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Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011

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Since early May 2011, an increased incidence of haemolytic uraemic syndrome (HUS) and bloody diarrhoea related to infections with Shiga toxin-producing *Escherichia coli* (STEC) has been observed in Germany, with most cases in the north of the country. Cases reported from other European countries had travelled to this area. First results of a case–control study conducted in Hamburg suggest an association between the occurrence of disease and the consumption of raw tomatoes, cucumber and leaf salad.

An unusually high number of cases of haemolytic uraemic syndrome (HUS) has been observed in Germany since early May 2011. This report presents the preliminary results of the investigation as of 26 May 2011

Haemolytic uraemic syndrome (HUS) is a serious and sometimes deadly complication that can occur in bacterial intestinal infections with Shiga toxin (syn. verotoxin)-producing *Escherichia coli* (STEC/VTEC). The complete clinical picture of HUS is characterised by acute renal failure, haemolytic anaemia and thrombocytopenia. Typically it is preceded by diarrhoea, often bloody. Each year, on average 1,000 symptomatic STEC-infections and approximately 60 cases of HUS are notified in Germany, affecting mostly young children under five years of age [1]. In 2010 there were two fatal HUS cases [1].

STEC are of zoonotic origin and can be transmitted directly or indirectly from animals to humans. Ruminants are considered to be the reservoir, especially cattle, sheep and goats. Transmission occurs via the faecal-oral route through contact to animals (or their faeces), by consumption of contaminated food or water, but also by direct contact from person to person (smear infection). The incubation period of STEC is between two and 10 days, the latency period between the beginning of gastrointestinal symptoms and enteropathic HUS is approximately one week.

Outbreak description

The Table lists the number of cases of HUS or suspected HUS notified to local health departments and communicated by the federal states to the Robert Koch Institute (RKI). Suspected HUS are included as the syndrome is a process and suspected HUS typically develops over the course of a few days into the full clinical picture.

Disease onset (regarding diarrhoea) in the 214 patients detected so far was between 2 and 24 May 2011. A total of 119 (56%) of the cases were communicated from four northern federal states (Hamburg, Schleswig-Holstein, Lower Saxony and Bremen). The highest cumulative incidence has been recorded in the two northern city states of Hamburg and Bremen. An additional 31 cases occurred in Hesse. They were connected to a catering company supplying the cafeterias of a company and a residential institution. It is likely that these cases constitute a satellite outbreak.

Besides the geographic clustering, the age and sex distribution of the cases is conspicuous: Of the 214 cases, 186 (87%) are 18 years of age or older (mostly young to middle-aged adults) and 146 (68%) are female. In the notification data for HUS cases from 2006 to 2010, the proportion of adults lay between 1.5% and 10% annually, and the sexes were affected equally.

Cases linked to this outbreak were also communicated from other European countries: On 25 May 2011, Sweden reported through the European Warning and Response System (EWRS) nine cases of HUS, four of whom had

travelled in a party of 30 to northern Germany from 8 to 10 May. Denmark reported four cases of STEC infection, two of them with HUS. All cases had a recent travel history to northern Germany. Another two HUS cases with travel history to northern Germany in the relevant period were communicated, one each by the Netherlands and by the United Kingdom.

So far two German HUS cases have died of the disease (both female, one in her 80s, one in her 20s) .

Laboratory investigations

Investigations at the National Reference Centre for Salmonella and other bacterial enteric pathogens at the RKI (Wernigerode) of isolates from two patients from Hesse and Bremerhaven suggests that the outbreak strain is an *E. coli* strain of serotype O104 with the following characteristics: Shiga toxin 2 (*vtx2a*, EQA nomenclature 2011, WHO Centre *E. coli* SSI Copenhagen)- producing, intimin (*eae*)-negative and enterohaemolysin (*hly*)-negative. The strain shows a high resistance to third generation cephalosporins (through extended spectrum beta-lactamases, ESBL, CTX-M-type), and a broad antimicrobial resistance to, among others, trimethoprim/sulphonamide and tetracycline.

Table

Cases of HUS and suspected HUS with onset of diarrhoea since 2 May 2011, Germany (n=214)

Federal State	Number of HUS cases and suspected-HUS cases	Cumulative incidence (per 100,000 population)
Hamburg	59	3.33
Bremen	11	1.66
Schleswig-Holstein	21	0.74
Mecklenburg-Vorpommern	10	0.61
Hesse	31	0.51
Saarland	5	0.49
Lower Saxony	28	0.35
North Rhine-Westphalia	31	0.17
Berlin	3	0.09
Baden-Württemberg	8	0.07
Bavaria	5	0.04
Thuringia	1	0.04
Rhineland-Palatinate	1	0.02
Brandenburg	0	0.00
Saxony	0	0.00
Saxony-Anhalt	0	0.00
Total	**214**	**0.26**

HUS: haemolytic uraemic syndrome.

Data as of 26 May 2011, 8am, communicated to the Robert Koch Institute by the federal states.

A further 13 isolates from Muenster, Paderborn, Hamburg and Frankfurt were analysed in the consulting laboratory for haemolytic uraemic syndrome in the Institute of Hygiene at the University hospital in Muenster. All were sequence-typed as ST678 (*stx1-, stx2+, eae-*, flagellin-coding gene *flicH4*), group HUSEC 41, also indicating serotype O104 [2,3]. Whether these results reflect the entire situation in Germany needs to be confirmed by the analysis of a greater number of isolates. As in the past most outbreaks of HUS in Germany and elsewhere were found to be connected with STEC O157 strains, the identification of serotype O104 in this context is highly unusual, although, *E. coli* O104 has previously been described as the cause of an outbreak in the United States in 1994 [4].

Investigation into the source of infection

The large number of persons suddenly affected, the geographical and demographic distribution as well as first interviews of patients suggested STEC-contaminated food as the vehicle of infection. Foods like raw milk and raw meat, which were identified as vehicles in former STEC outbreaks, appear not to be related to the current event. Preliminary results of a case–control study conducted by the RKI and the Hamburg health authorities demonstrate a significant association between disease and the consumption of raw tomatoes, cucumbers and leafy salads. This study collected food histories for the week before symptom onset for 25 patients hospitalised with HUS (n=20) or bloody diarrhoea with laboratory-confirmed STEC infection (n=5), who all had onset of disease between 9 and 25 May 2011. In addition, 96 controls matched by age, sex and residence were asked about their food consumption during the week before the interview. The food items they were asked about were those frequently mentioned in previous in-depth interviews of HUS cases. Consumption of each of the named food items was reported by around 90% of the cases in comparison to around 60% of the controls, yielding odds ratios between around 4 and 7, all statistically significant. Nevertheless it is possible that another or an additional food item is the source of infection. The results cannot necessarily be transferred to the whole of Germany because the study was limited to Hamburg.

Regarding the source of the suspicious food items the study showed a heterogeneous picture. It can be excluded that the source is a single shop or restaurant. Based on these findings, food trace-back investigations are currently ongoing.

Evaluation of the situation

The current events represent one of the largest described outbreaks of HUS/STEC worldwide and the largest in Germany, with a very atypical age and sex distribution of the cases. Incident cases of HUS or suspected HUS are continuing to be reported at least in Northern Germany, where the emergency room consultations for bloody diarrhoea remain elevated. Thus it has to be assumed that the source of infection is still

active. Many patients with bloody diarrhoea need to be admitted to hospital, and HUS patients often need intensive care with dialysis and/or plasmapheresis, which puts a severe strain on hospital resources in some areas. The epidemiological studies that were conducted in cooperation with regional and local health departments rapidly delivered important clues as to certain food items that could be linked to the outbreak. Further epidemiological studies, laboratory investigations and trace back of food items is needed to confirm these results and to narrow down the source of infection.

Recommendations for consumers and patients

Considering the ongoing outbreak that included many cases with a severe course of disease, the RKI and the Federal Institute for Risk Assessment (BfR) recommend to abstain from consuming raw tomatoes, cucumbers and leafy salads, especially in northern Germany, until further notice. Regular food hygiene rules remain in effect [5].

For persons with diarrhoea the importance of strict hand hygiene is emphasised. Patients with bloody diarrhoea should seek medical aid immediately. Physicians are reminded to initiate STEC stool diagnostics for these patients and to closely monitor them for the development of HUS. Patients suspected of developing HUS should be referred to appropriate stationary care.

Diagnostic laboratories are requested to send STEC isolates to the National Reference Centre for Salmonella and other bacterial enteric pathogens. The Protection Against Infection Act of 2001 renders both the laboratory confirmation of an STEC infection and the clinical diagnosis of HUS or suspected HUS notifiable to the local health department.

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False-negative results using *Neisseria gonorrhoeae porA* pseudogene PCR - a clinical gonococcal isolate with an *N. meningitidis porA* sequence, Australia, March 2011

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The gonococcal *porA* pseudogene is a popular target for in-house *Neisseria gonorrhoeae* PCR methods. With this study we present two novel findings: the first case of an *N. gonorrhoeae porA* pseudogene PCR false-negative result caused by sequence variation, and in the same organism, the first description of a clinical *N. gonorrhoeae* strain harbouring an *N. meningitidis porA* sequence.

In this report, we describe the first case of a *Neisseria gonorrhoeae* false-negative test result using an *N. gonorrhoeae porA* pseudogene PCR method, caused by sequence variation. Nucleic acid amplification tests (NAATs) are widely used for the detection of gonorrhoea, yet there are challenges for *N. gonorrhoeae* NAATs because of the considerable sequence variation and genetic exchange that is exhibited by the *Neisseria* genus. Many gonococcal NAATs are known to cross-react with commensal *Neisseria* strains necessitating the use of supplementary testing [1,2]. In addition, sequence-related false-negative results have also been reported for NAATs targeting certain gonococcal sequences. These include the *N. gonorrhoeae cppB* and *opa* genes [3,4].

In March 2011, a young man in his early 20s presented with anal pain to a sexual health clinic in Newcastle, New South Wales, Australia. The man reported having recently had numerous sexual contacts with men (MSM), some with overseas visitors, including from the United States, but reported no recent overseas travel. Pharyngeal and rectal swabs, as well as a urine sample were obtained and submitted for *N. gonorrhoeae* testing. The urine sample and rectal swab were tested by NAAT, and both swab samples were tested by bacterial culture. A summary of results is provided in the Table.

The rectal swab provided positive results for *N. gonorrhoeae* by Cobas4800 CT/NG testing (Roche Diagnostics, Australia) which targets a direct repeat

Table

Culture and NAAT results for *Neisseria gonorrhoeae* by anatomical site and type of sample, New South Wales, Australia, March 2011

Anatomical site / type of sample	*Neisseria gonorrhoeae* diagnostic methods			
	Culture	Cobas4800 CT/NG	LightCycler PCR (porA pseudogene)	TaqMan PCR (porA pseudogene)
Urine sample	NP	Negative	NP	NP
Rectal swab	Positive	Positive	Negative	Negative
Rectal isolate	NA	Positive	Negative	Negative
Pharyngeal swab	Positive	NP	NP	NP
Pharyngeal isolate	NA	Positive	Negative	Negative

NA: not applicable; NAAT: nucleic acid amplification test; NP: not performed.

region, DR-9, with a cycle threshold value of 27, and *N. gonorrhoeae* was subsequently isolated from both the pharyngeal and rectal swabs. The urine sample was negative by NAAT (Cobas4800). Following Australian public health laboratory network guidelines [2] which require supplementary testing for *N. gonorrhoeae* NAAT-positive samples, a DNA extract from the rectal sample was tested using a LightCycler-hybridisation probe-based PCR protocol targeting the gonococcal *porA* pseudogene [5]. The *porA* pseudogene is a target widely used for this purpose and has previously been shown to be highly conserved and specific to *N. gonorrhoeae* [5-8]. Negative results were obtained using the LightCycler method for both the DNA extract of the rectal swab as well as the clinical isolates cultured from the pharyngeal and rectal sites. When the rectal sample and the rectal and pharyngeal isolates were subsequently tested using a TaqMan-based *N. gonorrhoeae porA* pseudogene (*porA*-monoplex) assay, the results were also negative [8]. Testing of the clinical isolates using the Cobas4800 CT/NG assay provided positive results for *N. gonorrhoeae*, with cycle threshold values of 17 for both isolates.

The clinical isolates were further characterised phenotypically and genotypically. Both isolates were indistinguishable and were identified phenotypically [9] as *N. gonorrhoeae* by Gram stain, colonial morphology on modified New York City agar, oxidase, superoxol and rapid carbohydrate utilisation tests. The isolates were tested for prolyliminopeptidase (PIP) activity, auxotyped, serogrouped and the serovar determined by coagglutination reactions with 14 monoclonal reagents (Boule, Huddinge, Sweden). Both isolates tested positive for PIP, were prototrophs and belonged to a common serovar, 'Bropyst'. An identification of *N. gonorrhoeae* was also provided by the Bruker Biotyper matrix-assisted laser desorption ionisation time of

Figure

Sequence alignment of *porA* sequences of *Neisseria gonorrhoeae* FA1090 strain[a], *N. gonorhoeae porA*-variant[b] and *N. meningitidis* 278 strain[c]

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                                10        20        30        40        50        60        70        80        90
N.gonorrhoeae (FA1090)          TTGGCAGCTCGAGCAAGACGTATCCGTTGCCGGCGGCGGCGCGACCCGTTGGGGTAACAGGGAATCCTTTATCGGCTTGGCAGGCGAATT
N.gonorrhoeae (variant)         .........T.....................................AG.....C.....................................
N.meningitidis (278)            .........T.....................................AG.....C.....................................

                               100       110       120       130       140       150       160       170       180
N.gonorrhoeae (FA1090)          CGGCACGGCGCTCGCCGGTCGCGTTGCGAATCCGTTTGGCGATGCCAGCAAAGCCATTGATCCTTGGGACAGCAATAATAATGTGGCTTC
N.gonorrhoeae (variant)         ...T...CT..G.....................A.....A..........C.............................G..........
N.meningitidis (278)            ...T...CT..G.....................A.....A..........C.............................G..........

                               190       200       210       220       230       240       250       260       270
N.gonorrhoeae(FA1090)           GCAATTGGGTATTTTCAAACGCCACGACGGTATGCCGGTTTCCGTGCGTTACGATTCCCCCGGATTTTCCGGTTTCAGCGGCAGCATTCA
N.gonorrhoeae (variant)         ............................A..............A..C.....C..T..G.AC.....................G.C..
N.meningitidis (278)            ............................A..............A..C.....C..T..G.AC.....................G.C..

                               280       290       300       310       320       330       340       350       360
N.gonorrhoeae(FA1090)           ATTTGTTCCGAGTCAAAACAGCAAGTCCGCCTATACGCCTGCTACTTTCACGCTGGAAAGTAATCAGATGAAACCAGTTCCGGCTGTTGT
N.gonorrhoeae (variant)         ...C......GC.............................G...-----T.T.-....T.AGG.G...G..---T.TCA.G..........
N.meningitidis ( 278)           ...C......GC.............................G...-----T.T.-....TGAG..G...G..---T.TCA.G..........

                               370       380       390       400       410       420       430       440       450
N.gonorrhoeae (FA1090)          CGGCAAGCCGGGGTCGGATGTGTATTATGCCGGTCTGAATTACAAAAATGGCGGCTTTTTCGGAAATTATGCCCTTAAATATGCGAAACA
N.gonorrhoeae (variant)         .........C..A.............................................A...GC...G.GC......T.................
N.meningitidis (278)            .........C..A.............................................A...GC...G.GC......T.................

                               460       470       480       490       500       510       520       530       540
N.gonorrhoeae (FA1090)          CGCCAATGAGGGGCATGATGCTTTCTTTTTGTTCTTGCTCGGCAGAGCGAGTGAT------------ACCGATCCATTGAAAAACCATCA
N.gonorrhoeae (variant)         ..............G.............................C.G.......GAAGCCAAAGGT.........C..............
N.meningitidis (278)            ..............G.............................C.G.......GAAGCCAAAGGT.........C..............

                               550       560       570       580       590       600       610       620       630
N.gonorrhoeae (FA1090)          GGTACACCGCCTGACGGGCGGCTATGGGGAAGGCGGCTTGAATCTCGCCTTGGCGGCTCAGTTGGATTTGTCTGAAAATGCCGACAAAAC
N.gonorrhoeae (variant)         ........................A.......................................................................
N.meningitidis (278)            ........................A.......................................................................

                               640       650       660       670       680       690       700       710       720
N.gonorrhoeae (FA1090)          CAAAAACAGTACGACCGAAATTGCCGCCACTGCTTCCTACCGCTTCGGTAATACAGTCCCGCGCATCAGCTATGCCCATGGTTTCGACTT
N.gonorrhoeae (variant)         ..................................................G....T.................................
N.meningitidis (278)            ..................................................G....T.................................

                               730       740       750       760       770       780
N.gonorrhoeae (FA1090)          TGTCGAACGCAGTCAGAAACGCGAACATACCAGCTATGATCAAATCATCGCCGGTGTCGATT
N.gonorrhoeae (variant)         ..............................................................
N.meningitidis (278)            .A........G..A.A...G.....A..........C................C..T....
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[a] Genbank accession AJ223447.

[b] Pseudogene PCR negative strain from this study.

[c] Genbank accession GQ173789.

Forward and reverse primer targets of the TaqMan-based *Neisseria gonorhoeae porA* pseudogene PCR are represented by arrows at positions 262 to 284 and 324 to 250 respectively.

The probe target is represented by the box at position 298 to 323.

flight mass spectrophotometer (MALDI TOF MS) Maldi Biotyper (Bruker Biosciences Pty Ltd.). Antimicrobial resistance patterns for these isolates were characteristic of *N. gonorrhoeae*, as determined by the minimum inhibitory concentrations (MICs) using the agar plate incorporation method of the Australian Gonococcal Surveillance Programme and using the CDS Antibiotic Susceptibility criteria [10]. The clinical isolates had chromosomally-mediated penicillin resistance (MIC: 2.0 mg/L), quinolone resistance (ciprofloxacin MIC: 16.0 mg/L), decreased susceptibility to ceftriaxone (MIC: 0.03 mg/L) and sensitivity to azithromycin and spectinomycin. Both isolates were of multilocus sequence type (MLST) 1901 (*abcZ* 109, *adk* 39, *aroE* 170, *fumC* 111, *gdh* 148, *pdhC* 153, *pgm* 65). This is an *N. gonorrhoeae* MLST type previously observed in Australia and elsewhere [11,12] and representatives of this type have previously provided positive results by *porA*-pseudogene PCR in our laboratory (data not shown). Using *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST), both isolates harboured the previously described POR and tbpB types, 1297 and 983 respectively, which represented a novel NG-MAST type 5377. It should be noted that MLST and NG-MAST are not performed as a routine part of *N. gonorrhoeae* investigations in our laboratory, therefore isolates of these MLST and NG-MAST types may have previously been circulating in our population but not characterised.

To examine the basis of the *porA* pseudogene PCR false-negative results, a 773 base-pair fragment of the *porA* sequences of each isolate were amplified using primers CGGCTCGTTTATCGGCTT and GGTATTTCGTTTCAGCCAAGC and subjected to DNA sequencing. The *porA*-pseudogene PCR-negative *N. gonorrhoeae* strains from this study exhibited only 90% homology with the reference *N. gonorrhoeae* FA1090 strain (genbank accession number AJ223447) and had multiple mismatches and deletions evident in the primer and probe targets for the *porA* pseudogene Taqman-based-PCR (Figure) and for the LightCycler hybridisation probe-based method targeting the same region (data not shown).

Notably, genbank blast searching indicated that the *porA* sequence from the clinical isolates in this study were more similar to that of *N. meningitidis*, having 99% homology with *N. meningitidis* 278 strain (Genbank accession GQ173789). Only the last 60 bases of the 773 base *porA* sequence provided greater homology with *N. gonorrhoeae* than with *N. meningitidis*.

Conclusions

Overall the results show that the rectal and pharyngeal *N. gonorrhoeae* isolates from this patient were typical in terms of genotypic and phenotypic characteristics, except that they had acquired a meningococcal *porA* sequence presumably through horizontal genetic exchange and recombination. This is yet another example of the problems faced with molecular detection of *N. gonorrhoeae*, and with PCR-based diagnostics more generally. For *N. gonorrhoeae*, the problem is exacerbated by the fact that the species comprises numerous subtypes that exhibit considerable sequence diversity as well as propensity to mutate. Notably, the distribution of subtypes can vary geographically, temporally, and between patient groups. This has implications for the performance of *N. gonorrhoeae* NAATs: firstly, the performance may vary between patient populations because of the presence of different subtypes; but secondly, as in our case, the performance within a given population can suddenly change either due to the importation of new strains or mutation of currently circulating strains. In our opinion, the use of different methods, such as NAAT and bacterial culture in parallel, or multi-target NAAT assays provides the most suitable means of circumventing these problems, and to this extent we have previously described a duplex real-time PCR assay for detecting *N. gonorrhoeae* combining both the *porA* pseudogene and *opa* targets [8]. To date, we have not observed any other *N. gonorrhoeae* isolates with a meningococcal *porA* sequence in our laboratory and to our best knowledge this has also not been observed elsewhere. Given the propensity for gonococci to spread through populations, we consider it likely that this strain is more widespread. Further investigations including contact tracing and prospectively testing *N. gonorrhoeae* isolates by *porA* pseudogene PCR, are continuing.

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Outbreak of hand, foot and mouth disease caused by Coxsackie A16 virus in a childcare centre in Croatia, February to March 2011

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We describe an outbreak of hand, foot and mouth disease (HFMD) in a childcare centre in a district of Zagreb county, north-west Croatia. A total of eleven cases of HFMD occurred in the childcare centre and another nine were reported from nearby areas in the district. Coxsackie A16 virus was diagnosed in 13 clinical specimens obtained from 11 symptomatic and asymptomatic children. All cases resolved without complications.

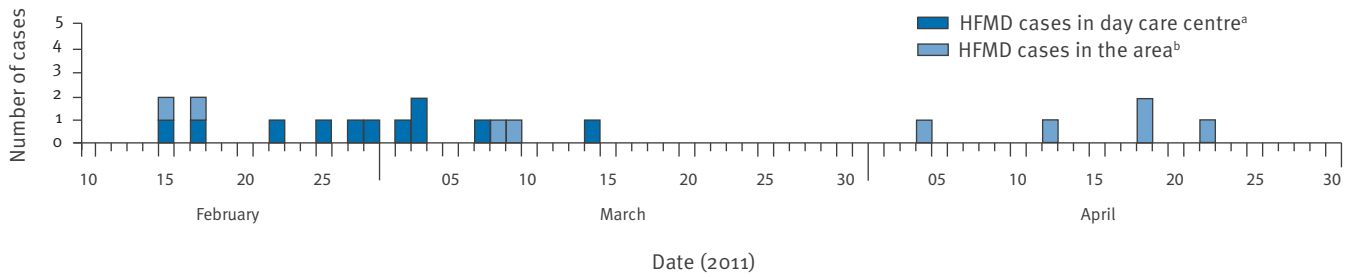
Outbreak description

On 2 March, the Department of Epidemiology of Zagreb County Institute of Public Health was contacted by a health professional from a childcare centre in Bregana, Samobor district, reporting four children with vesicular stomatitis and rash (hand, foot and mouth disease, HFMD) in the weeks before. The centre cares for 160 children at two locations and comprises eight classes (seven classes in the main building complex and one class in a house nearby). Three cases were from one class at the first location and a fourth case was from the nearby house.

On the same day that the cases were reported, the epidemiologist found another two children with the same symptoms in the same class of the main building complex. The epidemiologist immediately reported the cases to the Reference Epidemiology Centre at the Croatian National Institute of Public Health. The day after, mouth swabs and stools were collected for viral culture from all children in the two affected classes. After active case finding (parent questionnaire, paediatrician reports), another five cases were found in other classes of the same childcare centre (11 cases in total). In this investigation, only symptomatic individuals were considered as cases, irrespective of laboratory-confirmation. All cases presented with a mild clinical picture of disease, with no complications, and only four of the 11 children attending the childcare centre stayed at home until they recovered. The affected classes were neither closed, nor were infected children separated. The management of the childcare centre was rather instructed to carry out an extensive cleaning and disinfection of all surfaces, toys, furnishings, toilets and other objects, while parents were given

Figure

Cases of hand, foot and mouth disease, by date of onset, Croatia, February–April 2011 (n=20)



HFMD: hand foot and mouth disease.

[a] Three of 11 cases were laboratory-confirmed for Coxsackie A16 virus infection.

[b] Cases were clinically diagnosed.

written materials on disease transmission and how to avoid it. No further cases occurred in the childcare centre after 14 March.

The epidemiologist also identified nine cases, outside the childcare centre, retrospectively by active case finding. These cases were also preschool children, aged one to four years, living in the Samobor district, which comprises about 43,000 inhabitants. The nine cases were all diagnosed by four primary care paediatricians taking care of most of the children in the area. After the outbreak in the childcare centre was recognised, the epidemiologist in charge of the investigation checked with them all the records and asked to report further cases. None of the cases outside the childcare centre were laboratory-investigated.

Laboratory investigation

A total of 57 clinical specimens (21 stool specimens and 36 throat swabs) were collected from the first six HFMD cases and a further 29 asymptomatic children aged from 1.5 to 5.5 years who attended the same classes as the first six cases at the childcare centre, as well as from their nurse. Specimens were forwarded to the World Health Organization (WHO) National Polio Laboratory at the Department of Virology, Croatian National Institute of Public Health for analysis. After pre-treatment, faecal suspensions and throat swabs were inoculated on green monkey kidney (GMK), human rhabdomyosarcoma (RD(A)) and L20B (a recombinant mouse cell line expressing receptor for poliovirus) cell cultures, as a standard procedure for isolation of enteroviruses. Cell cultures were obtained from the WHO Labnet, National Public Health Institute, Helsinki, Finland. Viruses developed a cytopathic effect observed only on RD(A) cells, while there was no evidence of growth on GMK and L20B cells. Identification of Coxsackie A16 (CA16) virus was done by microneutralisation assay using pooled equine hyperimmune sera prepared at the National Institute of Public Health and Environment (RIVM) Bilthoven, the Netherlands [1]. Laboratory results of CA16 virus isolation are presented in the Table.

Table

Coxsackie A16 virus isolation from symptomatic and asymptomatic children and their asymptomatic nurse Croatia, February–April 2011 (n=57 specimens obtained from n=36 individuals

Clinical specimens	Stools positive/tested	Throat swabs positive/tested
Children with hand, foot and mouth disease symptoms (n=6)	2/2	1/6
Asymptomatic children and nurse (n=30)	8/19	2/30
Total (n=36)	**10/21**	**3/36**

A total of 13 clinical specimens obtained from 11 children were positive. They comprised three of the six symptomatic cases and eight of the 29 tested asymptomatic contacts (Table). Asymptomatic individuals with a positive laboratory result were not considered as cases and are therefore not included in the Figure. In two children with a positive throat swab, the respective stool samples also tested positive, and in one child with a positive throat swab, a stool sample was not collected.

HFMD is a syndrome characterised by vesicular stomatitis and cutaneous lesions of the distal extremities and it is usually caused by CA16 or enterovirus 71 (E71). These viruses are genetically closely related and are both serotypes of the human enterovirus A (HEV-A) species, *Picornaviridae* family [2].

HFMD has a worldwide occurrence. Outbreaks occur frequently among groups of children in childcare centres and schools [3]. Transmission is by faecal-oral route and by exposure to throat discharges or fluid from blisters. In the absence of cutaneous lesions, oral lesions of HMFD may be mistaken for aphthous ulcers or herpes simplex gingivostomatitis [4]. Although described, neurologic complications of HFMD caused by CA16 are rare when compared to the disease caused by E71 [5,6]. Diagnosis of HFMD is usually based on the clinical picture alone. Laboratory diagnosis is usually performed by virus isolation of cell culture from throat swabs and stool specimens.

Enterovirus infections are notifiable diseases in Croatia. Usually, there are few reports of sporadic cases of HFMD each year, diagnosed based on the clinical picture alone. There are also several different serotypes of enteroviruses isolated in Croatia each year [7] but laboratory confirmed cases caused by CA16 virus have not been registered until now.

Discussion

CA16 and E71 are prevalent in many parts of the world, especially in south-east Asia [5,6,8], but also in European countries [9,10]. In Croatia CA16 infections have not been documented until now, although these occur in neighbouring countries [9]. The traditional technique for detecting and characterising enteroviruses, also used in this laboratory, relies on viral isolation in cell culture followed by neutralisation using reference antisera. However, no single cell line exists that is capable of growing all human enteroviruses [2]. In 2004, RD(A) cells were introduced into the routine laboratory diagnostic algorithm for enterovirus culture together with previously used GMK and L20B cells. This has resulted in the detection of HEV-A viruses including CA16 and E71, which grow poorly, if at all, in the other two cell lines used in our laboratory. Immediate collection of clinical specimens by the epidemiologist during the investigation of this outbreak also contributed to the successful isolation of CA16.

CA16 and E71 are associated with sporadic cases and outbreaks of HFMD, and rare cases of acute neurological diseases. The HFMD outbreak reported here confirmed the previously observed characteristics of CA16 infection. An outbreak occurred in a childcare centre in children aged 1.5 to 5.5 years, who seem to be the most susceptible age group for CA16 infection. A seroprevalence study in Germany showed that two thirds of children aged 1 to 4 years do not posses neutralising antibodies to CA16 [10]. HFMD is a highly contagious disease, but the manifestation rate of infection is low. A large number of exposed children in this outbreak were infected as by laboratory confirmation (11/35). Most of the infected children were asymptomatic (8/11). The illness is usually mild without complications, as confirmed during this outbreak. The number of positive stool specimens versus throat swabs in symptomatic as well as asymptomatic children in this report confirmed that stool is the most appropriate clinical specimen for enteroviruses in which they survive for a long period.

In conclusion, a prompt reaction of the Epidemiology Service and the thorough investigation that followed, allowed the successful implementation of control measures which prevented further spreading of the HFMD outbreak in the childcare centre. CA16 appeared to have been circulating in the Samobor area from February, but as symptoms are mild or absent in most cases, many parents may not have sought medical help for their children.

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Coverage of human papillomavirus vaccination during the first year of its introduction in Spain

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The decision to introduce human papillomavirus (HPV) vaccination into the national immunisation programme in Spain was made in October 2007, recommending vaccination of girls aged between 11 and 14 years with three doses of HPV vaccine. All 19 regions of the country (17 Autonomous Communities and two Autonomous Cities) introduced HPV vaccination gradually into their immunisation programmes between November 2007 and the last school term of 2008. Eight regions administered the vaccine in healthcare centres and 11 in schools. In the first year of the introduction of HPV vaccination, coverage of the first and third doses was assessed, to determine the proportion of girls who did not complete the vaccination. On the basis of the available data, the Ministry of Health estimated that coverage for the first dose was 87.2% (range: 73.9–98.9%; 95% CI: 71.8 to 100) and 77.3% (range: 62.2–97.4%; 95% CI: 57.9 to 96.7) for the third dose. Higher uptake was observed when the vaccination was carried out in schools compared with healthcare centres, but the difference was not statistically significant. Negative messages in the media during implementation of the HPV vaccination programme may have had some influence on the attitudes of adolescent girls and/or their parents towards HPV vaccination and may be partly responsible for the observed vaccination dropout rate.

Background

Human papillomavirus (HPV) causes one of the commonest sexually transmitted infections and is also the main cause of cervical cancer in the world. About 70% of cervical cancer cases are associated with chronic infection with HPV types 16 and 18 [1]. HPV types 6 and 11 cause a high percentage of low-risk cervical dysplasia and more than 90% of genital warts. HPV infection is associated with age and early sexual debut [2].

In Spain there is no national cancer registry, therefore no direct information about the number of cervical cancer cases per year is available for the country as a whole. The International Agency for Research on Cancer uses local incidence data and national mortality data to estimate incidence and mortality due to cervical cancer in Spain. For 2008, a total of 1,948 new cases (age-standardised incidence rate of 6.3 cases per 100,000 population) and 712 deaths (age-standardised mortality rate of 1.9 per 100,000 population) were estimated for Spain [3]. In 2008, both HPV-specific incidence and mortality were among the lowest in southern Europe [4].

The European Commission granted a marketing authorization for two HPV vaccines in the European Union: the tetravalent Gardasil in October 2006 (which contains HPV types 16, 18, 6 and 11) and the bivalent Cervarix in September 2007 (containing HPV types 16 and 18) [5,6]. A three-dose vaccination course is recommended, at 0, 2 and 6 months for Gardasil [7] and 0, 1 and 6 months for Cervarix [8].

The main goal of the vaccination is to prevent infection by the HPV types included in the vaccine and to decrease acute and chronic disease burdens caused by the infection, particularly precancerous lesions and cancer.

Decision-making process for the introduction of a new vaccine into the National Immunisation Programme in Spain

Spain has a national health system with universal access funded from general taxation [9]. It is largely decentralised and the country's 19 regions (17 Autonomous Communities and two Autonomous Cities) are responsible for the management and delivery of vaccination programmes. The Interterritorial Council, coordinated by the Ministry of Health, is the decision-making body responsible for the coordination of all programmes within the national health system in order to ensure cohesion and equity. Resolutions are approved by consensus and materialise through recommendations.

The Commission on Public Health, also coordinated by the Ministry of Health, proposes public health programmes or makes recommendations on public health issues to the Interterritorial Council. The Vaccination Programmes and Registration Board (Vaccines Board) is the technical group of the Commission on Public Health that provides recommendations relating to

immunisation based on epidemiological, scientific and public health information. Approved recommendations are then incorporated into the National Immunisation Programme. Regions may also offer vaccines that are not in the national programme.

In February 2007 the Vaccines Board recommended the introduction of HPV vaccination. This recommendation was approved by the Commission on Public Health in September and by the Interterritorial Council in October that year [10]. The Council decided to include HPV vaccination within the framework of the country's cervical cancer prevention strategy. It recommended that the vaccine be introduced before the end of 2010, for girls between the ages of 11 and 14 years, in accordance with requirements, priorities and logistics of regional vaccination programmes and the availability and supply of vaccines in each region. In November 2007, the Commission on Public Health agreed that girls aged 14 years were the best cohort to vaccinate. No specific target coverage was set as an objective of the programme.

Implementation of HPV vaccination in Spain

After the consensus reached by the Interterritorial Council in October 2007, regions began to implement HPV vaccination gradually into their immunisation programmes, starting in November 2007. Three regions began the vaccination during the school winter term of 2007/08, six regions carried out the vaccination in the first term of 2008 and 10 in the last term of 2008.

The purchase and distribution of the vaccine were carried out independently by each region: 13 chose Gardasil and the other six chose Cervarix . Each region also covered all expenses related to the administration of vaccines in their territory and supported the introduction of the programme with publicity campaigns and communication strategies independently.

Most regions (n=13) chose to vaccinate girls aged 14 years, in line with the Commission on Public Health's decision. Three regions vaccinated girls aged 13 years, one vaccinated girls in their last year of primary education (aged 11–12 years) and two vaccinated girls in the first year of secondary education (aged 13–14 years). Two regions have temporarily extended the vaccination age in their territory: both have implemented the programme in schools and included young women attending three school years ahead of those included in the programme.

Eight regions administered the vaccine in healthcare centres and 11 in schools.

Estimation of HPV vaccination coverage

Monitoring of HPV vaccination coverage is carried out as part of the regular monitoring of coverage of administered vaccines included in the National Immunisation Programme. Predefined indicators are estimated annually at regional level and are sent to the Ministry of Health on a voluntary basis. The information is publicly available on the Ministry of Health web site [11].

Vaccination uptake is estimated by nominal registry in four regions and by an indirect method – using the total number of administered doses (reported by health care professionals) in the targeted female population – in the other regions. To estimate HPV vaccine coverage, the uptake of the three doses was assessed, but for the first year after the introduction of HPV vaccination, uptake of the first and third doses was included, to assess the dropout rate. Coverage was calculated as the proportion of the targeted population that received the first and the third doses of any HPV vaccine.

In the first year of HPV vaccine implementation (academic year 2008 to 2009), 14 regions sent information to the Ministry of Health about first-dose uptake and 18 regions about third-dose uptake in their territory (one region did not send any information on HPV vaccine uptake). The data indicated that coverage with one dose was 87.2% (range: 73.9–98.9%; 95% CI: 71.8 to 100) in the 14 regions that sent the information. Coverage decreased when looking at the proportion of girls receiving the three doses: in the 18 regions that sent the information, the coverage was 77.3% (range: 62.2–97.4%, 95% CI: 57.9 to 96.7). Only one region had a third-dose coverage lower than 70%, in six regions it was 70–80%, in five it was 80–90% and in six, over 90% coverage was achieved [11].

The observed difference between one- and three-dose uptake in regions reporting both sets of data was 9.9 percentage points, with high variability among the regions, ranging between 0.5 and 16.4 percentage points.

Regarding the venue of administration of the vaccines, coverage was 84.2% (range: 73.7–97.4%, 95% CI: 62.7 to 100) in regions that implemented the programme in schools and 70.1% (range: 62.2–85.7%, 95% CI: 36.2 to 100) for those that vaccinated in healthcare centres; however, the observed difference is not statistically significant.

Discussion and conclusion

During the first year of the HPV vaccination programme in Spain, vaccination with three doses was 77.3% for the targeted adolescent girls. Other countries of the European Union (EU) showed variable vaccination coverage of three doses of HPV vaccine in the respective targeted age groups during the first year: 44% in Belgium [12], 53.1% in Italy [13], and 80.9% in the United Kingdom [14]. In 2009, coverage of other vaccines administered in adolescents in Spain was 74.1% (for the vaccine against tetanus and diphtheria, administered at age 14–16 years) and 82.7% (for hepatitis B vaccine, administered at age 10–14 years) [11].

A dropout of 9.9 percentage points, between first-dose uptake (87.2%) and third-dose uptake (77.3%), has

been observed, but possible reasons for this have not been analysed. For hepatitis B vaccine, which is also administered in three doses, only information about coverage with three doses is gathered at central level so we do not know what the dropout rate is. Similar dropout figures between first- and third-dose uptake of HPV vaccines have been observed in the United Kingdom [14] and Italy [13], of 7.7 and 13.2 percentage points, respectively; nevertheless, efforts should be made to completely vaccinate girls that start the vaccination.

Although the anti-vaccination movement has had neither an important influence on vaccination uptake nor important coverage in the media in Spain, the introduction of HPV vaccination into the National Immunisation Programme created some controversy among the public and healthcare workers, which was covered by the media. Some healthcare workers claimed that the available information regarding efficacy and safety of the HPV vaccines had some important gaps and because of that, together with the high price of the vaccines, they questioned the benefit of introducing HPV vaccination [15].

During this time of public and professional uncertainty about HPV vaccination, two cases of status epilepticus with myoclonus were reported in two girls after administration of the second dose of Gardasil in schools in the same city, in February 2009 [16]. A resulting pharmacovigilance signal was notified to the European Medicines Agency and issues related to the cases were extensively covered by all media at regional and national level. This intense media attention lasted for two and a half months, until it was determined that the adverse events were not related to the vaccine (following investigation by the Spanish and European medicines agencies of the adverse events and the quality of the specific vaccine batch) [16,17]. The negative effect of the media coverage has not been analysed in detail but it is thought likely that it had some influence on the attitude of adolescent girls and/or their parents towards vaccination. This might have had been partly responsible for the observed dropout rate. In contrast, in the United Kingdom, where a similar dropout rate for HPV vaccination was found, positive media coverage surrounding the introduction of the vaccination programme has been considered as influencing public perceptions about acceptability of the vaccination and contributing to the good level of vaccination coverage [18]. Special attention should be paid to tailoring communication strategies in Spain to increase positive perceptions of HPV vaccination in adolescent girls and parents.

The observed difference of coverage according to the venue of HPV vaccination in Spain was not statistically significant. In the first year after HPV vaccine was introduced in England, a high level of vaccine uptake (80.1%) was achieved in eligible girls in the routine cohort (aged 11–13 years), who were mostly (94.2%) vaccinated through a school-based programme. Delivery through general practitioners resulted in lower uptake rates compared with those related to delivery through schools for each dose, and this was particularly noticeable for third-dose uptake [14]. The coverage for the routine cohort was higher than that seen in the 17–18-year-old catch-up cohort (31.8%), who were vaccinated in various venues (31.4% were vaccinated in schools, 60% in general practitioners' practices and 8.6% in community clinics). It was not stated whether the coverage differences seen according to venue of vaccine administration were statistically significant in this study in England [14]. Further data regarding regional strategies should be analysed in Spain to better understand the possible influence on coverage of the venue of implementation of immunisation programmes targeting adolescents.

As with other vaccines in the National Immunisation Programme, a high uptake is needed to maximise the results. The long-term impact of HPV vaccination is difficult to predict and the duration of immunity conferred by the vaccines is not known. A recent study modelling the impact of HPV vaccination in the United Kingdom has suggested that vaccinating a cohort of young women at 80% coverage will result in a 38–82% reduction in cervical cancer incidence and 44–100% reduction in anogenital warts incidence after 60 years of an ongoing vaccination programme if vaccine protection lasts 20 years on average [19]. In addition, it should be borne in mind that the HPV vaccination does not eliminate the need for cervical cancer screening, even for women vaccinated against HPV types 16 and 18, who will still be at risk from other high-risk types [20]

Parents' attitudes may also play a role in the uptake of HPV vaccination by adolescents, given that the vaccine prevents a sexually transmitted infection. The effect of social inequalities on the uptake of HPV vaccination has been shown in a prospective cohort study in Manchester, United Kingdom, which observed that parents who did not consent to their daughter's vaccination at school were from more deprived and ethnic minority backgrounds [21,22].

There are a couple of limitations in our analysis. First, overall national three-dose coverage was calculated without the information from one region, representing less than 5% of the Spanish population. It should be pointed out that coverage for other vaccines notified in previous years from this region did not fall into any of the coverage extremes, so the same may be true for HPV vaccination. Second, other information about the campaigns conducted at regional level, such as communication, media coverage and other recruitment strategies, has not been analysed and may also play a role in coverage besides the venue of administration.

In conclusion, in the first year of implementation of HPV vaccination in Spain, coverage was similar to that for other vaccines given to adolescents in the country.

Our findings demonstrate that the programme should be strengthened to reduce the dropout rate.

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