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Enterohaemorrhagic *Escherichia coli* O104:H4: are we prepared now?

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It is over. The outbreak of the enterohaemorrhagic *Escherichia coli* (EHEC) O104:H4 infection that had its major focus in Germany [1] and affected people in many other European countries has officially come to an end [2]. While the media coverage has been decreasing, the scientific community has been working to understand the reason why this dramatic outbreak occurred. We have learnt that the pathogen is not a totally new clone, but is a slight variant of a known – although rarely described – EHEC, called HUSEC-41 [3] with an extended-spectrum beta-lactamase (ESBL) resistance. Furthermore, the strain carries genes typically found in two types of pathogenic *E. coli*, the enteroaggregative *E. coli* (EAEC) and EHEC [4,5]. It specifically carries the genes for the classical haemolytic uraemic syndrome (HUS)-associated Shiga toxin 2.

Despite the efforts that have been made, major questions currently remain unanswered, such as why women were affected more than men, why the attack rate was so high, what the primary source was and what the reservoir is, how long people are carriers, what the importance of the ESBL resistance is, what the infectious dose is for this outbreak strain and what the role of secondary transmission is via symptomatic or asymptomatic carriers, directly to other persons or indirectly via an index source, such as food.

It is known that up to 15% of EHEC cases can be a result of secondary transmission arising from household contact with people who have sporadic EHEC infections [6]. In this issue of *Eurosurveillance*, two articles, Aldabe et al. [7] and Hauri et al. [8] report on secondary transmission during the EHEC O104:H4 outbreak. The first reports on a symptomatic man who transmitted EHEC to his wife and young daughter during the EHEC O104:H4 infection in France [7]. Interestingly, the EHEC that was isolated from the mother apparently lost its ESBL resistance, confirming the known mobility of plasmids carrying resistance genes. This fact should be taken into consideration in diagnostic laboratories if ESBL resistance of EHEC O104:H4 is used for primary selection of the pathogen from stools without using also non-selective enrichment and detection of Shiga toxin genes.

The second article [8] illustrates in detail the history of six possible household transmissions, two possible nosocomial and one possible laboratory transmission in the German State of Hesse, where satellite clusters occurred. These cases throw light on three crucial issues. First, secondary transmission of EHEC O104:H4 was shown not to be more frequent than expected. Second, the importance of microbiological serotyping was highlighted, as EHEC of other HUS-associated serogoups (O157, O91, and O103) were also identified during the outbreak. Serotyping data are rarely available, due to the need for time-consuming techniques usually only carried out in specialised reference labs. This shows the need for the development of rapid sero- and pathotyping methods for all HUS-associated *E. coli* strains. Third, infection control in hospitalised patients with EHEC infection needs specific consideration, as does laboratory safety in the handling of EHEC. It is not without reason that in most countries of the European Union EHEC is classified as a biosafety level (BSL)-3** microorganism (but no high-efficiency particulate air (HEPA) filter is required).

Both articles illustrate the importance of personal hygiene in preventing secondary transmission. In general, EHEC does not behave differently to any other organism transmitted via the faecal–oral route, but our 'preventive doors' for such organisms seem to stay wide open. We have become used to the fact that hundreds of thousands of Europeans have diarrhoea every year and a certain lack of basic hygiene seems to be acceptable, as usually nothing very severe happens. We often lack time for hand hygiene as we consider it not to be of great importance. However, diarrhoea is not a normal state. We forget that most enteropathogens are less infectious than EHEC or do not lead to such severe disease with such social visibility. This brings us to the biggest challenge. Circulating highly pathogenic and/or multiresistant microorganisms can be detected at a very early stage, before large outbreaks of disease occur. Preventive microbiology is a basis for preventive

medical advice and decision-making to protect people from infections. In future, European-wide coordination of preventive microbiology will be crucial for early detection of major health threats caused by infectious diseases. Its success will depend on our international and interdisciplinary efforts to foster protection against infection.

This outbreak is over. Let us get prepared!

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Ongoing measles outbreak in Romania, 2011

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Since January 2011 Romania has been experiencing a measles outbreak with 2,072 cases notified in 29 of the 42 Romanian districts. Most cases occurred in the north-western part of the country among unvaccinated children with the highest number of cases (893 cases) registered in children aged one to four years. This report underlines once more the need for additional measures targeting susceptible populations to achieve high vaccination coverage with two doses of measles-mumps-rubella vaccine.

Between January and June 2011, 2,072 measles cases were notified in 29 of the 42 Romanian districts with most cases registered in the north-western part of the country mainly among unvaccinated children. No measles-related deaths have so far been notified in 2011.

An outbreak of measles was first noticed in late August 2010 in the north-eastern part of Romania [1] and by the end of the year, 193 cases were registered in the whole country.

Measles is a statutorily notifiable disease since 1978 in Romania, and medical practitioners have to immediately report all suspected measles cases to the local public health authorities. At national level, the National Centre for Communicable Diseases Surveillance and Control in Bucharest collects and analyses all notifications of measles cases. National case-based notification was initiated in 1999 and the European Union (EU) case definition and case classification have been adopted since 2005 [2].

The monovalent measles-containing vaccine was introduced in 1979 in the Romanian immunisation schedule for children aged 9-11 months. In 1994, the second measles vaccine dose was introduced for children aged between six and seven years (first school grade). The combined measles-mumps–rubella (MMR) vaccine replaced the monovalent measles vaccine in 2004 and was recommended as a first dose for children aged 12-15 months. The second MMR vaccine has been recommended for children aged between six and seven years since October 2005.

Between 2000 and 2008 the national measles vaccination coverage for children aged between 18 and 24 months with the first dose of measles-containing vaccine was estimated at 97%-98% and for children aged seven years, the vaccination coverage with the second dose of measles-containing vaccine was estimated at 96%-98% [3]. In the last two years a constant decrease could be noticed in the measles vaccination coverage for children aged 12 months. According to the vaccination coverage reports, in 2009, the coverage for the first MMR vaccine dose was 85.1% (95% CI: 82.4–87.8) at the age of 12 months and reached the target of 95% (95% CI: 93.4–95.8) coverage for children aged 18 months. A high number of children remain unvaccinated not only in the hard-to-reach communities but also in the general population, due to parental refusal and scepticism regarding the benefits of the vaccination. Vaccination coverage for the second dose of MMR vaccine is reported every year by the school medical staff to the local health authorities after the school vaccination campaign. In 2010, the reported coverage for the second dose of measles-containing vaccine, calculated using the number of doses administrated divided by the total number of eligible children aged seven years was 93.4% (95% CI: 90.7–95.0).

Here we report an ongoing measles outbreak in Romania by analysing measles data available from 1 January to 30 June 2011. Descriptive analysis was performed using the national surveillance standardised form sent by the public health authorities of each district to the National Centre for Communicable Diseases Surveillance and Control.

Outbreak description

From the beginning of 2011 until 30 June, a total number of 2,072 measles cases were notified by the local public health authorities. The highest number of cases was registered among children aged between one and four years (893 cases), followed by the five-nine year-olds (445 cases) and the infants under one year of age (303 cases). Among the 10-14 year-olds there were 189 cases identified, 150 cases in those aged 20 years and above and 92 cases were registered among adolescents aged

between 15 and 19 years. Among the total number of cases, approximately half occurred in hard-to-reach communities. The monthly incidence increased from 131 cases registered in January to a peak of 515 cases in May, and decreased in June when the number of notified cases was 437 (Figure 1).

The laboratory confirmation was performed by detecting measles IgM antibodies in serum samples. Due to many local outbreaks, the laboratory confirmation was performed only in some of the first cases identified in a particular area until D4 genotype was confirmed. For those cases with clear epidemiological link with the outbreak, the epidemiological confirmation criteria were used.

Of the 2,072 notified measles cases, 898 were laboratory-confirmed, 1,161 were probable cases with documented epidemiological link and 13 were clinical measles cases for whom sera could not be obtained due to parental refusal.

RT-PCR techniques to detect measles virus nucleic acid were also used to confirm the first cases from some affected districts. Twelve viruses were genotyped by a nested RT-PCR reaction which targeted a 450 nt region at the C-terminus of the N protein (Nc region). All of them belonged to D4 genotype currently circulating in Europe [4].

Measles spread in 29 districts (including Bucharest) of a total of 42 and the geographical distribution shows a concentration of measles cases in the north-western part of the country (Figure 2).

Of the total number of 2,072 measles cases, 800 (38.6%) presented complications: 582 (72.8%) cases developed pneumonia, 203 (25.4%) diarrhoea, eight (1%) malnutrition, five (0.62%) convulsions and two (0.25%) encephalitis.

The median age was three years (range: three weeks – 43 years). The highest incidence (138.4 per 100,000 population) was in infants not eligible for vaccination (under one year of age), followed by the one to four year-olds (103.4 per 100,000 population) and the five to nine year-olds (42.3 per 100,000 population) (Figure 3). For the older age groups the incidence ranged between 17.1 per 100,000 population and 1.8 per 100,000 population.

Figure 1

Monthly incidence of notified measles cases, Romania, 1 January–30 June 2011 (n=2,072 cases)

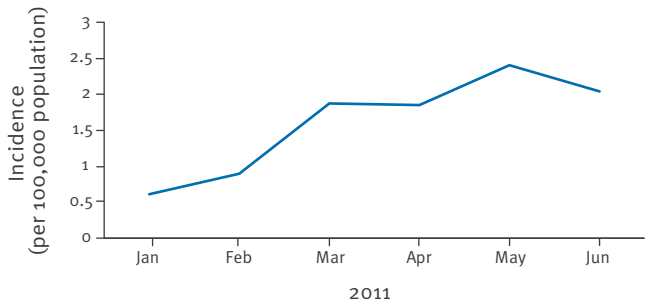
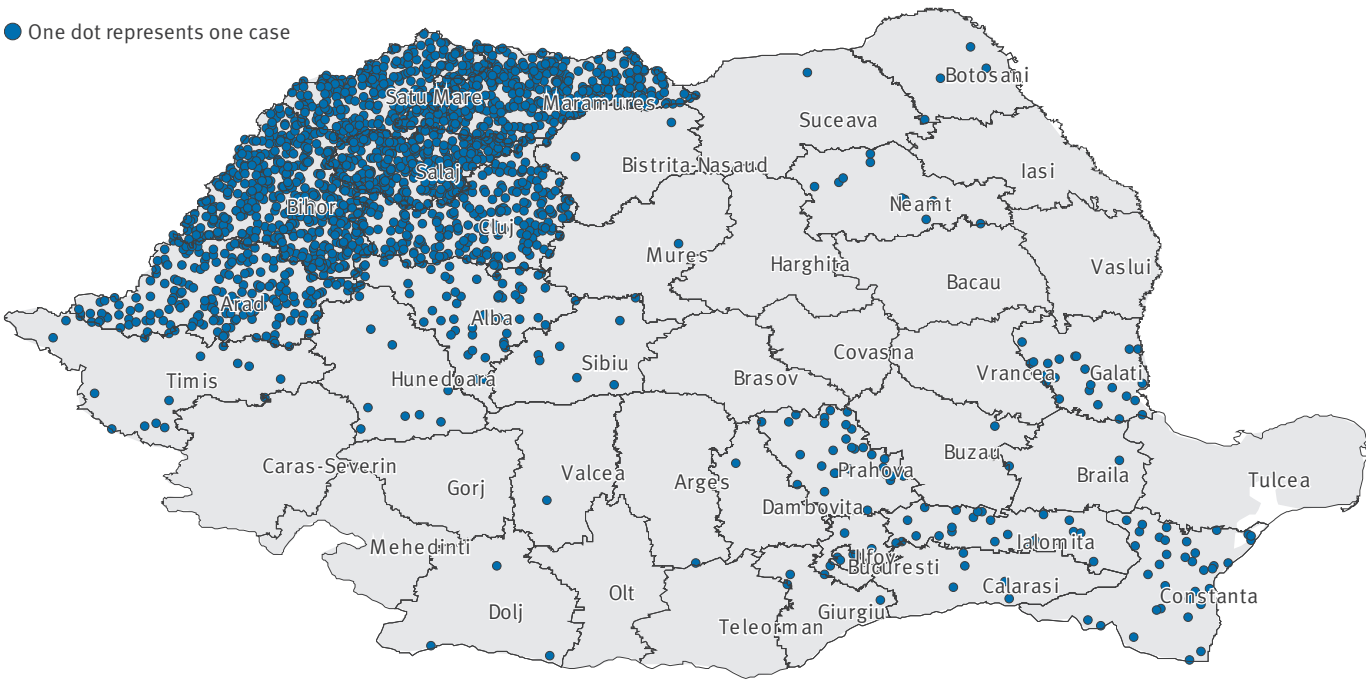


Figure 2

Distribution of notified measles cases, Romania, 1 January–30 June 2011 (n=2,072 cases)



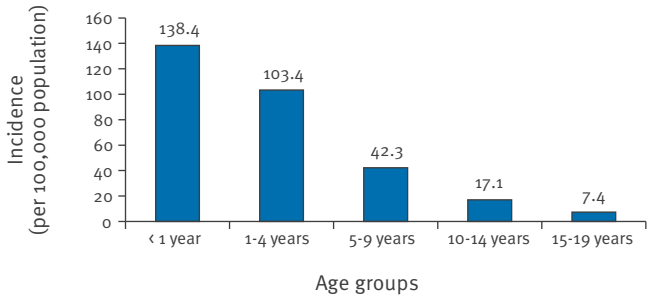
Most cases occurred among unvaccinated children representing 72.8% from the total number of cases registered during period mentioned above. Of these, only 19.8% were not eligible for MMR vaccination due to their age (under 12 months) (Figure 4).

Control measures

Several control measures have been implemented by the local health authorities in their efforts to stop this outbreak. An additional MMR vaccination campaign started in the affected areas targeting all children aged between seven months and seven years, irrespective of their measles vaccination status. Nevertheless, no change has yet been foreseen in the national immunisation schedule regarding the administration of the first dose of MMR vaccine. The MMR vaccine is supplied by the Ministry of Health and is offered free of charge through the routine immunisation services (family doctors) and special outreach teams. As of 30 June 2011, 4,500 children have been vaccinated with measles-containing vaccine, following this additional vaccination campaign. Active case finding was initiated by general practitioners in the areas most affected by the outbreak, as well as contact-tracing in hospitals and in the community. Other activities such as meetings with local public health representatives were undertaken by the national public health authorities in order to increase awareness on the ongoing outbreak not only among physicians but also in the general population.

Figure 3

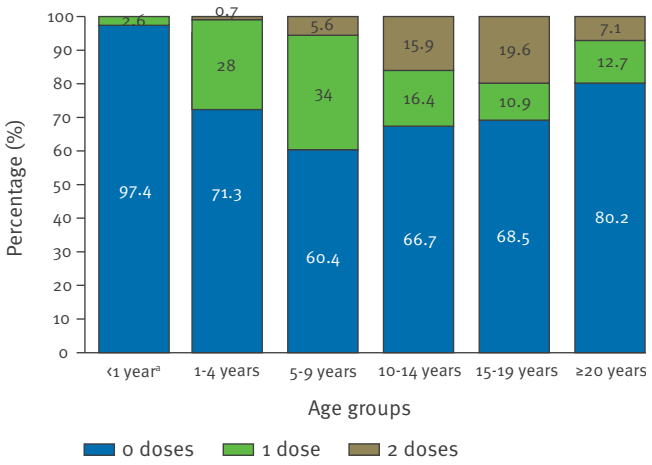
Measles incidence by age groups, Romania, 1 January–30 June 2011 (n=1,922 cases[a])



[a] Cases aged 20 years and above (n=150) are not included in the figure.

Figure 4

Percentage of measles cases by age group and vaccination status, 1 January–30 June 2011 (n=2,072 cases)



[a] 2.6% were vaccinated during the incubation period in the course of the additional vaccination campaign targeted at children aged seven months–seven years.

Discussion and conclusions

In Romania, the measles incidence dropped from 16.3 and 1.6 per 100,000 population in 2006–2007 respectively, to less than 0.1 per 100,000 population in 2008–2009 [5-7].

Despite the high national immunisation coverage with MMR vaccine reported during the last 10 years, this outbreak highlights the presence of pockets of individuals vulnerable to measles and particularly those members of hard-to-reach communities but not only. We observed that more parents, even among highly educated persons, lost their confidence in vaccination benefits for their children and this became an important problem that needs to be addressed. The current measles outbreak in Romania and in other European countries reveal the need for increased awareness on the declining confidence that people have in vaccination benefits for their children and for public health intervention focused in hard-to-reach communities. In addition, after the pandemic influenza A(H1N1)2009, a constant scepticism and refusal regarding vaccination in general could be noticed, not only in hard-to-reach communities, but also in the general population.

In areas and communities where vaccine coverage remains sub-optimum, large cohorts of susceptible people accumulate and represent a potential for large outbreaks. The large proportion of cases observed in infants suggests an intensely circulating measles virus [7].

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Evidence of enzootic circulation of West Nile virus (Nea Santa-Greece-2010, lineage 2), Greece, May to July 2011

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A West Nile virus (WNV) surveillance network including sentinel chickens was deployed in Thessaloniki county, Greece, from May to July 2011. For the first time in summer 2011, a chicken WNV isolate from 6 July was molecularly identified. The partial NS3 sequence was identical to that of the Nea Santa-Greece-2010 WNV lineage 2, detected in central Macedonia in 2010. This suggests that WNV is actively circulating in central Macedonia and that it may have overwintered in northern Greece.

During 2010, Greece underwent the second largest West Nile virus (WNV) epidemic in Europe in the last two decades with 262 clinical human cases and 35 fatalities [1]. WNV lineage 2 was identified in two pools of *Culex* mosquitoes (Nea Santa-Greece-2010 virus) [2] and in wild birds [3] that were sampled during the epidemic season of 2010 from areas in close proximity to human cases.

No active vector and arbovirus surveillance system was in place in Greece before the epidemic in 2010. We initiated a monitoring programme in 2011, from May to November, in order to understand subsequent transmission, to document virus activity, and to better assess the relative importance of vector species. A small scale mosquito and animal surveillance network was established in the county of Thessaloniki, one of the areas with the highest number of human cases during the epidemic of 2010 [1]. The long term objective of this project is to design within the following years an optimum, large scale arbovirus surveillance programme for Thessaloniki. We report here preliminary findings of the study that will have interest for public health authorities.

Methods

Sentinel chickens

Six chicken flocks (six chickens per flock) were placed in stationary cages along the western and eastern edges of Thessaloniki (three flocks on each side) to monitor WNV activity in areas suitable for potential enzootic transmission [4] (Figure 1). These areas combine abundance of mosquito larval sites (e.g. rice fields) and potential habitat for migratory birds (e.g. Axios River delta) that may serve as reservoir populations for WNV. The flocks were placed within or in close proximity to residential communities that experienced abundant mosquito activity. All chickens were confirmed WNV antibody negative prior to placement in the field. For each flock, the chicken cage was divided in six compartments so that each chicken would be kept separate from the others. Chickens were bled through the ulnar vein weekly (about 1ml of blood sample per chicken).

Mosquito population monitoring

Carbon-dioxide (dry ice) baited Centers for Disease Control (CDC) light traps (John W. Hock, Gainesville, United States (USA)) were deployed once a week at 28 sites in the Thessaloniki area beginning 20 May 2011 (Figure 1). Traps were located at approximately equal intervals in order to provide a geographically representative sampling.

Laboratory analysis

Chicken plasma (o.5 ml) and sera (o.25 ml) were collected for virus detection and serology, respectively. Serum samples were tested by ELISA for the detection of WNV-specific antibodies using a commercial ELISA kit (ID Screen West Nile Competition, IDVET, France). After the detection of seroconversion, RNA was extracted from selected plasma samples previously taken from the seroconverted birds. RNA extracts were examined using a one tube RT-PCR screening protocol employing a primer pair (WNPolUp: 5'-TTTTGGGAGATGGTGGATGARGA-3' and WNPolDo2: 5'-CCACATGAACCAWATGGCTCTGC-3') designed for the specific detection of WNV and targeting a 144 bp part of the nonstructural protein 5 (NS5) gene. Samples found positive by the RT-PCR screening protocol, were additionally subjected to RNA reverse transcription using random hexamers, followed by two PCR assays employing a primer pair (WN-NS3up1: 5'-GCTGGCTTCGAACCTGAAATGTTG-3'

and WN-NS3do1: 5'-CAATGATGGTGGGTTTCACGCT-3') targeting a 778 bp part of the nonstructural protein 3 (NS3) gene, and a nested primer pair (WN-NS3up2: 5'-GCAAGATACTTCCCCAAATCATCAAGG-3' and WN-NS3do2: 5'-TGTCTGGGATCTCTGTTTGCATGTC-3') targeting a respective 423 bp part. The nested PCR products were bidirectionally sequenced. The NS3 gene was selected for molecular characterisation because it is phylogenetically informative [5] and it encodes a protein residue (NS3-249) subject to adaptive evolution leading to increased viremia potential and virulence [6].

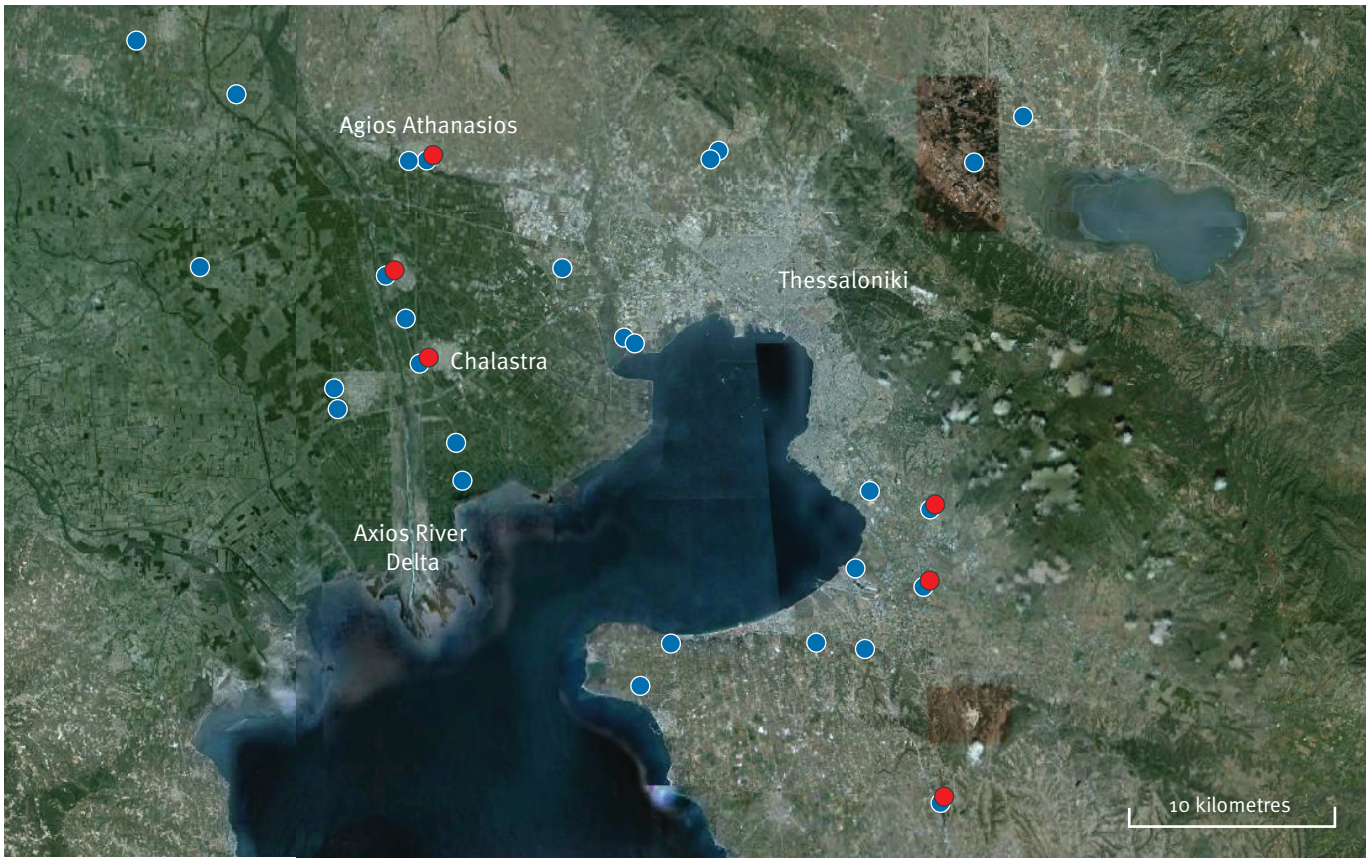
Results

Seroconversion of the first sentinel chicken was detected in the agricultural area of west Thessaloniki in the city of Chalastra (40°37'37.27"N, 22°43'45.05"E) (Figure 1) on 29 June. On 13 July a second chicken seroconversion was detected in the city of Agios Athanasios (40°43'0.59"N, 22°44'7.04"E), followed by a third chicken seroconversion on 20 July in the same area.

All prior samples collected from the three seroconverted chickens were tested using the one tube RT-PCR screening protocol targeting NS5. All RNA samples were negative except in the case of the sentinel chicken in the city of Agios Athanasios (40°43'0.59"N, 22°44'7.04"E) which seroconverted on 13 July. More specifically, a band of expected size was obtained from one PCR product derived from a sample taken from that respective chicken, one week before seroconversion (6 July). The specific RNA extract was subjected to nested PCR, targeting the partial NS3 gene sequence, which was subsequently determined. The sequence was deposited in GenBank database under accession number JN398476 and according to BLAST algorithm, it presented highest nucleotide sequence identity (99.73%) to that from Nea Santa-Greece-2010 virus derived from a *Culex* mosquito pool tested during the 2010 epidemic in Central Macedonia [7]. The inferred partial NS3 amino acid sequence was 100% identical to that of the Nea Santa-Greece-2010 WNV lineage 2. As in the Nea Santa-Greece-2010 virus NS3 sequence, the inferred NS3 residue 249 was determined to be proline, similar to several neuroinvasive lineage 1 WNV strains [6]. In contrast, all other investigated lineage 2 viruses have a NS3 protein with a histidine at this position [7].

Figure 1

Location of mosquito traps (n=28) and sentinel chicken flocks (n=6) for West Nile virus surveillance, Thessaloniki county, Greece, 2011



Mosquito trap location
Chicken flock location (collocated with mosquito trap)

Agios Athanasios and Chlalastra are the two cities where enzootic circulation of the virus was detected.

The cumulative number of *Culex* mosquitoes trapped weekly in the agricultural area of Thessaloniki (Figure 2) was low (n=142) during the last week of May and the first two weeks of June. The population rapidly increased during the second half of June, with a peak (n=23,867) at the end of the month. During the following two weeks, the population decreased and then started building up again during the third week of July. So far, the most prevalent mosquito species in both residential and agricultural areas (rice-fields) were *Culex pipiens* followed by *Culex modestus* Ficalbi. It should be noted that *C. modestus* populations started building up significantly in early July. Testing for WNV in mosquitoes is in progress but no results are available at this stage of the surveillance programme.

Discussion

This is the first report of enzootic circulation of WNV Nea Santa-Greece-2010 in Greece during 2011, one year after the WNV epidemic in Greece. The virus in 2010 was detected in Nea Santa from a *Culex* mosquito pool [2], and in 2011 we detected an identical isolate (molecular characterization based on NS3 gene) in the agricultural area of west Thessaloniki in the city of Agios Athanasios, approximately 21 km southwest of Nea Santa. The 2011 Greek WNV isolate shows close genetic relationship to the lineage 2 goshhawk-Hungary-2004 strain that emerged in Hungary in 2004 but differs from the latter in that it maintains the amino acid substitution H249P found in the Nea Santa-Greece-2010 isolate, which may be associated with increased virulence [7]. WNV lineage 1 strains are distributed in north Africa, Europe, America, Asia and Australia, whereas lineage 2 are mostly distributed in south Africa and Madagascar. Due to increased illness and death caused by WNV lineage 1 compared to lineage 2 in the past, lineage 2 strains were previously considered to be less virulent. However, recent evidence from Africa and Hungary demonstrated that lineage 2 strains may also result in severe disease [8,9].

Up to now, no WNV genomic sequences have been published from the human cases during the 2010 epidemic and there is no direct evidence to incriminate the WNV Nea Santa-Greece-2010 strain as the cause of the 2010 human epidemic. The discovery of the same strain in sentinel chickens in 2011 suggests that the virus was able to overwinter in this region, consistent with current opinion on the endemicity of WNV in Europe [10]. Specifically, the reoccurrence of WNV in continuous years in the same places in Romania and Italy, involving humans and equines, is likely linked to the endemicity of the infection in the areas rather than to a new introduction of the virus [10]. This situation appears to parallel that experienced in California, USA, which has a similar climate (warm temperate, seasonal winter rainfall) [11], where WNV was introduced in 2003, quickly spread throughout the state, and became endemic with the ability to overwinter in a cycle between winter mosquitoes and birds.

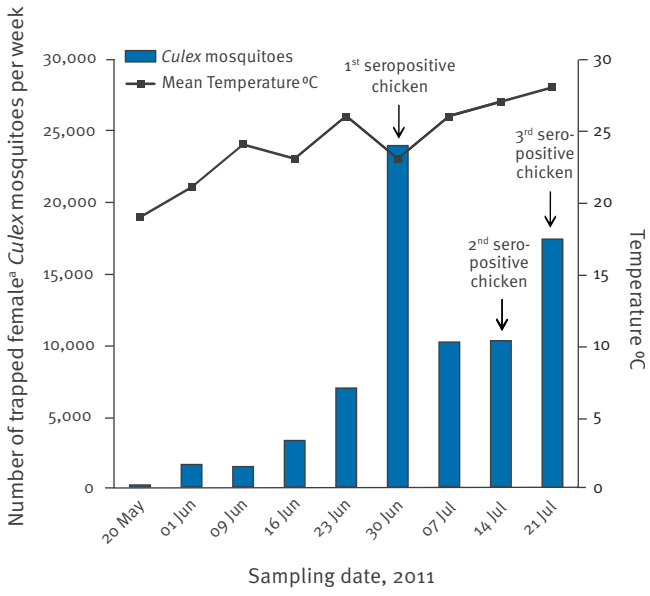
Transmission in the sentinel chickens occurred immediately after the first significant *Culex* population peak, as has been observed in WNV outbreaks [11,12]. Two of the principal WNV vectors in Europe, *C. pipiens* and *C. modestus* [13], are highly abundant in the agricultural area of Thessaloniki and both species may be associated with the transmission of the virus. In Greece, so far, the virus has been isolated from two pools of *C. pipiens* mosquitoes during the epidemic of 2010 [2]. More studies are needed to increase our knowledge on the role of the aforementioned species in the enzootic, epizootic and tangential (e.g. to humans) transmission of WNV in Greece.

Monitoring disease activity by using sentinel animals can provide critical information regarding periods of increased transmission. Surveillance networks involving sentinel animals and mosquitoes have been used in many parts of the world as an early warning system aiming to identify periods and locations of elevated risk of WNV disease transmission [14,15,16]. The rationale behind these surveillance networks is (i) to increase our understanding of the epidemiology of arboviruses, (ii) to identify the circumstances favourable to the appearance of the disease in humans before this occurs, and (iii) to guide mosquito control efforts in time and space to reduce the impact or likelihood of an epidemic.

Arbovirus surveillance systems can be expensive and labour intensive, with weekly monitoring of chickens. This is nevertheless feasible and these systems have been successfully established in some regions [16]. In urban centres of increased vulnerability to mosquito

Figure 2

Culex female mosquito abundance in the agricultural area of west Thessaloniki, Greece, 2011 (n=74,909)



[a] Female mosquitoes were targeted by mosquito traps and accidental male mosquito collection was negligible (<0.1%).

borne epidemics, such as Thessaloniki (approximately 1 million inhabitants, in close proximity to prolific mosquito breeding environments), there is a need and demand for such systems and the benefits associated with their successful deployment outweigh the associated costs. This is the first active arbovirus surveillance system in place in Thessaloniki and in order to optimise its use, extensive data are required in the following years. These data could help create a useful disease surveillance tool that may increase our understanding of the disease transmission cycle and help the local authorities to design a local WNV response plan based on the disease transmission levels. It is encouraging, that through a small scale surveillance system, like the one described in this paper, we were able to detect WNV enzootic circulation in Greece before the onset of any human cases for the year of 2011.

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Household transmission of haemolytic uraemic syndrome associated with *Escherichia coli* O104:H4, south-western France, June 2011

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Following the outbreak of haemolytic uraemic syndrome (HUS) on June 2011 in south-western France, household transmission due to *Escherichia coli* O104:H4 was suspected for two cases who developed symptoms 9 and 10 days after onset of symptoms of the index case. The analysis of exposures and of the incubation period is in favour of a secondary transmission within the family. Recommendations should be reinforced to prevent person-to-person transmission within households.

Introduction

On 30 June 2011, an outbreak of haemolytic uraemic syndrome (HUS) and bloody diarrhoea was reported among attendees of an open day event at a children's community centre that took place on 8 June in a town near Bordeaux, south-western France [1]. The identified strain was Shiga toxin 2-producing ***Escherichia coli*** O104:H4, with the same characteristics as the strain that caused the recent outbreak in Germany [2,3,4]. As of 26 July 2011, 15 cases of bloody diarrhoea have been observed in relation with this event, nine of whom have developed HUS. An investigation was conducted to identify the vehicles of infection and to guide control measures. Preliminary results of interviews and trawling questionnaires suggested sprouts as the vehicle of transmission. Here we describe the two cases of HUS for whom household transmission of *E. coli* O104:H4 was suspected. The possibility of person-to-person transmission of *E. coli* O104:H4 has also been reported in the Netherlands [5].

Case descriptions

Patient A

On 18 June, a man in his 40s was admitted in a hospital near Bordeaux, with abdominal pain and bloody diarrhoea of two days. Stool samples were sent to the National Reference Centre for *E. coli* and *Shigella* in Paris. Four days after admission, the patient left the hospital and returned home. On 27 June, he was hospitalised again in the nephrology department of Bordeaux University Hospital with a diagnosis of HUS. Test results from stool samples showed the presence of *E. coli* O104:H4 possessing the *stx2* gene, encoding Shiga toxin 2. The strain was negative for the gene coding for intimin (*eae*) but positive for *aggR* which regulates the expression of aggregative adherence fimbriae. The antimicrobial resistance pattern of the strain was similar to than seen in the outbreak strain in recent *E. coli* O104:H4 outbreak in Germany: ampicillin-resistant (R), cefotaxime-R, ceftazidime-R, imipenem-sensitive (S), streptomycin-R, kanamycin-S, gentamicin-S, sulfamethoxazole-R, trimethoprim-R, cotrimoxazole-R, tetracycline-R, chloramphenicol-S, nalidixic acid-R, and ciprofloxacin-R. The PCR analysis indicated the presence of the $bla_{\text{CTX-M-15}}$ gene, encoding an extended-spectrum beta-lactamase (ESBL), and the presence of the bla_{TEM} gene, encoding a penicillinase. After treatment, the patient gradually recovered from HUS. He returned home on 6 July having received specific hygiene guidelines from the hospital, notably recommendations about hand-washing.

Patient A had participated in the open day event on 8 June, accompanied by his three-year-old child (Patient B). His wife and his second five-year-old child did not attend. Patient A's interview revealed that he had consumed three kinds of sprouts (fenugreek, mustard, and rocket) during the event.

Patient B

The three-year-old child of Patient A presented first symptoms of illness on 26 June, i.e. 18 days after the

open day and nine days after the onset of symptoms in Patient A. At onset, the child had abdominal pain followed after three days by bloody diarrhoea. Shiga toxin 2-producing *E. coli* O104:H4 was isolated from stool samples. Some bacterial colonies produced the ESBL, whereas others only the penicillinase. However, both types of colonies were also resistant to streptomycin, sulfamethoxazole, trimethoprim, cotrimoxazole, tetracycline, and nalidixic acid, as observed for other outbreak isolates [1]. On 3 July, the child developed anaemia, haemolysis and high urine protein-to-creatinine ratio, compatible with HUS and was admitted to the paediatric department of Bordeaux University Hospital. On 4 July, thrombocytopenia developed. The child gradually recovered after treatment and was discharged from hospital on 8 July.

Patient B had participated in the open day event but, according to Patient A, did not consume sprouts. As the children had no access to the buffet unless accompanied by an adult, it is unlikely that the child has eaten any sprouts without the father's knowledge.

Patient C

On 2 July, a woman in her 30s, wife of Patient A and mother of Patient B, was admitted to Bordeaux University Hospital with bloody diarrhoea of six days, i.e. she had had the first signs of disease on 27 June, respectively 10 and 1 days after the onset of illness in Patient A and Patient B. Shiga toxin 2-producing *E. coli* O104:H4 was isolated from her stool samples. It was resistant to ampicillin, streptomycin, sulfamethoxazole, trimethoprim, cotrimoxazole, tetracycline, and nalidixic acid, but susceptible to extended-spectrum cephalosporins (i.e. only production of the penicillinase). The absence of the CTX-M-15 ESBL compared to the isolates from Patient A and some isolates from Patient B might be due to the mobilisation of insertion sequences usually present in the vicinity of the $bla_{\text{CTX-M-15}}$ gene. This might be in relation to the absence of selective pressure by this class of antimicrobials. Patient C developed symptoms of anaemia, haemolysis, thrombocytopenia and proteinuria on 8 July. After treatment, she recovered gradually and she left the hospital on 12 July.

Patient C had not attended the open day event and not consumed any type of sprouts during the two months previously.

Family members

The five-year-old child spent the whole period at home. It did not develop any symptoms but was admitted to the paediatric department of Bordeaux University Hospital for observation from 3 to 4 July.

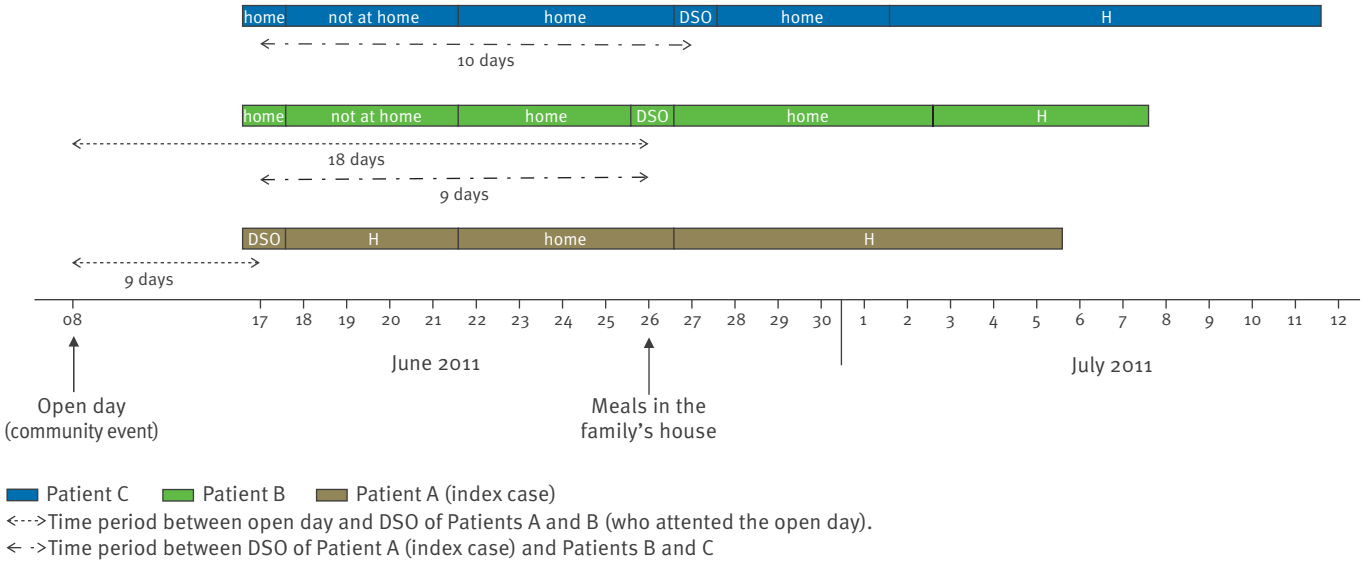
Two other relatives of Patients A, B and C shared meals in the family's house on 26 June. One of them stayed in the family's house between 29 June and 3 July and complained of severe fatigue. The other relative developed mild diarrhoea on 28 June. A rectal swab was performed for both with an O104 serology for the relative with severe fatigue. Both relatives' stool samples were negative for the presence of *E. coli* O104:H4.

Hypothesis of transmission

We hypothesised that Patients B and C both probably acquired HUS by secondary transmission from Patient A because they developed illness 9 and 10 days, respectively, after Patient A's symptom onset.

Figure

Two cases of probable household transmission of haemolytic uraemic syndrome due to *Escherichia coli* O104:H4, south-western France, June 2011



DSO: date of symptom onset; H: hospitalised (the day of discharge was not counted as day of hospitalisation).

Although Patient B attended the open day event, food-borne transmission from the sprouts to patient B is unlikely because she reportedly did not eat any sprouts. Moreover, an incubation period of 18 days would be unusually long. Both Patients B and C had spent time with Patient A on the day when he first experienced symptoms and after his return from hospital. During his first hospitalisation, his family did not stay in Bordeaux and had no contact with him.

A recent study by the German outbreak investigation team showed that the median incubation period of *E. coli* O104:H4 in that outbreak was eight days (interquartile range: 7–9 days) [3]. Therefore, two scenarios can be considered for the household described here: transmission on 17 June when Patient A first presented symptoms at home, or transmission between 22 and 26 June after his return from hospital. Between 22 and 26 June, patient A had symptoms of diarrhoea and severe fatigue; during this period, he had prepared some meals.

Recommendations for hygiene and infection control

Following the detection of the outbreak on 22 June 2011, recommendations for hygiene and infection control were disseminated, starting on 24 June, to the general population and to the participants of the open day through several press releases, the website of the Ministry of Health, and local physicians. Furthermore, following the probable household transmission described here, a letter was sent on 5 July to the participants of the open day. This letter stressed the importance of personal hygiene measures, and safe food preparation practices, to reduce the risk of transmission. No other secondary cases in connection with the community event have been reported to date.

Here, as in the household transmission of *E. coli* O104:H4 reported in the Netherlands [5], the index case was an adult. In our episode, one of the two secondary cases was also an adult. A review of 90 confirmed outbreaks caused by classical *E. coli* O157, showed that a lower median age of the index case was associated with a higher rate of secondary cases and that young children were most likely to become infected [6]. The unusual transmission from adult to adult observed in our episode is in line with the preponderance of cases in adults reported in the German outbreak [3]. This unusual pattern could be attributable to the specific properties of this strain [4,7]. Measures to prevent secondary transmission among adults should be strictly implemented.

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Agreement of patients

Written consent of patients to this report has been obtained.

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Secondary transmissions during the outbreak of Shiga toxin-producing *Escherichia coli* O104 in Hesse, Germany, 2011

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During the recent outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 in Germany most cases notified in the State of Hesse (6 million inhabitants) were linked to satellite clusters or had travelled to the outbreak area in northern Germany. Intensified surveillance was introduced to rapidly identify cases not linked to known clusters or cases and thus to obtain timely information on possible further contaminated vehicles distributed in Hesse, as well to describe the risk of secondary transmission among known cases. As of 2 August 2011*, 56 cases of haemolytic uraemic syndrome (HUS) including two fatal cases, and 124 cases of STEC gastroenteritis meeting the national case definitions have been reported in Hesse. Among the 55 HUS and 81 STEC gastroenteritis cases that met the outbreak case definition, one HUS case and eight STEC gastroenteritis cases may have acquired their infection through secondary transmission. They include six possible transmissions within the family, two possible nosocomial and one possible laboratory transmission. Our results do not suggest an increased transmissibility of the outbreak strain compared to what is already known about *E. coli* O157 and other STEC serotypes.

Introduction

On 19 May 2011, the public health authority of Frankfurt, Hesse, and the Robert Koch Institute (RKI), Germany´s national public health authority, were informed about clusters of cases of haemolytic-uraemic syndrome (HUS) in Frankfurt and Hamburg [1,2]. These were the first notified cases of an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) serotype O104:H4. Between 1 May and 20 July 2011, 727 HUS cases and 3,039 STEC cases with diarrhoea have been reported in Germany [3]. Epidemiological evidence suggested that STEC-contaminated sprouts were the vehicle of infection. Trace back studies carried out by the German Enterohaemorrhagic *E. coli* Task Force and authorities of Lower Saxony identified one sprout-producing farm in Lower Saxony (Establishment A) as being the most likely source of sprouts contaminated with STEC O104. In a second step, forward tracing established that all 41 case clusters identified at that time in Germany were linked to consumption of sprouts originating from Establishment A [4].

The outbreak strain has been microbiologically characterised in detail [5]: All outbreak strains investigated belonged to serotype O104:H4 and contained the stx_2 gene, encoding Shiga toxin 2 (Stx2). The *eae* gene, encoding adhesion intimin, and *astA*, encoding enteroaggregative *E. coli* Shiga toxin 1 (Stx1) were not present. All isolates displayed an extended-spectrum beta-lactamase (ESBL) phenotype.

The highest HUS incidences were reported from the northern German states of Hamburg, Schleswig-Holstein, Bremen, Mecklenburg-Vorpommern and Lower Saxony, the so-called northern German outbreak area. Aside from satellite clusters in Hesse and eastern North Rhine-Westphalia, most of the HUS cases from other states could be linked to travel-related exposure in the outbreak area [1].

Identified clusters in Hesse included patrons of cafeterias run by Company A and guests of two private parties. Sprouts served in the cafeterias and sprouts used in a salad brought by one of the guests from northern Germany to Party A were traced back to Establishment A. Foods supplied to Party B were prepared by a caterer who is likely to have acquired the infection through person-to-person transmission.

The first possible secondary cases were reported in Hesse at the end of May, and local public health authorities were requested on 1 June 2011 to systematically collect and report information on possible secondary cases to the Hessian state health office. Starting on 7 June, testing for the outbreak strain of stool samples from patients notified with STEC infection and from symptomatic household members of outbreak cases

was offered free of charge at the state health office and performed according to RKI recommendations [6]. The aim was to rapidly identify cases not linked to known clusters and thus obtain timely information on possible further contaminated vehicles distributed in Hesse, as well as to describe the risk of secondary transmission among known cases.

Here we present data on reported cases of STEC gastroenteritis and HUS in Hesse with symptom onset since 1 May 2011. We provide additional information on possible secondary outbreak cases and on cases without an epidemiological link to identified clusters or known outbreak cases.

Methods

Hesse is one of the 16 German states, with a population of 6.0 million, subdivided into 26 counties.

We extracted from the Hessian database for notifiable diseases all cases of STEC gastroenteritis and HUS meeting the national case definitions with disease onset on or after 1 May 2011. Data were extracted as of 2 August 2011 and further updates are to be expected. Disease onset was defined as the onset of diarrhoea, regardless of whether the HUS developed at a later date. We summarised data available at local public health authorities on exposures of cases, including possible epidemiological links to known cases or clusters, and on laboratory reports. For (possible) outbreak cases without epidemiological link (as defined below) we contacted the primary diagnosing laboratory or the national reference or consulting laboratories to obtain additional information on diagnostic tests done and their results.

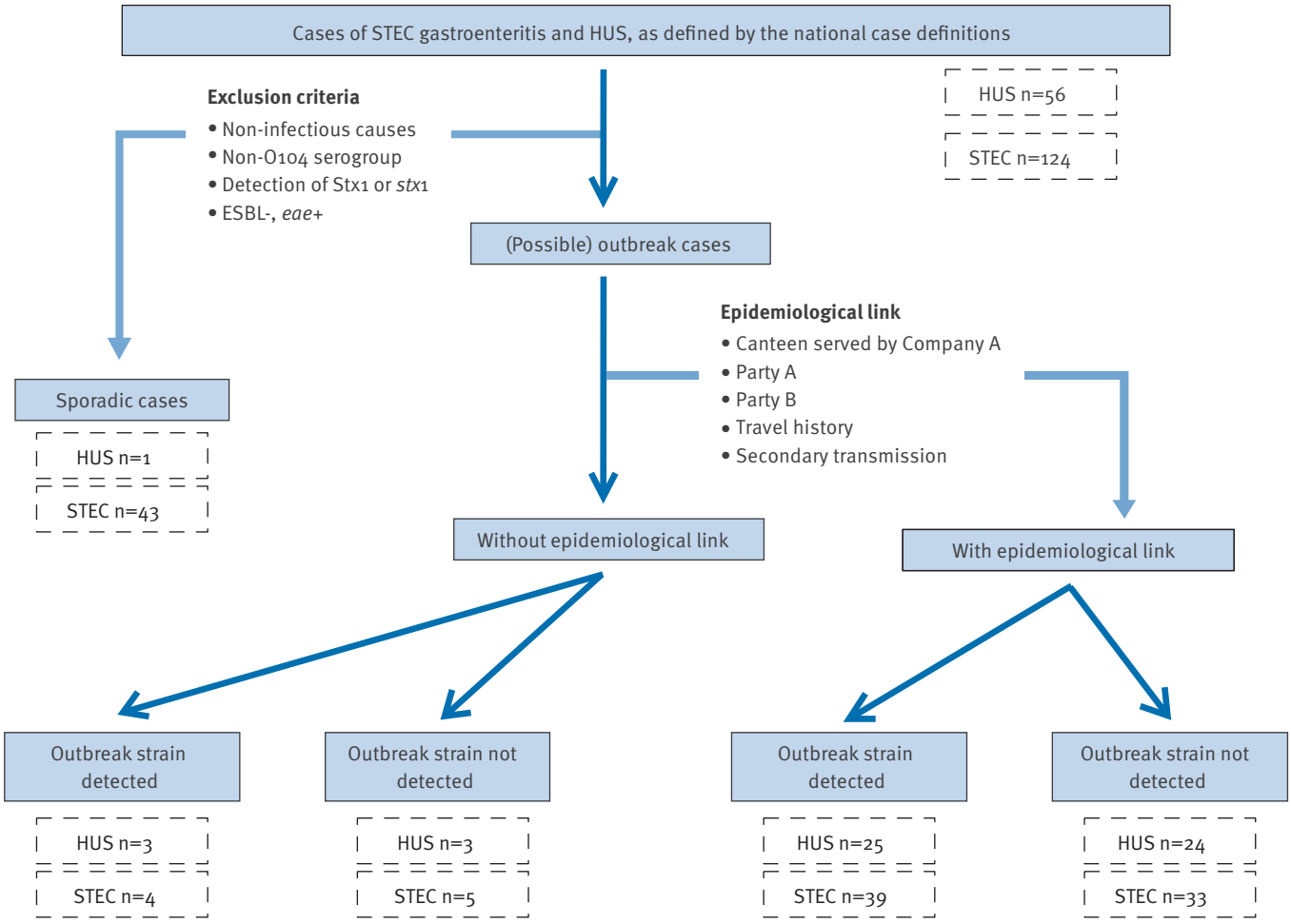
Data analysis was done with STATA (StataCorp LP, United States, version 11.2). For statistical comparisons the Mann-Whitney U test was used for age distribution and the Pearson chi-square test for proportions.

STEC gastroenteritis and HUS case definitions of the German surveillance system

According to the German Protection against Infection Act of 2001, the detection of a Shiga toxin (Stx) in *E. coli*

Figure 1

STEC gastroenteritis and HUS cases and criteria for sporadic cases and outbreak cases with or without epidemiological link, Hesse, Germany, 1 May–2 August* 2011 (n=180)



Eae: adhesion intimin; ESBL: extended-spectrum beta-lactamase; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing Escherichia coli; Stx: Shiga toxin.

isolates or of a Shiga toxin gene (*stx*) in stool enrichment culture or isolates must, by law, be reported by diagnosing laboratories to local health departments [2]. The German case definition of STEC gastroenteritis (without HUS) requires the presence of at least one of the following symptoms: diarrhoea (three or more loose stools in a 24-hour period), abdominal cramps, or vomiting in addition to a laboratory confirmation (as defined above) or an epidemiological link to laboratory-confirmed case. Physicians are required to report clinical symptoms compatible with diarrhoea-associated HUS in a patient. The German case definition of HUS comprises thrombocytopenia (platelet count of ‹150,000 per mm^3), haemolytic anaemia, and acute renal dysfunction [7]. Reported cases of HUS or STEC infection are investigated and recorded by the local health department and, if case definitions are met, the reports are forwarded electronically, without identifying information, through the state to the federal level.

Outbreak case definitions

For cases fulfilling the case definition for STEC gastroenteritis or HUS we further distinguished between sporadic cases and outbreak cases. To define sporadic cases we used a set of exclusion criteria based on laboratory results [8,9]: detection of a non-O104 serogroup, of Stx1 or its encoding gene stx_1, detection of *eae*, or of an *E. coli* strain not displaying an ESBL phenotype. Therefore, by definition, outbreak cases included possible outbreak cases, i.e. cases without any epidemiological link to known cases and for which the outbreak strain could not be detected in a stool sample (Figure 1).

Outbreak cases were considered epidemiologically linked if they were patrons of a canteen served by Company A, guests of Party A or B, if they had travelled to the northern German outbreak area during their incubation period or were linked to an STEC O104:H4-cluster outside Hesse, or if they were thought to have acquired their infection through secondary transmission. Secondary transmissions included contacts of epidemiologically linked persons as defined above and possible nosocomial and laboratory transmission.

For surveillance purposes, the RKI defined combinations of at least two laboratory results to be sufficiently specific for the outbreak strain [9]. For example, in case of detection of the stx_2 gene in an ESBL-positive isolate or detection of stx_2 gene and serotype O104, detection of the outbreak strain was assumed. The RKI requested all local public health authorities to interpret laboratory results and to forward reports accordingly.

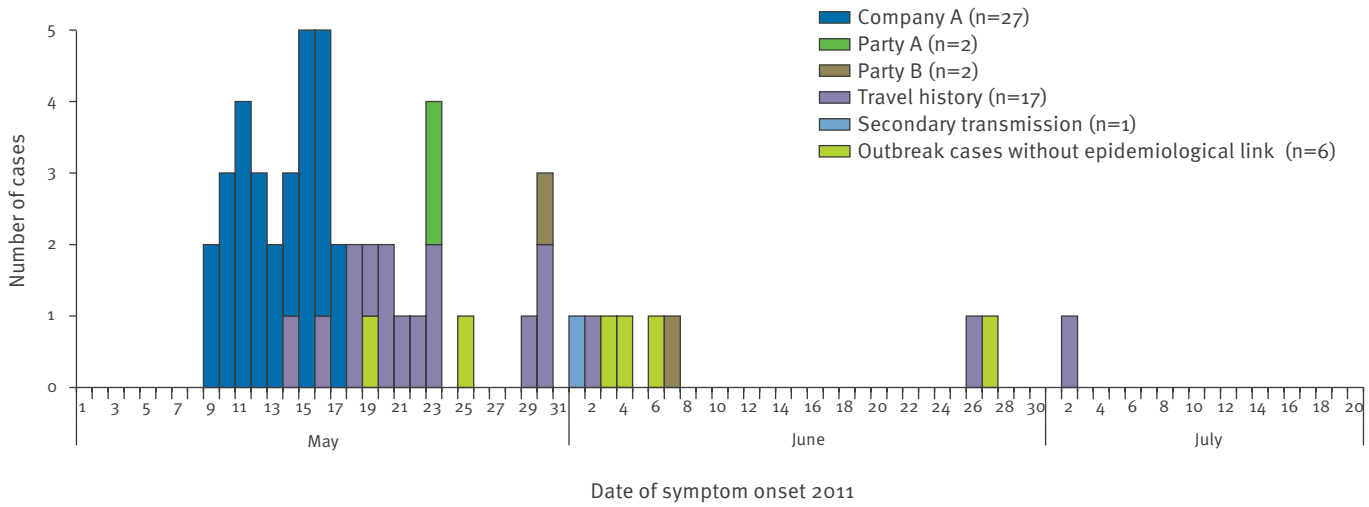
Results

As of 2 August 2011*, 56 HUS cases, including two fatal cases, and 124 STEC gastroenteritis cases meeting the national case definitions were reported in Hesse, with onset dates of 1 May or later (Figures 2 and 3). Of these, 55 HUS cases and 81 STEC gastroenteritis cases met the outbreak case definitions (Figure 1).

Among the 55 HUS outbreak cases, 49 were epidemiologically linked: 27 cases linked to Company A, two cases to Party A, two cases to Party B, 17 cases with travel history and one case of secondary transmission.

Figure 2

Epidemic curve of HUS cases meeting the case definition for (possible) outbreak cases, Hesse, Germany, 1 May–21 July 2011 (n=55)



HUS: haemolytic uraemic syndrome.

(Possible) outbreak cases include epidemiologically linked cases (canteen served by Company A, Party A and B, exposure history to the northern German outbreak area, secondary transmission) and epidemiologically unlinked cases. Date of hospitalisation was used when date of onset of diarrhoea was not available.

The age of epidemiologically linked HUS cases ranged from 21 to 75 years (median: 39 years). The six epidemiologically unlinked HUS cases were 5, 7, 41, 42, 64 and 73 years-old. Thirty-four of the 55 cases were female. The sporadic case was a four year-old girl with STEC O157 infection.

Among 81 STEC gastroenteritis outbreak cases, 72 were epidemiologically linked to the outbreak: 27 cases linked to Company A, no case at Party A, eight cases at Party B, 29 cases with travel history and eight cases with possible secondary transmissions. For 43 of the 81 cases the outbreak strain could be detected in a stool sample (Figure 1). Sporadic cases had symptom onsets from 3 May until 8 July 2011. For 15 of the 43 sporadic cases information on the identified serotype was available: five were serotype O157, three were serotype O91, and two were serotype 103. The median age of all patients reported to have STEC gastroenteritis was 44 years and did not differ significantly between outbreak and sporadic cases (44 and 42 years, respectively). Among the STEC gastroenteritis cases, 44 of 77 outbreak cases and 26 of the 43 sporadic cases were female. Information on sex was missing for four outbreak cases.

The outbreak strain was detected in stool samples of four of the nine epidemiologically unlinked STEC gastroenteritis cases. They are described below together with the unlinked HUS cases.

Cases with epidemiological link: possible secondary transmissions

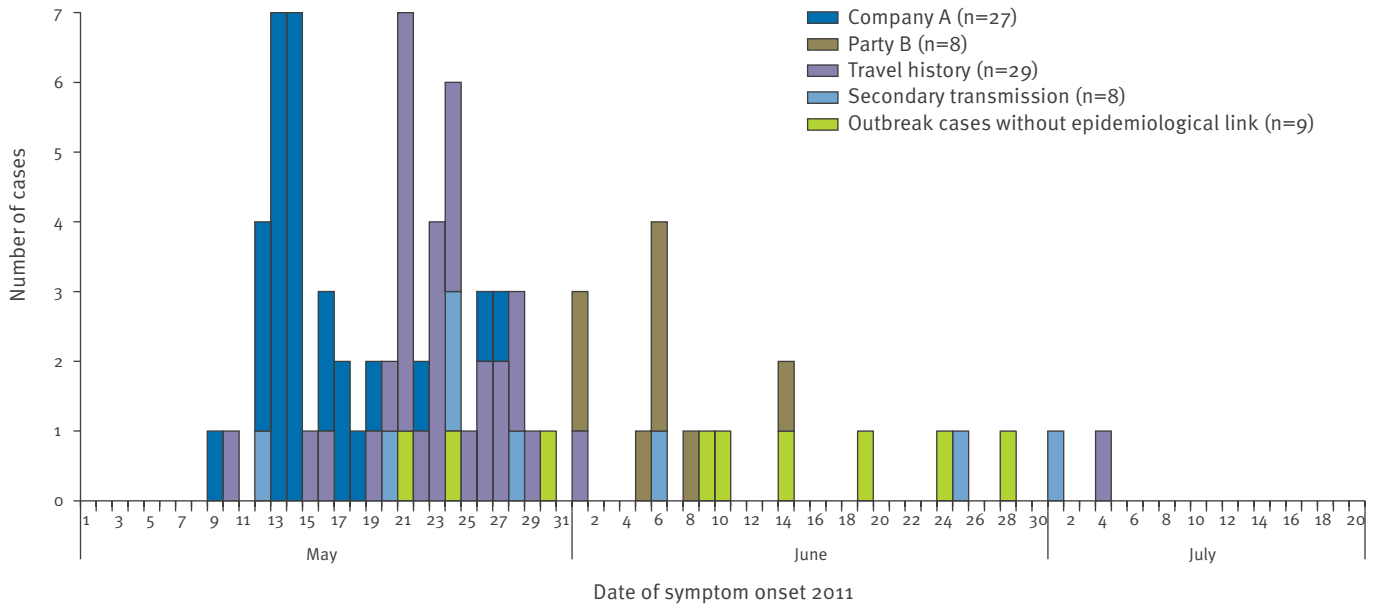
Among outbreak cases, eight of the 81 STEC gastroenteritis cases and one of the 55 HUS case were possible secondary cases. They included six transmissions within the family, two nosocomial and one laboratory transmission. The strength of the epidemiological and laboratory evidence linking these cases to their respective index cases or the known clusters differs. Therefore, these possible secondary transmissions are described in detail.

Family 1

On 24 May 2011, a woman in her 40s, whose husband had eaten at a cafeteria served by Company A, fell sick with bloody diarrhoea and stomach cramps. She was hospitalised on 26 May 2011 and subsequently the outbreak strain was isolated from a stool sample. On 27 May 2011, the local public health authority took stool samples from the husband, and the one and eight year-old children. Stool samples were tested in a private microbiology laboratory using broth enrichment culture for STEC and an ELISA test for Stx1/2. They were repeatedly negative for the husband and the eight year-old child. Stool samples of the one year-old child had a positive ELISA Stx1/2 result in all three samples. No further laboratory tests were done. The father reported light stomach pain but no diarrhoea on 18 May 2011 and for the one year-old child light non-bloody diarrhoea some time before symptom onset of the mother. No foods sold at the Frankfurt canteen were eaten by

Figure 3

Epidemic curve of STEC gastroenteritis cases meeting the case definition for (possible) outbreak cases, Hesse, Germany, 1 May–21 July 2011 (n=81)



STEC: Shiga toxin-producing Escherichia coli.

(Possible) outbreak cases include epidemiologically linked cases (canteen served by Company A, Party B, exposure history to the northern German outbreak area, secondary transmission) and epidemiologically unlinked cases. Date of hospitalisation was used when date of onset of diarrhoea was not available.

the mother and the two children and no travel to other outbreak areas was reported.

Family 2

A woman in her 30s fell ill on 12 May 2011 with bloody diarrhoea and was hospitalised on the same day. During her hospital stay she had a colonoscopy, which included clearing of the colon of solid matter. She was discharged on 14 May and subsequently readmitted on 23 May 2011. Starting on 25 May 2011, three stool samples were taken, but all tested negative for STEC. She and her husband had eaten meals at a canteen served by Company A during the two weeks before symptom onset. The husband and a two year-old child did not report any gastrointestinal symptoms. After being discharged from the hospital the family left for vacation and no further stool samples could be taken before their departure.

The woman's mother was hospitalised on 1 June 2011 for HUS. The outbreak strain was isolated from a stool sample. She had visited her daughter during her first hospital stay and taken case of her two year-old grandchild on five days from 16 to 25 May 2011. The grandmother attended the child in the household of her daughter, who reported having used a separate toilet.

The grandmother's husband fell ill with diarrhoea on 6 June 2011 and was hospitalised the following day. The outbreak strain was isolated from a stool sample. He had not visited his daughter or grandchild. He and his wife had not travelled to northern Germany.

Family 3

On 14 May 2011, a woman in her 40s living in an On 14 May 2011, a woman in her 40s living in an assisted accommodation became ill with bloody diarrhoea and was hospitalised for HUS on 17 May 2011. She had eaten meals prepared in a cafeteria served by Company A. Her mother, a woman in her 70s, assisted in caring for her in the first days after symptom onset. The mother fell sick with bloody diarrhoea on 28 May 2011 and was hospitalised on the same day. The outbreak strain was isolated from stool samples from both patients.

Family 4

A woman in her 20s became ill with bloody diarrhoea on 10 May and subsequently developed HUS. She had eaten meals from Company A during the two weeks before symptom onset. On 13 May 2011 she moved to the house of her mother, who took care of her until the daughter's hospitalisation on 15 May. The mother, in her 50s, developed bloody diarrhoea on 24 May 2011 and was hospitalised on 26 May 2011 for STEC gastroenteritis. The outbreak strain was isolated from stool samples from both patients.

Nosocomial transmission 1

A man in his 70s became ill with bloody diarrhoea on 28 May 2011. *E. coli* was isolated from a stool sample and confirmed as the outbreak strain at the national reference centre. The patient had been hospitalised from 12 to 16 May 2011 with a diagnosis of diverticulitis. He reported generally eating only at home. During the incubation period he had not eaten sprouts and not travelled to northern Germany. He did not know of any diarrhoeal illness among his family members or acquaintances or any link to known clusters or the outbreak-associated cafeterias. No further outbreak cases are known to have been hospitalised on the same ward or among the staff. However, given the long incubation period of the outbreak strain, nosocomial transmission cannot be excluded with certainty.

Nosocomial transmission 2

A woman in her 30s was hospitalised until 10 June 2011 for a neurological diagnosis unrelated to the STEC outbreak. She had had meals in a canteen served by Company A and became ill on 17 May 2011 with STEC gastroenteritis. Isolation precautions were followed in the hospital, given that at the time of hospitalisation she was a known asymptomatic carrier of the outbreak strain. Nevertheless, the patient once spread faeces on the ward during a delirious episode. A man in his 20s was an inpatient of the same ward on 9 and 10 June 2011. He continued to be hospitalised until 25 June 2011 when he developed bloody diarrhoea. An stx_2+, stx_1- *E. coli* of an ESBL phenotype was isolated from a stool sample and confirmed as the outbreak strain at the national reference centre.

Laboratory infection

A woman in her 20s fell ill with bloody diarrhoea on 1 July 2011. She had been in contact with the outbreak strain during the incubation period while working in a microbiology laboratory. The outbreak strain was isolated from her stool sample. She had not travelled to northern Germany and not eaten sprouts during the incubation period. She lives in an area without known outbreak clusters and had no known link to Company A or the two private parties.

Cases without epidemiological link

Among outbreak cases, nine of the 81 STEC gastroenteritis cases and six of the 55 HUS cases had no epidemiological link to known clusters or possible secondary cases (Figures 1–3). Among epidemiologically unlinked outbreak cases, the outbreak strain was detected in stool samples of three HUS and four STEC gastroenteritis cases. The three HUS cases fell ill on 19 and 25 May and 6 June 2011. They were 7, 42 and 73 years-old. None of them had recently travelled to northern Germany or had any known contact to outbreak cases or known clusters. Only one of them reported having eaten sprouts once during the incubation period. The four STEC gastroenteritis cases with the outbreak strain detected in a stool sample fell ill on 21 May, and on 9, 24 and 28 June 2011. They were 10, 24, 32 and 55 years-old. None of them reported having eaten sprouts, any recent travel to northern Germany or known contact to outbreak cases or known clusters. Of the seven epidemiologically unlinked cases with the outbreak

strain detected in a stool sample, five live (four cases) or work (one case) in the two cities in Hesse with the highest incidences of outbreak cases.

Discussion

As of 2 August 2011, a total of 55 HUS and 124 STEC gastroenteritis outbreak cases have been reported in Hesse. Among these cases, at least nine cases may have acquired their disease through transmissions within the family, nosocomial or laboratory transmission. These nine cases are a minimum estimate of possible secondary transmissions. Given the long incubation period of this pathogen (median eight days) [2], distinction between co-primary cases and secondary transmission is difficult for family members with a common exposure history. Whenever we were unable to distinguish between co-primary and secondary person-to-person transmission, cases were categorised as co-primary, i.e. epidemiologically linked to the northern German outbreak area, cafeterias served by Company A, or to Party A or B. Therefore, while misclassification of secondary cases as co-primary cases is possible, we know of no cases that occurred more than 10 days apart among family members linked to the northern German outbreak area or the two private parties. In addition, risk of secondary transmissions within Hesse may have been reduced if travel-associated cases became sick and were hospitalised while still on travel outside Hesse. It has previously been shown that hospitalisation of STEC cases reduces the risk of household transmissions [10,11].

All six transmissions within the family described here were linked to Company A. Three of these six transmissions occurred among non-regular household members, i.e. family members who had moved in temporarily to provide or receive assistance during sickness. Many cases linked to cafeterias served by Company A live in small households of one or two persons and without children. This may have contributed to limiting the number of secondary cases, especially among children. The presence of siblings and the young age of the index cases has been associated with increased transmission risk [10-12], and transmissions between families have been described previously in outbreak settings [13].

While the outbreak strain was present in stool samples of five secondary cases within families, it could only be detected in stool samples of three of the four respective index cases. In Family 1, the index case – who had eaten at a cafeteria served by Company A – reported only light stomach pain for one day during the two weeks before symptom onset of his wife. It remains unclear if the stomach pain was related to an STEC infection, if he had an asymptomatic STEC infection, or if – in our view less likely – he was not infected. The first cases linked to Company A fell ill on 9 May 2011 and the index case's stool samples may have become negative by the time they were first tested (on 27 May). For non-outbreak STEC infections, identification in patient´s faeces late in the illness has been shown to be difficult [14].

Secondary transmissions frequently occur in outbreaks caused by *E. coli* O157 [15] and have been described to occur in 4–15% of households following sporadic infection [11]. In a population-based study in Scotland, 11% of O157 cases were identified as secondary [12]. In addition, nosocomial and laboratory-acquired infections with *E. coli* O157 have been reported [16,17]. They underline the need for strict adherence to standard infection control precautions [18].

Several episodes of secondary transmissions and asymptomatic carriage have already been described for the recent O104 outbreak in Germany [19-21]. In the three instances where we could calculate a serial interval, the time span between symptom onset of primary and secondary cases was 14, 14 and 20 days, confirming previous reports for the outbreak strain [21] and for *E. coli* O157 [10] that, considering the relatively long incubation time for the outbreak strain, household transmission occurs early during disease.

We have here described seven epidemiologically unlinked cases for whom the outbreak strain could be detected in a stool sample. Several possibilities may explain these seven cases: (i) our definition of outbreak strain may have been too generic, i.e. the *E. coli* strains identified may not have been outbreak strains, (ii) these infections may have been acquired from foodborne transmission, and (iii) secondary transmission. Several of the laboratory results for the seven epidemiologically unlinked cases have been confirmed by the national reference and consulting laboratories (while others are pending) and we have no further evidence suggesting that contaminated foods were circulating in Hesse outside the identified clusters. Direct or indirect secondary transmission among non-close contacts may therefore be the most likely explanation for most of these seven cases.

We extracted from the Hessian database for notifiable diseases only data on cases meeting the national case definitions for STEC gastroenteritis and HUS. Data cleaning and analysis on asymptomatic cases and on cases with symptoms not meeting the national case definition (e.g. only one episode of loose stool) from the restaurant outbreaks is still ongoing.

From 3 May to 8 July 2011, 43 non-outbreak cases of STEC gastroenteritis were reported in Hesse. In comparison, during the five-year period from 2006 to 2010, only 76 cases of STEC gastroenteritis were reported. Serogroup O157 was the most commonly detected serogroup in the European Union in 2008 and 2009, representing about 52% of STEC cases with known serotypes [22]. Reported cases represent a subset of infections in the community [23] and testing for STEC infection increased considerably during the outbreak. Therefore, in outbreak settings, the timely distinction

between sporadic and outbreak cases is important in orienting further investigations and control measures of public health and veterinary authorities.

The current outbreak strain is a very rare serogroup in humans in Europe and worldwide [22]. This and its other unique characteristics may be part of the reason why the possible nosocomial and laboratory infections were identified and why we considered the unlinked cases as probable secondary transmissions. Adult age of index patients and transmission among non-regular household members are particular characteristics of the described secondary transmissions. They should not be interpreted as indicative of a particular high transmissibility of the outbreak strain. The majority of the transmissions involve patients residing in different counties. We believe that the particular characteristics of the outbreak strain together with the structure of the German surveillance system (including local and state levels and a national level) facilitated the identification and description of possible secondary transmissions.

In conclusion, the outbreak strain can be easily transmitted but our preliminary results do not suggest an increased transmissibility of the outbreak strain compared to what is already known about *E. coli* O157and other STEC serotypes.

*Authors' correction:

On request of the authors the phrase "As of 21 July" was changed to "As of 2 August" in the abstract and the first sentence of the results. This date was also corrected in the title of Figure 1. These changes were made on 1 Sept 2011.

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Usutu virus – potential risk of human disease in Europe

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Usutu virus (USUV) is an African mosquito-borne flavivirus, member of the Japanese encephalitis antigenic group. This avian virus is transmitted by arthropod vectors (mainly mosquitoes of the *Culex pipiens* complex). It is well known that free-living birds, including migratory species, have the potential to disperse certain pathogenic microorganisms. Usutu virus has recently been introduced to Europe and is spreading through Austria, Hungary, Italy, Spain and Switzerland, causing disease in birds and humans. Like West Nile virus, USUV may become a resident pathogen in Europe and the consequences for public health should be considered. Many different biotic and abiotic factors affect the survival of the virus in a new environment and influence the efficiency of its geographical dispersal. In this article, we consider the possibility of including USUV infections among the vector-borne diseases to be monitored in Europe.

Background

Usutu virus (USUV) is an African mosquito-borne virus of the family *Flaviviridae*, genus *Flavivirus*, belonging to the Japanese encephalitis serocomplex [1]. From an ancestral *flavivirus* with a bird/mosquito natural cycle evolved the different flaviviral species present today, such as USUV and West Nile virus (WNV) in Africa, Asia and Europe, Japanese encephalitis virus (JEV) in Asia, Murray Valley encephalitis virus in Australia and Saint Louis encephalitis virus in the American continent. USUV was originally isolated from a mosquito (*Culex neavei*) in 1959 in South Africa. Further USUV strains were detected from different bird and mosquito species in Africa in subsequent years, but human disease (rash and fever) has only been reported once, in the Central African Republic [2,3]. In the past, USUV was not considered as a potential threat for humans because the virus had never been associated with severe or fatal diseases in animals or humans, and it had never before been observed outside tropical and subtropical Africa.

Avian, horse and vector surveillance

In the summer of 2001, USUV emerged in Austria, causing deaths in several species of resident birds, especially among birds of the order *Passeriformes* [4-6]. In the following years, the virus has been detected in dead birds and/or mosquitoes in several countries, including Hungary (2005) [7], Italy (2009) [8], Spain (2006 and 2009) [9,10] and Switzerland (2006) [11]. USUV infection has also been demonstrated serologically in wild bird hosts in the Czech Republic (2005) [12], England (2001–2002) [13], Germany (2007) [14], Italy (2007) [15], Poland (2006) [16], Spain (2003–2006) [17] and Switzerland (2006) [18] (Figure). The recurrence of the virus over several years in Austria (2001–2006) [19], Hungary (2003–2006) [7], Italy (2006–2008) [8] and Spain (2006, 2009) [9,10] suggests either frequent reintroduction of the virus or, more likely, persistence of the transmission in the affected areas, possibly through overwintering mosquitoes. Comparisons of pathologic alterations revealed similar lesions in birds infected in the Austrian, Hungarian, Italian and Swiss USUV outbreaks, and these findings were supported by partial nucleotide sequence analysis with >99% identity between the viruses which emerged in Vienna in 2001, in Budapest in 2005, and in Zurich and Milan in 2006. A one-time introduction of USUV from Africa to Europe (Vienna) is therefore highly likely, and this particular strain has since been spreading in Central Europe [11]. However, a two-year study carried out in 2008 to 2009 in Italy to monitor the USUV circulation within the West Nile Disease (WND) national surveillance plan suggests a different scenario [20]. In that work, sentinel horses and chickens, wild birds and mosquitoes were sampled and tested for serological and virological evidence of USUV. Seroconversion in sentinel animals proved that the virus had circulated in Italy in these two years. In addition, the study demonstrated USUV infection in horses for first time in Europe. Sequence comparison of USUV detected from different species in different counties showed that two different strains of USUV are likely to have circulated in Italy between 2008 and

2009, and these strains have adapted to new hosts and vectors to become established in new areas.

Recent human cases and clinical characteristics

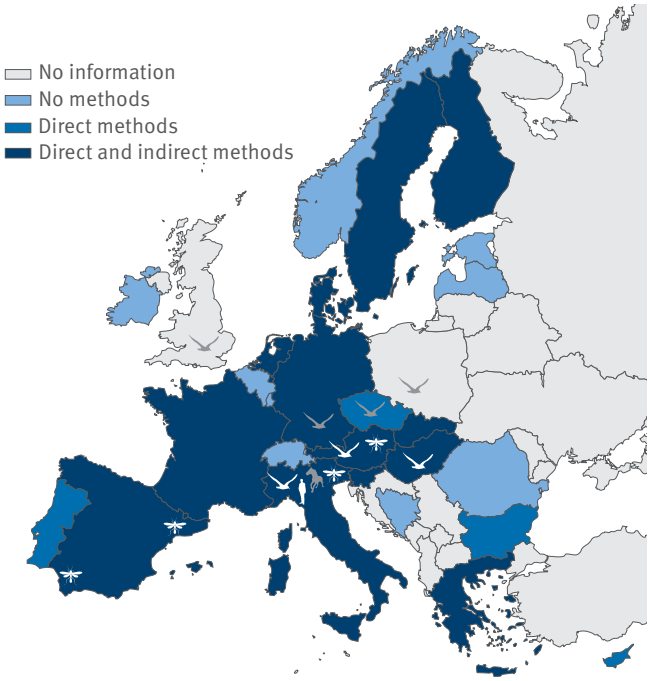
In the end of the summer 2009, the virus was associated with neurological disorders in two immunocompromised patients (both had received blood transfusions) in Italy [21,22]. In addition, USUV was isolated from the blood obtained from one of these subjects during the acute stage of disease. The patients were detected concurrently with the active surveillance programme of blood and organ donations that the public health authority of the Emilia Romagna region had initiated in August 2008, based on several veterinary and entomological reports of WNV circulation in north-eastern Italy [23]. The two infections could be consistent with local transmission, either directly through a mosquito bite or indirectly through an infected donor. Both patients had in common that they were immunosuppressed and had received blood transfusions in the same period of time (August 2009). As transmission for WNV through blood products and transplantation has been documented [24,25], screening for WNV was performed of blood samples and organ donations from 15 June to 31 October, with negative results. The two patients were the first human cases of USUV neuroinvasive illness described worldwide. The common clinical symptoms were persistent fever of 39.5 °C, headache and neurological disease (impaired neurological functions). One patient developed a fulminant hepatitis, a pathology that had been described previously in rare cases of WNV infection [26,27]. In both patients, the clinical picture was similar, with a clear involvement of the central nervous system, resembling the related WNV neuroinvasive disease. Whether this new tropism was associated with new characteristics of the infecting viruses, with a possible inoculation route through transfusion, and/or to the underlying diseases of the patients still remains unclear, but these findings reinforce the need for further investigations. The partial sequences obtained from cerebrospinal fluid (CSF) and plasma samples of these patients were more than 98% identical with the viruses that had emerged in Vienna and Budapest (in 2001 and 2005, respectively) [21,22]. In a recent phylogenetic study of sequences of USUV strains obtained in Italy in 2009 from mosquitoes, birds and humans, the sequences obtained from human hosts clustered with the sequences obtained from birds, which would indicate an endemic distribution of USUV in Europe [20].

Diagnostics

Clinical suspicion of USUV infection requires laboratory confirmation. Within laboratory methods, we can distinguish between direct methods (detecting the virus by cell culture or genomic amplification) and indirect methods (detect the antibody response to the infection). Serological diagnosis of USUV infections in humans will require an approach similar to the one used for WNV. Although there is a lack of experience about USUV infection in humans, it is assumed that its incubation period will be two to 14 days, that USUV will be detectable in CSF and serum in the acute stage of the disease, and that IgM antibodies will appear five days after onset of fever, in analogy to the current knowledge about the pathogenesis of WNV-related illness in humans. Antibodies may persist in serum for many months after infection [28]. Diagnosis of USUV will not be easy, particularly in areas where circulation along with others cross-reacting *flaviviruses* occur. That is the case for WNV and tick-borne encephalitis virus in several European countries [29]. Until more specific diagnostic methods are developed and made available for diagnostic laboratories, antibody detection could be carried out using cross-reacting ELISA methods designed for WNV diagnosis. It is also expected that cross-reactivity will be higher for IgG than for IgM detection; consequently, development of tests for USUV-specific IgM is needed more urgently. As an already available alternative, acute and convalescent sera should be tested for seroconversion of IgG antibodies using in-house or commercial ELISA tests based on WNV antigens. Cross-reactions can be resolved by parallel titrations against various *flaviviruses* in assays for neutralising antibodies, which are more specific

Figure

Diagnostic capacities for Usutu virus in European countries in the ENIVD network and detection of the virus in mosquitoes, birds, horses and/or humans



ENIVD: European Network for Diagnostics of Imported Viral Diseases.

Colour code indicates diagnostic capacities: direct methods detect the virus by cell culture or genomic amplification, indirect methods detect the antibody response to the infection.

Animal symbols indicate detection of Usutu virus in these species: geographical distribution is indicated either by virus detection (species in white) or by evidence of neutralising antibodies (dark grey).

than ELISAs but can be performed only in specialised laboratories that can handle hazardous viruses [30].

The possibility of USUV to infect and cause severe neurological syndromes in humans makes it necessary to develop new affordable and rapid molecular methods for its detection. Recently, a specific real-time RT-PCR assay has been developed to identify USUV in human plasma, serum and CSF samples. This technique has allowed the detection of USUV in three CSF specimens that were collected in the summers of 2008 and 2009 from 44 patients with suspected meningoencephalitis and were negative for WNV [31]. However, serological testing is still needed and important to identify infection after the viraemic stage. In Europe, most of the countries are prepared for detecting USUV genome in human or bird samples (Figure), generally using cross-reactive or generic methods for detecting *flavivirus*es. More specific techniques are required, especially for those countries with direct evidence for WNV and/or USUV circulation (Austria, Belarus, Bulgaria, Czech Republic, France, Hungary, Italy, Moldova, Portugal, Romania, Russia, Slovakia, Spain and Ukraine) [32], and new methods are being designed to identify and distinguish USUV from other arboviruses, particularly from members of the JEV group that have been circulating in Europe [31,33]. In fact, a false-positive result of a WNV RT-PCR was reported in Italy in 2009 in a patient with viraemia caused by USUV [23].

Surveillance and control

The number of recent notifications of mosquito-borne diseases in the European Union in 2010 is a reason for concern. These events involved different types of pathogens like WNV, USUV, dengue virus, chikungunya virus and *Plasmodium* sp, some of which are considered typical for tropical areas. This current situation triggered a request from the European Commission for a risk assessment [34]. The overall objective of this consultation was to acquire a comprehensive understanding of the transmission potential for mosquito-borne diseases in Europe in order to propose recommendations for preparedness actions. The final conclusion was to develop a tool for decision making in WNV infection preparedness and control, which would guide countries through the complexities of responding to any alerts or outbreaks of this disease.

In Europe, WNV re-emerged in Romania, where it was first associated to neurological disease [35]. Since then, the virus has been detected with increasing activity in several European countries [36], including Italy, where it was circulating at least in 2008 and 2009, with eight and 16 human cases, respectively, of West Nile neuroinvasive disease [37]. Because of WNV circulation in Italy with neuroinvasive cases in humans and horses [38,39], a regional surveillance plan was implemented starting from 2008 [40]. Thanks to, these WNV surveillance activities antibodies against WNV and USUV were detected in Italy in 2009 in sentinel animals (horses and chickens), wild birds and provided evidence of cocirculation of WNV and USUV in mosquitoes and birds in the same area [20,41,42].

That five human USUV infections have recently been detected in areas where an effective surveillance for WNV exists, suggest that this disease may also be under-recognised in some other areas where the surveillance for WNV is lacking or poorly implemented. Both viruses seem to be able to cause neurological disease in humans under certain circumstances. The emergence of USUV in Europe, even if not presently considered a major threat warrants the enhancement of surveillance plans for neuroinvasive illness during the summer season, corresponding to the peak of activity of potential vectors. The extension of surveillance to *flavivirus*es other than WNV will require new diagnostic procedures and the development of more specific serological tests that can be used in the field [42]. As WNV and USUV viruses share many eco-epidemiological and virological characteristics, WNV surveillance programmes could be easily adapted to survey also USUV in birds, horses, mosquitoes and human samples. This approach should be based on the development of adequate and standardised differential laboratory diagnosis using validated methods (serological and molecular) enabling the differential detection of WNV and USUV infections, especially in those countries with demonstrated co-circulation of both viruses (at least Austria, Hungary, Italy, and Spain). A specific real-time RT-PCR assay to identify USUV in human plasma, serum, and CSF that has been developed [31] is very helpful for donor screening and diagnostics. Some of the molecular techniques designed to detect WNV can also amplify the signal for USUV due to false positive results by lack of specificity in the technique.

A surveillance programme for USUV in Europe could be very similar to national surveillance systems for WNV that are already implemented in some countries in Europe. In fact, in those European countries which have implemented a national WNV surveillance plan, this could be used in parallel for USUV surveillance. These programmes consist of human, veterinary and entomological surveillance. The objective of passive and active human surveillance systems would be the early detection of infection in humans. This activity should be performed by serology and/or detection of the viral genome in blood and cerebrospinal fluid from all suspected cases suffering from acute meningoencephalitis. In this regard, it would be important to raise the awareness of clinicians for this emerging disease, which may improve the sensitivity of the surveillance system. Since the diagnosis of encephalitis is of general importance, the inclusion of USUV diagnostics for differential diagnosis in cases of unknown origin should be considered for extended screening of aetiologies. Key requirements for a possible future surveillance study at European level have already been suggested [30]. Animal surveillance should be performed on the basis of passive and active surveillance of horses and non-migratory wild birds. Entomological

surveillance should be based on the weekly to monthly (frequency depending on local resources) collection of mosquitoes in fixed stations and at sites where USUV activity has been demonstrated ascertained in birds, humans or horses.

As suggested by Chvala et al. [5], mosquito monitoring and screening of wild birds are suitable to detect USUV circulation and could replace surveillance of dead birds when bird mortality drops because of herd immunity. Although virological surveillance (with molecular techniques) may be preferable over serological monitoring because it avoids cross-reactions with other flaviviruses, they are impeded by short-lived viraemia, when serology is still possible due to long-lasting serum antibodies. Sera reacting to both WNV and USUV were detected in other studies using tests with low specificity such as haemagglutination inhibition [19] or ELISA [15]. Plaque reduction neutralisation has to be performed to confirm positive sera, but this test is complex, costly, time-consuming and not accessible for laboratories lacking high biocontainment facilities.

As for WNV, surveillance of wild birds and vectors will be used in the coming years to forecast the spread of USUV. The information gathered will be used to develop actions to prevent virus transmission, such as vector monitoring and control, information campaigns to improve personal protection, and screening tests for donor blood, tissue and organs.

Conclusions

In Europe the risk exists that potential emerging infectious diseases, such as those caused by WNV or USUV, will not be recognised in time by existing surveillance infrastructures of the various European countries [43]. As treatments for USUV and WNV are not available, there is a need to strengthen surveillance. Circulation of USUV in Austria, Hungary, Italy and Spain during consecutive years and seroconversions reported recently in sentinel animals and detection of virus in wild birds in Italy, show that these territories are suitable to support USUV circulation between vectors and vertebrate hosts, as well as overwintering, enabling the establishment of endemic cycles. This indicates a need to organise standard surveillance measures and early warning systems to detect WNV and USUV activity, and to assess the risk for public health. Establishing a European surveillance system by grouping the existing resources and introducing a standardised reporting and diagnostic system is essential for future preparedness and response. This surveillance system should be sensitive and able to detect USUV and WNV circulation at an early stage. A multidisciplinary approach should be considered when evaluating the risk of USUV and WNV transmission, and the contribution of the different components (mosquitoes, birds, horses, humans) should be carefully assessed.

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