this virulent cytotoxin [25]. The strain also has a distinct set of virulence factors, including three regions encoding serine protease autotransporters of enterobacteriaceae (SPATEs), which promote colonisation, mucosal damage, and subsequent discharge of Stx into the circulation [26]. STEC O104:H4 harbours three plasmids, of which the two larger are of particular interest. The larger plasmid (ca. 88 kb), designated pESBL, contains the genes bla CTX-M-15 [type of β-lactamase (class A)] encoding extended-spectrum β-lactamase agar (ESBL) CTX-M-15 and bla TEM-1 [type of β-lactamase (class A)] encoding TEM-1 β-lactamase [27, 28]. This plasmid encodes resistance of the strain to a broad spectrum of antimicrobials. The smaller plasmid (ca. 75 kb) contains virulence loci typical for EAEC, including aggR encoding master regulator of EAEC plasmid virulence genes, and several genes under transcription control of AggR. It also contains the aggABCD cluster, encoding aggregative adherence fimbriae I (AAF/I), which mediate the characteristic aggregative adherence of this strain to intestinal epithelial cells [28, 29]. The *eae* gene, encoding adhesion intimin, (an intestinal adherence factor), astA, encoding enteroaggregative E. coli Shiga toxin 1 (Stx1) ehx (encoding enterohemolysin toxin) [30] and haemolysin A (hlyA) [27, 31], were not present. Isolates containing the outbreak strains were resistant to ampicillin, third generation cephalosporins, streptomycin, nalidixic acid, tetracycline, and co-trimoxazole, but were susceptible to carbapenems, ciprofloxacin, chloramphenicol, kanamycin, and gentamicin [32]. However, it is not known when the outbreak strain acquired the gene encoding CTX-M-15-type ESBL. To date, only two STEC strains with an ESBL-producing phenotype have been reported in the literature, a CTX-M-3-type ESBL-producing STEC O26:H11 strain and CTX-M-18-type ESBL-producing STEC O26:H11 strain; both in 2005 [16].

Summing up, *E coli* O104:H4 is Shiga toxin-positive (stx2a), intimin-negative (eae), enterohaemolysin-negative (hyl) and adheres to enteroaggregative *E. coli* (EaggEC). Thus, it possesses genetic characteristics that could explain its virulence, with an unusual combination of virulence factors, being both shigatoxin-producing and enteroaggregative [1, 7]. The German outbreak strain harboured a unique combination of EHEC and EAEC genomic features. These data suggest a new *E. coli* pathotype EAHEC that has EHEC and EAEC ancestors [2]. In contrast to STEC/VTEC strains, which have an animal reservoir, mostly ruminants, EAggEC strains probably only have a human reservoir [33].

HAEMOLYTIC UREMIC SYNDROME – SYMPTOMS, PATHOGENESIS AND TREATMENT

HUS is characterised by the triad of acute renal failure, (with high urea and creatinine levels), haemolytic anaemia, (haemoglobin < 10 g/dL), with fragmented erythrocytes (schizocytes), and thrombocytopenia (platelets < 150,000/mm^3). High lactate dehydrogenase (LDH) is also observed, often involving the central nervous system [31, 34, 35, 36]. The median incubation period, (from exposure to the onset of diarrhoea), was 8 days, and the interval from the onset of diarrhea to the diagnosis of the HUS was 5 days. Bloody diarrhoea (80%) and abdominal pain (78%) were the most common symptoms observed in infected cases, whereas vomiting (19%) and low-grade fever (7%) were less frequently reported [16]. Pancreatic failure and cardiac involvement are less frequent during the acute phase of HUS (2%) [35]. Overall, the incidence of HUS in STEC infections is around 6-9%, and 15% in children, whereas in the STEC-O104:H4 outbreak it rose to 25% [37]. HUS-causing O104:H4 strains are more closely related to typical EAEC than to EHEC strains [38]. Unlike children, adults with HUS have high mortality rates; up to 86% in the elderly [39]. HUS is a complication of infection with Shiga-toxin-producing bacteria, although other infections, such as *Salmonella enteritidis* and *Streptococcus pneumonia* [35] and inheritable abnormalities in complement regulatory proteins can cause the disorder[40]. Despite its low mortality rate (5-7%), it is associated with significant morbidity; 50% of patients require extra-renal depurative techniques and 5-10% have renal sequelae [37].

The augmented adherence to intestinal epithelium might facilitate systemic absorption of Shiga toxin in infected cases, and explains the high frequency (25%) of progression to HUS, and the serious consequences after infection, e.g. organ damage and death [16]. After its adherence to enterocytes, STEC produces attaching-effacing lesions through its plasmidic encoded proteins [37]. Stx are composed of an enzymatically-active A subunit and a pentameric B subunit. The B subunits form a doughnut-shaped structure with a central pore, and

bind to the glycosphingolipid globotriaosylceramide (Gb3), which is expressed at the surface of endothelial cells, leading to subsequent internalization of the toxin. The A subunit is able to inhibit elongation of the peptide chain during protein synthesis, resulting in eukaryotic cell death, tissue damage and organ failure [30]. New findings suggest that STX also directly interferes with the alternative complement pathway, and it is this that forms the key pathogenic mechanism [37]. The histological lesions are characterised by thickening of arteriole and capillary walls, with swelling and detachment of the endothelial cells from the basement membrane and the accumulation of fluffy material in the subendothelium and intraluminally, together with increased platelet aggregation. Thrombi affect the microcirculation and in HUS microthrombi are present primarily in the kidneys, which often leads to renal complications [41]. Thrombocytopenia, (platelet count of <150,000 per mm^3), is the first abnormality seen in STEC infections, whether the patient develops HUS or not, and lasts up to one week [30]. Microangiopathic haemolytic anaemia is a result of a traumatic rupture due to endothelial thrombi and is the most persistent abnormality, usually preceding renal failure [37]. The renal involvement ranges from haematuria, (usually microscopic), and proteinuria, to severe renal failure and oliguria that occur in 50% of cases. Hypertension is common [42]. Dialysis is initiated to correct metabolic abnormalities when required. The short-term renal prognosis is generally favourable. However, the risk of renal failure 20 years after recovery from Stx-HUS is not insignificant, and renal histology showing a glomerular microangiopathy affecting > 50% of glomeruli, arterial microangiopathy, and/or cortical necrosis is the best indicator of long-term prognosis [43]. Moreover, the simultaneous involvement of other microvascular beds, to greater or lesser extents, in the heart, brain and pancreas, is recognised and has been reported in all subtypes of HUS [44]. The neurological symptoms ranged from mild disorientation to qualitative and quantitative alterations of consciousness [45]. CNS pathology associated with HUS includes seizures, encephalopathy and brain infarction [46], and may also affect cortico-subcortical areas and in so doing determines motor and neurocognitive outcomes, thus decreasing the patients' quality of life [35]. Neurological symptoms are observed, such as irritability, drowsiness, cortical blindness, hemiparesis or coma [47], double vision, dysphasia, hyperreflexia and apraxia, to the loss of adverse effects reflexes or repeated epileptic seizures requiring intubation and mechanical ventilation [45]. These symptoms may be a consequence of thrombotic occlusions, (coagulative necrosis due to microthrombosis without haemorrhage), microangiopathic changes of small vessels, (oedema and focal haemorrhage), direct injury from the toxin, ischaemia-reperfusion injury, hypertension and metabolic dysregulation, (e.g. hypoglycaemia, dehydration, acid-base disturbances, changes in serum osmolarity or azothemia) [37]. Neurologic evaluation at the acute phase and during follow-up is crucial to diagnose CNS damage and prevent medium- and long-term sequelae [35].

TREATMENT

Most people recover without any specific treatment in 5–10 days. The WHO recommends that in general, treatment of HUS with antibiotics and antidiarrhoeals is not recommended for patients infected with EHEC. Such treatments have been reported to actually increase the likelihood of complications. The WHO recommends people who are experiencing the characteristic symptoms to seek medical attention and not self-medicate. For patients with severe HUS, blood transfusions and dialysis might be needed to support failing kidneys [1]. Optimum therapy for HUS is still controversial [48]. This syndrome, together with the further effects of toxin and complement complex formation, must be managed and addressed urgently by using a multi-targeted approach [49]. HUS treatment is based on hydro-electrolytic management, peripheral and central venous pressure must be monitored, and cardiac function closely controlled; renal function control is especially important, as well as caloric intake adjustment [35]. HUS with severe renal insufficiency or brain impairment, and for atypical HUS is mostly treated by plasma therapy which involves the addition of fresh plasma to newborns, (plasmapheresis or plasma exchange is carried out). This removes the antibodies in the blood which damage the ADAMTS13 enzyme [50]. Several mechanisms might account for the effectiveness of plasma exchange, including early toxin removal from the circulation [51]. Some claim that antibiotic treatment of children with STEC has been associated with an increased risk of overt HUS [52]. Antibiotic-induced injury to the bacterial membrane might favour the acute release of large amounts of preformed toxin. If antibiotic therapy is deemed clinically appropriate, carbapenems should probably be regarded as a first treatment choice [48]. Several randomised and controlled trials have been carried out in children with HUS where the findings showed that none of the interventions, including transfusion of fresh frozen plasma, administering heparin, Shiga toxin-binding agents or steroids, was better than supportive therapy on its own for all-cause mortality outcome or long-term neurological or renal sequelae (panel). Another form of therapy under consideration is treatment with Eculizumab, an anti-C5 monoclonal antibody [53] component of the complement system, which inhibits the uncontrolled complement activation [45, 54]. Eculizumab binds to C5 and prevents the generation of C5a and the formation of the membrane attack complex [43] that amplifies vascular damage on exposure to Shiga-toxin [51]. Eculizumab is also effective in atypical forms of HUS caused by underlying defects in complement regulation [26].

THE EPIDEMIOLOGICAL SITUATION IN GERMANY FOLLOWING THE OUTBREAK OF *E. COLI* O104:H4 CONTAMINATION IN FOOD

On 26 May 2011, the German Rapid Alert System for Food and Feed (RASFF) issued a notification that Enterohaemorrhegic *E. coli* strains (EHEC) had been identified in four cucumber samples taken at the time when the countrywide incidence of HUS and STEC infections had been significantly increasing since 21 May. Studies at the Robert Koch Institute demonstrated that afflicted patients had eaten significantly more raw tomatoes, cucumbers and lettuce compared to healthy controls. As a result, the German Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment) publicly recommended that eating these vegetables should be avoided at the present time because they constitute a potential source of the pathogen, and that

more care is needed with hygiene procedures, especially in the northern parts of Germany [1].

Related instances of illness and infection were also noted in other countries of the EU, including Sweden, Denmark, Holland, UK, France, Austria, Poland, Spain, Luxemburg, Greece, Czech Republic and Norway. A document informing the public about how to avoid infection from the strain responsible for the German outbreak was prepared by the ECDC and EFSA; proper personal hygiene, (e.g. washing hands), before/after preparing food was stressed. The sources of contamination were at first traced to vegetables, and consequently a large number of such samples were analysed, (e.g. from patient's homes, marketplaces, distribution centres, etc.). Nevertheless, the infection source could not be identified; a major difficulty being the long disease incubation period because patients were hospitalised 8-10 days after becoming infected [1, 2].

The first indications that the infection source could be sprouts, (in particular mixtures containing lentils, alfalfa seeds, fenugreek and adzuki beans), showed that it originated from organic cultivation in Bienenbüttel Gaertnerhof in Lower Saxony. These sprouts had been supplied to all catering facilities in which people who became sick had eaten and the relevant products were therefore withdrawn from the market. Despite intensive sampling of seeds, seedlings and from production equipment, analyses did not confirm the presence of STEC/VTEC bacteria. During this time, the number of new cases recorded decreased [5, 20].

The German authorities, however, did not rescind their recommendations for restricting consumption of vegetables as the possibility of second wave infections might be expected. Further sampling and analyses were performed which included several hundred samples of products, raw materials and environmental samples from a manufacturer in Lower Saxony, where account was taken of the location of production, water supply and sewage disposal. Sprouts from other manufacturers were also investigated. However, after analysing the 853 samples taken to this time, no EHEC bacteria had been detected. Nevertheless, after analysing supply routes, patient feedback, and observing where new cases had occurred, the indications were that the EHEC infection has spread from a particular farm in Lower Saxony where in many cases the distribution of seedlings and seeds matched the geographical pattern. Finally, on 11 June 2011, the German authorities reported that a strain of EHEC 0104: H4 Had been identified in samples taken from the seedling household waste of one of the families in which two members were diagnosed with EHEC 0104: H4. The first analysis of these samples was performed by a laboratory in Krefeld and demonstrated the presence of EHEC 0104: H4. The result was subsequently confirmed by the National Reference Laboratory at the Federal Institute for Risk Assessment. The German authorities also notified that the sprouts had come from an organic farm in Bienenbüttel Gaertnerhof in Lower Saxony [1, 2, 5].

THE EPIDEMIOLOGICAL SITUATION IN FRANCE FOLLOWING THE OUTBREAK OF *E. COLI* O104:H4 CONTAMINATION IN FOOD

On 24 June 2011, the French authorities informed that EHEC O104: H4 cases had been detected and confirmed, consisting of two cases of infection with *E. coli* producing Shiga toxin (STEC), 9 cases of HUS, and four cases where HUS was not suspected. Eleven of the patients had attended the same event in the district of Bordeaux. Infection caused by strain O104: H4 was confirmed in 12 out of 15 cases. Epidemiological investigations showed that the potential source of infection were from fenugreek seed sprouts. Phenotypic and genotypic characteristics of *E. coli* O104: H4 isolates obtained from French and German cases were found to be similar. The French authorities immediately began tracing the distribution pathways of sprouts and seeds suspected of causing the disease where a British company was identified as being the supplier. Thanks to the efforts of British Food Control bodies, the UK authorities established that the organic fenugreek seeds had originally been purchased in Germany, from the importer who had imported seeds from Egypt, and who had also delivered these seeds to the other EU member countries. One of the German distributors to customers in the EU was found to have supplied the infected product to Poland, the recipient being a company from the Małopolska Province. The District Sanitary Inspection in Kraków then attempted to establish the distribution list of fenugreek seeds and mixtures in Poland. As a result, 16 packages of grains containing fenugreek were immediately withdrawn from the market. It was not possible, however, to identify all the individual customers who had previously bought these products. Nonwithstanding, those found by the SSI to have consumed the seeds showed no signs of illness. Furthermore, subsequent analyses by the SSI of the withdrawn packages of fenugreek seeds and seed mixtures did not show the presence of a strain of *E. coli* O104: H4. Despite this, the withdrawn products were destroyed in accordance with Commission Decision (2011/402/UE) [13].

THE POSITION OF THE EC REGARDING THE OUTBREAKS IN GERMANY AND FRANCE

Laboratory analysis of samples from infected people performed by EC, EFSA, ECDC and the WHO traced the most likely common source to a batch of fenugreek seeds imported from Egypt. As a result, the EC Bureau of Food and Veterinary Office conducted an audit in Egypt for possible confirmation. Certain shortcomings were identified in the manufacturing process, where E. coli O104: H4 contamination of the seeds may have arisen through contact with human or animal faeces. Such problems were not observed in the production of fresh vegetables or legumes intended for human consumption. Thus, the threat to food security was considered to be confined only to the sprouting fenugreek seeds, and the re-importation of other produce could be permitted. The Polish State Sanitary Inspectorate undertook national checks with the producers and distributors of the seeds intended for sprout manufacture on the Polish market, and it was determined that most had originated from domestic sources. The rest came either from other EU countries, i.e. Italy, Germany, UK, or from third countries, China, USA, Mexico, Canada, Turkey, Peru, Argentina, India and Thailand. There were none directly from Egypt [1, 13, 16].

THE SITUATION IN POLAND – ACTIONS BY THE STATE SANITARY INSPECTORATE (SSI)

Following instructions from the Chief Sanitary Inspectorate in Poland at the end of May 2011, the SSI began additional monitoring due to concerns over the possibility of infection/illness cases appearing in Poland, originating from abroad, where increased incidences of HUS caused by verotoxin, (from STEC) had been detected from the outbreaks in Germany and France, as well as cases in other EU countries. Microbiological analysis was therefore undertaken on numerous samples of fruit and vegetables, particularly imported cucumbers from Spain, (Almeria and Malaga provinces), and Germany. Domestic samples were also analysed from fresh produce sold primarily in hypermarkets, supermarkets, local markets and fruit and vegetable markets, which included cucumbers, tomatoes, spinach, radishes, lettuce, carrots, endive salad, cabbage and sprouts, as well as chopped and whole ready-to-eat vegetables. Particular attention was paid to organic farm produce. Weekly sampling of five samples/vegetable type was performed in each province – a total of 720. Subsequently, the intense checking of produce from Spain was stopped as the threat was then perceived to be negligible; however, extra vigilance was maintained for all produce coming from Germany, irrespective of the country of ultimate origin.

Samples were tested according to EC regulations. A critical value of an increase in >10cfu/g of single-turquoise colonies on agar plates with TBX medium was set to merit further detailed molecular analyses in order to exclude the verotoxigenic strain of E. coli. This low value being a reflection of the fact that a low dose of serotype O104: H4 is highly infective, (<1,000 cells), thus a small number of pathogens can cause serious illness in people. All further work on identification was performed at the National Veterinary Institute – National Research Institute in Puławy in accordance with decisions by the Chief Sanitary Inspector and the Minister of Agriculture and Rural Development. Control measures were targeted at the sources used for on sale products or those used in the production or processing of fresh fruit and vegetables. Product traceability systems were also checked for suppliers and customers, the conditions of transporting and storage of fresh fruits and vegetables, as well as the correctness of technological processes in which fruits and vegetables are to be used as raw material. In addition, checks were made to see whether Good Manufacturing Practice had been followed and whether the principles of hygiene in sales/production had been adopted; including staff personal hygiene and how they have been incorporated into established Principles of Good Hygiene Practice in factories.

Information and education campaigns were instigated aimed at raising awareness of the potential sources of threat, how food should be safely prepared for consumption and maintenance of good personal hygiene. Departments responsible for this sanitary supervision were Food Hygiene, Nutrition and Consumers, Epidemiology, Health Education and Health Promotion, and Health of Children and Youth. Advice was provided to commercial facilities and caterers during inspection, (including distributing educational material), on how best to reduce or avoid infection in the consumption of fresh fruit and vegetables. In similar fashion, the general public were also informed and educated. Recommendations on basic hygiene practice and exercising care when preparing/consuming imported fresh fruit and vegetables for the general public were made available on the websites of the Chief Sanitary Inspectorate, Provincial Sanitary-Epidemiological Stations and selected Sanitary-Epidemiological Stations. Travellers and visitors from Germany were likewise targeted as were physicians/GPs who were sent relevant information on verotoxigenic *Escherichia coli*. The websites contained a leaflet 'Five Steps to Food Safety' which was also distributed in public buildings, city and municipal offices, food/nutritional establishments handling food and especially to mass catering facilities, including small restaurants, food outlets in market stalls, and on tourist routes – in railway and bus stations and all other public places, as appropriate.

THE REPORTING OF RESULTS IN POLAND

The National Focal Point of the SSI provided daily, detailed reports on the number of inspections undertaken, (specifying vegetable type and country of origin), and results from laboratory analyses to the RASFF system, (Rapid Alert System for Food and Feed), and the EWRS, (Early Warning and Response System). Information on developments in Germany was also received by the former.

On 2 June 2011, the Russian Federation (RF) introduced a ban on imports of vegetables from EU member states. Subsequently this was revised on 22 June in Moscow where the EU and RF concluded an agreement to restore the export of vegetables to the RF, on the condition that EU member countries attach export certificates to all shipments that the vegetables are free of the *E. coli* O104: H4 bacteria. In Poland, the competent authority empowered with issuing such certificates was the State Plant Health and Seed Inspectorate; however, the certification solution proved to be short-lived following the 26 July 2011 announcement made by the Robert Koch Institute in Berlin that the epidemic outbreak of *E. coli* O104 infection: H4 STEC in Germany had stopped. The RF then dropped its certification requirement. In Poland, this applied from 10 August 2011, when the SSI ceased performing the additional checks/tests.

These checking procedures nevertheless demonstrated that the majority of plants, fruit and vegetables used for consumption/cooking were domestic products delivered by local suppliers or farmers. In all, during the period 31 May 2011 – 10 August 2011 the SSI carried out 24,550 inspections which consisted of collecting 2,872 samples of fresh fruit and vegetables because of the *E. coli* outbreak, including 670 samples from Germany, Spain and Poland and 2,202 due to the aforementioned temporary RF requirements. There were however no cases in which the verotoxic strain of *E. coli* 0104 had been identified.

Future sampling plans for 2012 will now entail taking 800 vegetable samples to test for the presence of enterohaemorrhagic *E. coli* O157 and the verotoxigenic *E. coli* O104 H4 serotype which is stipulated to be mostly from lettuce, cucumbers, spinach and carrots, as well as 800 samples of sprouts and seeds. This was prepared by the National Institute of Public Health – National Institute of Hygiene as part of the 'Sampling Plan' in the official food control system of monitoring by the SSI and performed according to current recommendations of the EFSA Biohazard Panel. These point out that monitoring of microbial contamination of the food

should not be limited to testing for the presence of the O157 serotype, but should be extended to other types, such as verotoxigenic O104, O91, O103 in keeping with current knowledge. All Provincial Sanitary Inspectorates are to be responsible for collecting and pre-testing vegetable samples prior to testing for enterohaemorrhagic *E. coli*.

IN CONCLUSION

Infectious diseases emerging throughout history have included some of the most feared plagues of the past. New infections continue to emerge today, while many of the old plagues are with us still. These are global problems. Emerging infectious diseases can be defined as infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range. Among recent examples are hantavirus pulmonary syndrome [55, 56], Lyme disease [57, 58, 59, 60] and hemolytic uremic syndrome [22, 61] caused by certain strains of *Escherichia coli* (eg. serotype O104:H4). Specific factors precipitating disease emergence can be identified in virtually all cases. These include ecological, environmental or demographic factors that place people at increased contact with a previously unfamiliar microbe or its natural host or promote dissemination. These factors are increasing in prevalence; this increase, together with the ongoing evolution of viral and microbial variants and selection for drug resistance, suggests that infections will continue to emerge and probably increase and emphasizes the urgent need for effective surveillance and control.

Effective control over such outbreaks is therefore a vital public health task. Early and rapid detection are necessary, including molecularly elucidating the pathogen. Identifying the contagion mode also needs to be fast, as does actions to limit the outbreak once it occurs, i.e. removal of the offending food product(s) from market circulation and ensuring the public is well informed, as well as measures preventing secondary spread, e.g. focusing on asymptomatic carriers. The recent spate of outbreaks also affords an opportunity to improve upon/design therapies for treating the STEC disease during its stages, including biomarkers of risk which may indicate the severity of the disease. Targets for antimicrobial therapy could include Stx phage induction, Shiga toxin production, virulence and resistance gene transfer, intestinal adherence, among others [62]. In addition, an appropriate surveillance strategy is essential, coupled with the most effective detection methods where developments in molecular subtyping should be exploited in the future.

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