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Fifth European Antibiotic Awareness Day on 18 November - joining forces to reduce antibiotic resistance

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Antibiotics are medicines that are beneficial for mankind and their use in treatment and prevention of bacterial diseases has greatly contributed to reducing the overall burden of such infections. However, already since antibiotics have been used in clinical practice, emergence and spread of antibiotic resistance has represented an obstacle for the effective treatment of infected patients. A growing number of resistance mechanisms and antibiotic-resistant strains have been described and related infections have been increasing in numbers. These trends have been identified as a threat and have thus been watched closely by scientists and public health experts globally to have a clear picture of the magnitude of the problem and its impact on public health, and to identify and implement appropriate control measures.

Extensive coverage in the scientific and general literature illustrates the importance of antibiotic resistance in research and public health practice. On 14 November 2012, entering the simple term 'antibiotic resistance' in PubMed and Google Scholar resulted in the retrieval of 133,163 and over 1,300,000 related publications, respectively. Antibiotic resistance limits the number of options for effective treatment of infected patients. In extreme cases such as infections with carbapenemase-producing *Enterobacteriaceae*, alternatives for treatment are limited to only few antibiotics that often are old, have side-effects and limitations for their use.

Antibiotic-resistant bacteria are often responsible for healthcare-associated infections. This is obviously related to antibiotic prescribing practices in hospitals and other healthcare settings, and to poor compliance with infection control measures to prevent spread and patient-to-patient transmission of these bacteria. In this week's issue, *Eurosurveillance* publishes an article by Zarb et al. in which the authors present the results from a pilot study using a new point prevalence protocol for healthcare-associated infections and antimicrobial use in European acute care hospitals. The results from participating hospitals in 23 countries show that 7.1% of patients had a healthcare-associated infection, and 34.6% received at least one antimicrobial agent [1].

Healthcare-associated infections and antibiotic resistance are closely related issues that concern patients, physicians, healthcare providers and public health experts. Due to their associated morbidity and mortality, they lead to a high strain on individuals and health systems. For example it is estimated that in the European Union (EU) alone, the excess hospital stay attributable to selected common multidrug-resistant infections in hospitals amounts to 2.5 million days and 25,000 patients die each year as a result of these infections [2].

Acknowledging the importance of the subject, the EU Commission formulated a strategy against Antimicrobial Resistance and EU Health Ministers adopted Council recommendations on the prudent use of antimicrobial agents in human medicine already in 2001 [3,4] and many more initiatives followed. Last year, the European Commission released its Action plan against the rising threats from antimicrobial resistance [5]. Another initiative is the European Antibiotic Awareness Day (EAAD) that provides a platform and support for national campaigns on the prudent use of antibiotics [6]. This European health initiative, coordinated by the European Centre for Disease Prevention and Control (ECDC) in Stockholm, has grown over the years and new important partners have joined. The first EAAD took place on 18 November 2008 and has been marked at the same date also in the following years. While the first year saw 32 countries participating, in 2012, over 40 countries have started or will launch activities around 18 November when the fifth EAAD takes place. Moreover, in 2012, the World Health Organization Regional Office for Europe supports the campaign actively for the first time and a range of activities have also been organised this week in the United States, in Canada and in Australia [7-9].

In the run up of the day, and in previous years much activity has been ongoing in mass and social media that should have resulted in increasing awareness of the problem of antibiotic resistance and the need to use

antibiotics prudently, i.e. only when indicated, among the general public and among health professionals.

An initial evaluation of EAAD took place in 2009, showing strong political and stakeholder support [10], however, in a next step it will be important to measure the success of the initiative and see whether awareness has been transformed into action. Such action could be indicated for example by more adequate prescribing by doctors and less self-medication by patients, and as a result less antibiotic consumption. Obtaining the respective data and attributing them to efforts associated with the EAAD as one important element in the fight against antibiotic resistance is a challenge. Visible results can only be expected over time and evaluation may require specific studies and analyses. *Eurosurveillance* will keep on following the evolution of the EAAD and publish articles that contribute to give insight into the situation and related issues connected with antimicrobial resistance and healthcare-associated infections.

References

1. Zarb P, Coignard B, Griskeviciene J, Muller A, Vankerckhoven V, Weist K, et al. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro Surveill. 2012;17(46):pii=20316. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20316
2. European Centre for Disease Prevention and Control (ECDC) / European Medicines Agency (EMEA). Joint Technical Report. The bacterial challenge: time to react. Stockholm: ECDC; Sep 2009. Available from: http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf
3. European Commission. Communication from the Commission of 20 June 2001 on a Community strategy against antimicrobial resistance. [Accessed 15 Nov 2012]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:52001DC0333:EN:HTML
4. Council of the European Union. Council Recommendation of 15 November 2001 on the prudent use of antimicrobial agents in human medicine (2002/77/EC). Official Journal of the European Communities, 2002 Feb. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:034:0013:0016:EN:PDF
5. European Commission. Communication from the Commission to the European Parliament and the Council. Action plan against the rising threats from Antimicrobial Resistance. COM (2011) 748. Available from: http://ec.europa.eu/dgs/health_consumer/docs/communication_amr_2011_748_en.pdf
6. European Centre for Disease Prevention and Control (ECDC) European Antibiotic Awareness Day (EAAD). Stockholm: ECDC. [Accessed 15 Nov 2012]. Available from: http://ecdc.europa.eu/en/eaad/Pages/Home.aspx
7. Centers for Disease Control and Prevention (CDC). Get Smart About Antibiotics Week. Atlanta: CDC. [Accessed 15 Nov 2012]. Available from: http://www.cdc.gov/getsmart/index.html
8. AntibioticAwareness.ca [Internet]. Antibiotic Awareness Week. [Accessed 15 Nov 2012]. Available from: http://antibioticawareness.ca/
9. NPS Medicinewise [Internet]. Antibiotic Awareness Week. [Accessed 15 Nov 2012]. Available from: http://www.nps.org.au/bemedicinewise/antibiotic_resistance/antibiotic_awareness_week
10. Earnshaw S, Monnet DL, Duncan B, O'Toole J, Ekdahl K, Goossens H, et al. European Antibiotic Awareness Day, 2008 – the first Europe-wide public information campaign on prudent antibiotic use: methods and survey of activities in participating countries. Euro Surveill. 2009;14(30):pii=19280. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19280

The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use

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A standardised methodology for a combined point prevalence survey (PPS) on healthcare-associated infections (HAIs) and antimicrobial use in European acute care hospitals developed by the European Centre for Disease Prevention and Control was piloted across Europe. Variables were collected at national, hospital and patient level in 66 hospitals from 23 countries. A patient-based and a unit-based protocol were available. Feasibility was assessed via national and hospital questionnaires. Of 19,888 surveyed patients, 7.1% had an HAI and 34.6% were receiving at least one antimicrobial agent. Prevalence results were highest in intensive care units, with 28.1% patients with HAI, and 61.4% patients with antimicrobial use. Pneumonia and other lower respiratory tract infections (2.0% of patients; 95% confidence interval (CI): 1.8–2.2%) represented the most common type (25.7%) of HAI. Surgical prophylaxis was the indication for 17.3% of used antimicrobials and exceeded one day in 60.7% of cases. Risk factors in the patient-based protocol were provided for 98% or more of the included patients and all were independently associated with both presence of HAI and receiving an antimicrobial agent. The patient-based protocol required more work than the unit-based protocol, but allowed collecting detailed data and analysis of risk factors for HAI and antimicrobial use.

Introduction

Healthcare-associated infections (HAIs) and antimicrobial resistance are well known major public health threats. The European Centre for Disease Prevention and Control (ECDC) proposed in 2008 that the total burden of HAIs should be measured regularly and in a standardised manner throughout the European Union (EU) [1]. The initial steps towards standardisation of surveillance of HAIs in Europe had been carried out on surgical site infections and infections in intensive care units by the 'Hospitals in Europe Link for Infection Control through Surveillance (HELICS)' project, from 2000 to 2003 [2-6].

Subsequently, HELICS implemented standardised surveillance of HAIs in 2004 and 2005, and later as part of the 'Improving Patient Safety in Europe (IPSE)' network from 2005 to 2008 [7] which was transferred to ECDC in July 2008. Continuous surveillance, especially prospective active surveillance, is the gold standard [8]. However, repeated point prevalence surveys (PPSs) represent a more feasible alternative for hospital-wide surveillance of all HAIs, while still allowing the estimation of disease burden by HAIs in acute hospitals, and helping to prioritise areas requiring interventions [9]. Based on a review of 30 national or multicentre PPSs in 19 countries that had been carried out between 1996 and 2007 and included a total of 837,450 patients, ECDC estimated in 2008 the prevalence of HAIs in EU acute care hospitals to be on average of 7.1% [1].

However, major methodological differences between these PPSs made comparison between countries impossible [1,10-13]. When coordination of the IPSE network was transferred to ECDC in July 2008, ECDC recommended that surveillance in the EU should include all types of HAIs. Subsequently, the ECDC prepared a protocol for a PPS of HAIs in acute care hospitals, which was finalised in March 2011 [14].

Although most antimicrobials are prescribed in the community [15], the selective pressure they exert is

much higher in hospitals, where the proportion of patients receiving antimicrobial agents is much higher there than in the community [16]. This is considered to be the main reason why microorganisms isolated from hospital infections show more resistant profiles than microorganisms from community infections [17]. Various hospital PPSs on antimicrobial use were carried out in the last three decades [18-22]. Also these PPS varied greatly in aims, protocols and populations surveyed, thus making comparison of their results difficult. The 'European Surveillance of Antimicrobial Consumption (ESAC)' project initiated standardisation of the methodology for measuring antimicrobial consumption across Europe [23-26]. This methodology has proven feasible and reliable [24,25,27]. In view of the transition of the ESAC network to ECDC in July 2011, the ESAC methodology for PPS of antimicrobial use was integrated as part of an ECDC protocol for PPS of HAIs and antimicrobial use in acute care hospitals. Combined PPSs of HAIs and antimicrobial use had also previously been carried out in different populations [28-32], but again with large methodological differences between surveys.

The main aim of this ECDC pilot PPS was to test a common European methodology for PPSs of HAIs and antimicrobial use in acute care hospitals before its implementation across the EU, with the specific objectives to estimate the total burden of HAIs and antimicrobial use and disseminate the results at local, regional, national and EU level. The ECDC pilot PPS protocol met the objectives of the Council Recommendation of 9 June 2009 on patient safety, including the prevention and control of HAIs (2009/C 151/01), and specifically article II.8.c of this recommendation, i.e. "to establish or strengthen active surveillance at institution, regional and national level" [33]. In addition, the ECDC pilot PPS also met the objectives of Council Recommendation of 15 November 2001 on the prudent use of antimicrobial agents in human medicine (2002/77/EC) [34].

Methods

Participating countries and hospitals

In January 2010, ECDC invited all national contact points for HAI surveillance and/or experts designated as national expert for the ECDC PPS to participate in the pilot PPS study and enter at least one institution qualified as acute care hospital according to national definitions. Two or more hospitals per country were preferred to allow testing of both the patient-based ('standard') and unit-based ('light') version of the protocol in the same country. In total, 23 countries (22 EU Member States and one EU enlargement country) participated in the survey with 66 hospitals and including 19,888 patients.

The number of hospitals per country was: Belgium (n=7 hospitals), Bulgaria (n=2), Croatia (n=2), Cyprus (n=3), Czech Republic (n=2), Estonia (n=2), Finland (n=16), France (n=3), Germany (n=1), Greece (n=1), Hungary (n=2), Italy (n=4), Latvia (n=2), Lithuania (n=3), Luxembourg (n=1), Malta (n=1), Poland (n=1), Portugal (n=2), Romania (n=1), Slovakia (n=2), Slovenia (n=2), Spain (n=5), and the United Kingdom, Scotland (n=1).

The national contact points acted as national PPS coordinators and invited hospitals to participate on a voluntary basis. As this was a pilot survey, we did not aim for a representative sample of hospitals in the countries. It was recommended to include both large and small hospitals in order to test the feasibility of the protocol in different settings. Information on the size and type (primary, secondary, tertiary and specialised) of each hospital was collected through a specific hospital questionnaire. National questionnaires were used to collect data on the number of acute care hospitals and beds for the entire country and by hospital type.

Case definitions

European case definitions for HAIs were used where these had been developed previously by HELICS or other European projects [35-38], whereas case definitions from the National Healthcare Safety Network (NHSN, formerly NNIS) at the United States Centers for Disease Control and Prevention (CDC) were used otherwise [39,40]. In the HAI section, data on microorganisms and the respective resistant phenotype were collected. Only results that were already available on the date of the survey were included.

For the purposes of this protocol, an infection was defined as active on the day of the survey when:

1. signs and symptoms were present on the date of the survey;
OR
2. signs and symptoms were no longer present but the patient was still receiving treatment for that infection on the date of the survey. In this case, the symptoms and signs occurring from the start of treatment until the date of the survey were checked to ascertain that the infection matched one of the case definitions of HAI.

An active infection was defined as healthcare-associated (associated to acute care hospital stay only, for the purpose of this protocol) when:

1. the onset of the signs and symptoms was on Day 3 of the current admission or later (with Day 1 the day of admission);
OR
2. the signs and symptoms were present at admission or became apparent before Day 3, but the patient had been discharged from an acute care hospital less than two days before admission;
OR
3. the signs and symptoms of an active surgical site infection were present at admission or started before Day 3, and the surgical site infection occurred within 30 days of a surgical intervention (or in the case of

surgery involving an implant, a deep or organ/space surgical site infection that developed within a year of the intervention);
OR
4. the signs and symptoms of a *Clostridium difficile* infection were present at admission or started before Day 3, with the patient having been discharged from an acute care hospital less than 28 days before the current admission.

For antimicrobial use, the Anatomical Therapeutic Chemical (ATC) classification system of the World Health Organization Collaborating Centre for Drug Statistics Methodology was used [41]. Antimicrobial agents for systemic use within the ATC groups A07AA (intestinal anti-infectives), D01BA (dermatological antifungals for systemic use), J01 (antibacterials for systemic use), J02 (antimycotics for systemic use), J04AB02 (rifampicin) and P01AB (nitroimidazole-derived antiprotozoals) were included. Antiviral agents and antimicrobials for the treatment of tuberculosis were not included.

As in the former ESAC hospital PPS protocol [23-26], antimicrobial treatment was recorded if, at the time of survey, the antimicrobial agent was still prescribed on the treatment chart. In the case of surgical prophylaxis, any single dose of an antimicrobial agent given within the 24-hour period before 8:00 am on the day of the survey was recorded. This time window for surgical prophylaxis allowed making the distinction between single dose prophylaxis, one day prophylaxis, or prophylactic doses given over more than one day.

Data collection and inclusion criteria

Two data collection protocols were available for use by participating hospitals. The first was patient-based: Denominator data, including risk factors, were collected for each individual patient irrespective of whether the patient had a HAI and/or received antimicrobials. The patient form for this protocol also included more detailed information, such as the presence of invasive devices, the specialty area of the patient's disease or consultant in charge of the patient and the McCabe score (the McCabe score classifies the severity of underlying medical conditions) [42]. The second protocol was unit-based: Denominator data were aggregated at ward level, and a patient form was used only for patients with a HAI and/or receiving antimicrobials. For both protocols, data were also collected at both ward level (ward name and specialty) and hospital level, including hospital type, size and whether or not any wards were excluded from the survey.

Each participating hospital had to choose one of the two data collection protocols. For each ward, all patients registered on the ward census before 8:00 am and not discharged from the ward at the time of the survey were assessed. Patients who were temporarily absent from the ward (e.g. for medical imaging, endoscopy, surgery) were included in the survey. Day admissions, outpatients (including patients attending the hospital for haemodialysis) and patients at the Accident and Emergency department were excluded. In addition, given that the agreed objective of the EU-wide ECDC PPS was to estimate the burden of HAIs and antimicrobial use in acute care hospitals only, long-term care units in acute care hospitals were excluded from the survey; however, long-term patients within an acute care ward were included. It was recommended that each participating hospital should include all eligible patients in the survey. Despite this recommendation, five of the 66 hospitals excluded one or several wards that were eligible for inclusion, because the hospital staff considered that being exhaustive was not needed for a pilot study.

The ECDC pilot PPS protocol recommended that personnel experienced in reading patient charts/notes and in identifying HAIs (e.g. infection control professionals, clinical microbiologists, infectious disease physicians) should act as survey team leaders in the hospitals. To obtain better information, collaboration with the clinical team in charge of patient care was recommended rather than exclusively reading the patient chart/notes and laboratory results. The number and type of healthcare workers (HCWs) performing the PPS in the hospital was assessed by questionnaire.

Data collectors in the hospital were trained by the national PPS coordinators to become familiar with the protocol and case definitions. Training material in English language was provided by ECDC through a contract with the Health Protection Agency, London (contract ECD.1842).

Time window

The ECDC pilot PPS had to be carried out any time between May and October 2010. The ideal duration of a 'point' prevalence survey is a single day but this was not feasible for the majority of participants due to the size of the hospital and/or the lack of trained personnel. To ensure feasibility of the survey, the maximum total time allowed to complete data collection in each hospital was three weeks and preferably not more than two weeks. Each individual ward, and if possible each respective department (e.g. all medical wards), had to be surveyed on the same day.

Data entry

Each country was free to organise its own system for data entry and processing, as long as all variables were collected in accordance with the ECDC methodology. It was not possible for a hospital to use a mixture of the patient-based and unit-based protocols. Most hospitals entered their data directly into an adapted version of the ESAC WebPPS located on the server of the University of Antwerp [24,25]. Only one country (Slovenia), participating with two hospitals, used its local software, whilst Belgium used the WebPPS installed on the server of the Belgian Scientific Institute for Public Health (WIV-ISP) in Brussels. Belgian data were uploaded on the WIV-ISP server and were

later incorporated into the European data set at the University of Antwerp. Data from Slovenia were converted by ECDC and then transferred to the University of Antwerp for incorporation into the central database.

Feasibility and workload

An additional feasibility questionnaire was sent to the national contact points of the 23 participating countries and to the corresponding 66 hospital contact points. At the national level, we requested information about whether a list of hospitals by type (primary, secondary, tertiary and specialised) and size was available, thus assessing the feasibility of a systematic sampling design using these variables in future surveys. National contact points were also asked to give any other feedback regarding the feasibility of obtaining a representative sample of hospitals in their country. In addition, data about the workload needed for training, data collection and data entry were requested both at the national and hospital level. The number and type of HCWs involved in the survey were also collected.

Data analysis

Data were analysed at the University of Antwerp and at ECDC using Stata 10.1 (StataCorp Texas, US). Binomial exact confidence limits were calculated where appropriate. Risk factor analysis was performed separately for HAIs and for antimicrobial use using multiple logistic regression. Presence of a peripheral and central vascular catheter were excluded from the multiple logistic regression model since the time relationship between insertion of a catheter and start of parenteral antimicrobial use cannot be deduced from the protocol. In both models, p values below 0.05 were considered as statistically significant. Individual hospital reports (Microsoft Excel spreadsheets) summarising the hospital's prevalence figures, compared to the aggregated prevalence figures of all participating hospitals in the country, were produced by ECDC using Stata 10.1 and sent to the national contact points for further distribution and feedback to the hospital contact points. We did not receive any feedback from the hospitals that these reports were not concordant with local hospital data.

Results

A total of 19,888 patients from 66 hospitals in 23 countries were included in the ECDC pilot PPS. Fifty hospitals used the patient-based protocol and 16 hospitals used the unit-based protocol.

Hospital characteristics were available for 65 hospitals. University or other teaching hospitals (defined as 'tertiary' hospitals in the protocol) represented 52.3% of participating hospitals, secondary hospitals 24.6%, primary hospitals 15.4% and specialised hospitals 7.7%, with an average hospital size of 614 beds, 431 beds, 215 beds and 300 beds, respectively. The overall average hospital size in the study sample was 483 beds (median: 400 beds). At national level, only 13 countries (representing 29 hospitals in the study sample) were able to provide national numbers of hospitals by type. Tertiary hospitals represented 7.7% of all acute care hospitals in these countries, secondary hospitals 31.1%, primary hospitals 49.3% and specialised hospitals 11.9%. The total number of hospitals in these 13 countries was 2,609 with on average 298

Table 1

Prevalence of healthcare-associated infections and antimicrobial use in surveyed patients, by specialty, during the ECDC pilot point prevalence survey, 2010 (n=19,888)

Specialty	Surveyed patients		Patients with HAI[a]		Patients with antimicrobial use[b]	
	n[c]	%[d]	n[c]	%[e]	n[c]	%[e]
Surgery	6,653	33.5	518	7.8	2,584	38.8
Medicine	7,833	39.4	505	6.4	2,888	36.9
Paediatrics	1,024	5.1	38	3.7	310	30.3
Intensive care	915	4.6	257	28.1	562	61.4
Obstetrics and Gynaecology	1,711	8.6	32	1.9	313	18.3
Geriatrics	502	2.5	33	6.6	117	23.3
Psychiatry	828	4.2	2	0.2	18	2.2
Other/mixed	422	2.1	23	5.5	83	19.7
All specialties	**19,888**	**100**	**1,408**	**7.1**	**6,875**	**34.6**

ECDC: European Centre for Disease Prevention and Control; HAI: healthcare-associated infection.

[a] Patients with a least one HAI.
[b] Patients receiving at least one antimicrobial agent.
[c] Number of patients in category.
[d] Percentage of total (column percent).
[e] Percentage within category (category percent).

beds (median: 261 beds), for a total population of 160 million inhabitants in 2010.

Healthcare-associated infections

Overall, 7.1% patients had at least one HAI, ranging from 0.2% in psychiatry to 28.1% in intensive care departments (Table 1). The prevalence of HAIs was 5.8% in primary hospitals, 6.3% in secondary hospitals, 7.4% in tertiary hospitals and 7.8% in specialised hospitals.

The most common type of HAI was pneumonia and other lower respiratory tract infections, representing 25.7% of all reported HAIs (Table 2). The second most frequently reported type of HAI was surgical site infection (18.9%), followed by urinary tract infection (17.2%), bloodstream infection (14.2%) and gastro-intestinal infection (7.8%). *Clostridium difficile* infections represented 1.4% of all HAIs. On average, there were 1.09 HAIs per infected patient (or a total of 1,531 HAIs in 1,408 patients with HAI). The median length of stay before onset of HAI acquired during the current hospitalisation (n=1,159) was 12 days (range: 4–65 days). Of 372 (24%) HAIs present at admission, 58% were associated with a previous stay in the same hospital.

For 59.1% of the HAIs, a positive microbiology result was available, ranging from 40.3% for gastro-intestinal infections to 94.0% in bloodstream infections (Table 3).

The most commonly isolated groups of microorganisms were Gram-negative non-*Enterobacteriaceae* in pneumonia (36.5%), *Enterobacteriaceae* in urinary tract infections (63.8%) and Gram-positive cocci in surgical site infections (54.3%). Overall, the most commonly isolated microorganism was *Escherichia coli* (15.2% overall, and 37.1% in urinary tract infections), followed by *Staphylococcus aureus* (12.1% overall and 21.5% in surgical site infections).

Carbapenem resistance was reported in 3.2% of *Enterobacteriaceae*, 23.4% of *Pseudomonas aeruginosa* and 20.4% of *Acinetobacter* spp. The percentage of meticillin-resistant *S. aureus* (MRSA) was 34.2% and that of glycopeptide-resistant *Enterococcus* spp. was 5.4%.

Antimicrobial use

A total of 6,875 patients (34.6%) received at least one antimicrobial agent at the time of the survey, ranging from 2.2% in psychiatry to 61.4% in intensive care departments (Table 1). The prevalence of antimicrobial use was 36.2% in primary hospitals, 32.1% in secondary hospitals, 35.7% in tertiary hospitals and 28.7% in specialised hospitals. Analysing the antimicrobial agents used by main indication (treatment, surgical prophylaxis and medical prophylaxis) revealed differences in the use of different antimicrobial classes (Table 4).

Pneumonia or other lower respiratory tract infection was the most common indication (29.2%) for antimicrobial treatment, and accounted for 31.6% of intentions for treatment of community infection, and 24.8% of intentions for treatment of hospital infection.

The most widely used antimicrobial agents at ATC 4th level were combinations of penicillins with beta-lactamase inhibitors (16.3%), mainly for treatment intention (18.0%). For surgical prophylaxis, first- and second-generation cephalosporins were mostly chosen: 26.8% and 20.0%, respectively. For medical prophylaxis, fluoroquinolones, primarily ciprofloxacin, were the most widely used antimicrobial agents.

Table 5 summarises the indications for antimicrobial use, their route of administration and whether the reason for antimicrobial use was indicated on the patient chart. Community infection was the most common treatment intention (41.3%), followed by hospital infection (24.0%). Surgical prophylaxis (17.3%) was prolonged for more than one day in 60.7% of cases. Medical prophylaxis accounted for 13.5% of antimicrobial use. The parenteral route of administration was used for 71.9% of administered antimicrobial agents. A reason was included in the chart of 69.3% of the patients on antimicrobials (Table 5).

Risk factors

Data from the 50 hospitals that used the patient-based protocol, including patient characteristics and risk factors, are shown in Table 6. Using multiple logistic regression, the presence of an HAI was independently associated with age (highest adjusted odds ratio in children under five years-old, $p<0.001$), male sex ($p<0.05$), length of stay before onset of HAI (p for trend<0.001), the McCabe score (p for trend<0.001), the number of invasive devices (urinary catheter and intubation) before onset of infection (p for trend<0.001) and surgery since admission ($p<0.001$). Antimicrobial use was independently associated with age (highest adjusted odds ratio in the age category 1–4 years, $p<0.001$), male sex ($p<0.001$), the McCabe score (p for trend<0.001), the number of invasive devices (urinary catheter and intubation, p for trend <0.001), length of stay in the hospital (p for trend<0.05) and surgery since admission ($p<0.001$).

Feasibility

Thirteen countries (Belgium, Bulgaria, Cyprus, Estonia, France, Greece, Italy, Lithuania, Malta, Portugal, Romania, Slovakia and Spain) responded to the national feasibility questionnaire. Fifty hospitals responded to the hospital feasibility questionnaire.

Overall, the average number of HCW involved in data collection, excluding ward staff, was six, with a maximum of 21. In five hospitals, one single HCW was involved in the data collection process. Ward staff was involved in 20 hospitals. On average per hospital, 3,7 different types of HCW were involved in the survey for

Table 2

Prevalence of healthcare-associated infections and antimicrobial use in surveyed patients, by specialty, during the ECDC pilot point prevalence survey, 2010 (n=19,888)

Type of infection	HAIs				Antimicrobial use (treatment only)a					
					All treatment intentions[b]		Treatment intended for community infection		Treatment intended for hospital	
	n patients[c]	% patients [95% CI][d]	n HAIs[e]	Relative % HAIs[f]	n intentions	Relative %	n intentions	Relative %	n intentions	Relative %
Pneumonia or other lower respiratory tract infection	392	2.0 [1.8–2.2]	394	25.7	1,328	29.2	922	31.6	382	24.8
Surgical site infection	290	1.5 [1.3–1.6]	290	18.9	–[g]	–[g]	–[g]	–[g]	–[g]	–[g]
Urinary tract infection	263	1.3 [1.2–1.5]	264	17.2	679	14.9	412	14.1	237	15.4
Bloodstream infection (BSI)[h]	216	1.1 [0.9–1.2]	217	14.2	219	4.8	67	2.3	145	9.4
Gastrointestinal infection	118	0.6 [0.5–0.7]	119	7.8	593	13.0	466	16.0	117	7.6
Skin and soft tissue infection	59	0.3 [0.2–0.4]	59	3.9	646	14.2	357	12.2	279	18.1
Bone or joint infection	38	0.2 [0.1–0.3]	39	2.5	154	3.4	92	3.2	60	3.9
Eye, ear, nose or mouth infection	47	0.2 [0.2–0.3]	47	3.1	211	4.6	170	5.8	41	2.7
Systemic infection[h]	40	0.2 [0.1–0.3]	40	2.6	668	14.7	318	10.9	334	21.7
Cardiovascular system infection	26	0.1 [0.1–0.2]	26	1.7	76	1.7	40	1.4	36	2.3
Central nervous system infection	15	0.1 [0.0–0.1]	15	1.0	67	1.5	54	1.8	12	0.8
Catheter-related infections without bloodstream infection	11	0.1 [0.0–0.1]	11	0.7	–[g]	–[g]	–[g]	–[g]	–[g]	–[g]
Reproductive tract infection	10	0.1 [0.0–0.1]	10	0.7	65	1.4	49	1.7	16	1.0
Missing/unknown	0	NA	NA	NA	65	1.4	39	1.3	25	1.6
Total	**1,408**	**7.1 [6.7–7.5]**	**1,531**	**100**	**4,552**	**100**	**2,919**	**100**	**1,539**	**100**

CI: confidence interval; ECDC: European Centre for Disease Prevention and Control; HAI: healthcare-associated infection; NA: not applicable.

[a] This table does not include antimicrobials used for prophylaxis or for unknown indications (shown in Table 5).

[b] The category "Treatment intended for infections acquired in long-term care facilities" represented 2.0% of all treatment intentions and is not shown in the table.

[c] Number of patients with HAI (site-specific number)

[d] Percentage of patients with HAI (site-specific prevalence)

[e] Number of HAIs.

[f] Percentage of total number of HAIs (relative percentage)

[g] For used antimicrobials, the types of infection 'surgical site infection' and 'catheter-related infection without bloodstream infection' were not specifically recorded and could be included within the category 'skin and soft tissue infection'.

[h] Includes catheter-related infections with positive blood culture, and neonatal bloodstream infections and clinical sepsis. For used antimicrobials, some bloodstream infections (bacteraemia) may have been included in the category 'systemic infection'.

Table 3

Distribution of microorganisms isolated in healthcare-associated infections, by main type of infection, ECDC pilot point prevalence survey, 2010 (n=1,165)

	All types of infection	Pneumonia or other lower respiratory tract infection	Surgical site infection	Urinary tract infection	Bloodstream infection	Gastrointestinal infection
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
HAIs and microorganisms						
HAIs, total	1,531 (100)	394 (25.7)	290 (18.9)	264 (17.2)	200 (13.1)	119 (7.8)
HAIs with microorganisms	905 (59.1)	191 (48.5)	172 (59.3)	187 (70.8)	188 (94.0)	48 (40.3)
Microorganisms, total	1,165 (100)	249 (100)	247 (100)	210 (100)	228 (100)	65 (100)
Major groups of microorganisms						
Gram-positive cocci	410 (35.2)	46 (18.5)	134 (54.3)	39 (18.6)	95 (41.7)	21 (32.3)
Enterobacteriaceae	404 (34.7)	80 (32.1)	58 (23.5)	134 (63.8)	79 (34.7)	18 (27.7)
Gram-negative bacteria, non-*Enterobacteriaceae*	226 (19.4)	91 (36.5)	36 (14.6)	29 (13.8)	30 (13.2)	7 (10.8)
Fungi	69 (5.9)	23 (9.2)	5 (2.0)	7 (3.3)	17 (7.5)	4 (6.2)
Top 15 microorganisms (accounting for (92.4% of total number microorganisms)						
Escherichia coli	177 (15.2)	24 (9.6)	29 (11.7)	78 (37.1)	29 (12.7)	10 (15.4)
Staphylococcus aureus	141 (12.1)	26 (10.4)	53 (21.5)	2 (1.0)	26 (11.4)	5 (7.7)
Pseudomonas aeruginosa	131 (11.2)	44 (17.7)	24 (9.7)	21 (10.0)	17 (7.5)	6 (9.2)
Enterococcus spp.	114 (9.8)	4 (1.6)	33 (13.4)	32 (15.2)	21 (9.2)	11 (16.9)
Coagulase-negative staphylococci	97 (8.3)	3 (1.2)	33 (13.4)	3 (1.4)	38 (16.7)	1 (1.5)
Klebsiella spp.	94 (8.1)	22 (8.8)	7 (2.8)	30 (14.3)	25 (11.0)	3 (4.6)
Candida spp.	56 (4.8)	15 (6.0)	3 (1.2)	6 (2.9)	16 (7.0)	3 (4.6)
Enterobacter spp.	49 (4.2)	13 (5.2)	10 (4.0)	6 (2.9)	10 (4.4)	1 (1.5)
Acinetobacter spp.	49 (4.2)	18 (7.2)	5 (2.0)	5 (2.4)	9 (4.0)	1 (1.5)
Streptococcus spp.	45 (3.9)	13 (5.2)	11 (4.5)	2 (1.0)	4 (1.8)	4 (6.2)
Proteus spp.	35 (3.0)	5 (2.0)	6 (2.4)	15 (7.1)	4 (1.8)	0 (0)
Anaerobic bacilli	24 (2.1)	1 (0.4)	5 (2.0)	0 (0)	5 (2.2)	11 (16.9)
Serratia spp.	17 (1.5)	11 (4.4)	1 (0.4)	0 (0)	5 (2.2)	0 (0)
Other *Enterobacteriaceae*	17 (1.5)	3 (1.2)	0 (0)	1 (0.5)	4 (1.8)	3 (4.6)
Stenotrophomonas maltophilia	16 (1.4)	11 (4.4)	3 (1.2)	0 (0)	1 (0.4)	0 (0)
Citrobacter spp.	15 (1.3)	2 (0.8)	5 (2.0)	4 (1.9)	2 (0.9)	1 (1.5)

ECDC: European Centre for Disease Prevention and Control; HAI: healthcare-associated infection.

The table only shows details for the main infection types. The total also includes all other HAI types.

Table 4

Distribution of antimicrobial agents (ATC 4th and 5th levels) by main indication for use, ECDC pilot point prevalence survey, 2010 (n=9,588 antimicrobial agents)

	All indications	Treatment	Surgical prophylaxis	Medical prophylaxis
	n (%)	n (%)	n (%)	n (%)
Antimicrobial agents, total	**9,588 (100)**	**6,365 (100)**	**1,654 (100)**	**1,293 (100)**
Top antimicrobial agents at ATC 4th level (accounting for 93.1% of use)				
Combinations of penicillins, incl. beta-lactamase inhibitors (J01CR)	1,566 (16.3)	1,147 (18.0)	217 (13.1)	145 (11.2)
Fluoroquinolones (J01MA)	1,293 (13.5)	948 (14.9)	133 (8.0)	168 (13.2)
Second-generation cephalosporins (J01DC)	900 (9.4)	475 (7.5)	330 (20.0)	76 (5.9)
Third-generation cephalosporins (J01DD)	701 (7.3)	521 (8.2)	94 (5.7)	67 (5.2)
First-generation cephalosporins (J01DB)	599 (6.2)	121 (1.9)	444 (26.8)	23 (1.8)
Carbapenems (J01DH)	583 (6.1)	503 (7.9)	25 (1.5)	37 (2.9)
Imidazole derivatives (J01XD)	494 (5.2)	278 (4.4)	151 (9.1)	51 (3.9)
Glycopeptide antibacterials (J01XA)	449 (4.7)	365 (5.7)	41 (2.5)	31 (2.4)
Aminoglycosides (J01GB)	427 (4.5)	277 (4.4)	72 (4.4)	69 (5.3)
Triazole derivatives (J02AC)	424 (4.4)	246 (3.9)	11 (0.7)	153 (11.8)
Penicillins, extended spectrum without anti-pseudomonal activity (J01CA)	289 (3.0)	200 (3.1)	18 (1.1)	65 (5.0)
Combinations of sulfonamides and trimethoprim, incl. derivatives (J01EE)	252 (2.6)	70 (1.1)	7 (0.4)	163 (12.6)
Lincosamides (J01FF)	232 (2.4)	183 (2.9)	38 (2.3)	11 (0.9)
Macrolides (J01FA)	185 (1.9)	144 (2.3)	4 (0.2)	26 (2.0)
Beta-lactamase-resistant penicillins (J01CF)	160 (1.7)	138 (2.2)	16 (1.0)	5 (0.4)
Nitroimidazole derivatives (P01AB)	134 (1.4)	102 (1.6)	17 (1.0)	9 (0.7)
Beta-lactamase-sensitive penicillins (J01CE)	133 (1.4)	90 (1.4)	9 (0.5)	32 (2.5)
Other antibacterials (J01XX)	102 (1.1)	80 (1.3)	4 (0.2)	11 (0.9)
Top antimicrobial agents at ATC 5th level (accounting for 70.8% of use)				
Amoxicillin and enzyme inhibitor (J01CR02)	1,045 (10.9)	696 (10.9)	193 (11.7)	104 (8.0)
Cefuroxime (J01DC02)	866 (9.0)	466 (7.3)	318 (19.2)	63 (4.9)
Ciprofloxacin (J01MA02)	844 (8.8)	607 (9.5)	100 (6.0)	113 (8.7)
Metronidazole (J01XD01)	493 (5.1)	277 (4.4)	151 (9.1)	51 (3.9)
Cefazolin (J01DB04)	473 (4.9)	57 (0.9)	396 (23.9)	12 (0.9)
Piperacillin and enzyme inhibitor (J01CR05)	432 (4.5)	374 (5.9)	19 (1.1)	36 (2.8)
Ceftriaxone (J01DD04)	396 (4.1)	282 (4.4)	52 (3.1)	47 (3.6)
Vancomycin (parenteral) (J01XA01)	376 (3.9)	310 (4.9)	36 (2.2)	26 (2.0)
Meropenem (J01DH02)	375 (3.9)	322 (5.1)	9 (0.5)	29 (2.2)
Fluconazole (J02AC01)	319 (3.3)	201 (3.2)	11 (0.7)	96 (7.4)
Levofloxacin (J01MA12)	310 (3.2)	246 (3.9)	13 (0.8)	34 (2.6)
Gentamicin (J01GB03)	265 (2.8)	151 (2.4)	62 (3.7)	46 (3.6)
Sulfamethoxazole and trimethoprim (J01EE01)	235 (2.5)	66 (1.0)	7 (0.4)	150 (11.6)
Clindamycin (J01FF01)	228 (2.4)	183 (2.9)	34 (2.1)	11 (0.9)
Imipenem and enzyme inhibitor (J01DH51)	141 (1.5)	120 (1.9)	11 (0.7)	7 (0.5)

ATC: Anatomical Therapeutic Chemical; ECDC: European Centre for Disease Prevention and Control; HAI: healthcare-associated infection.

The category "Unknown indication" represented 2.9% of the total and is included in the first column.

Table 5

Antimicrobial use: prevalence, indication, route of administration and reason in patient charts/notes, ECDC pilot point prevalence survey, 2010 (n=6,875 patients)

	Patients with antimicrobial use[a]		Antimicrobial agents	
	n	%[b] [95% CI]	n	Relative %[c]
Total	**6,875**	**34.6 [33.8–35.4]**	**9,588**	**100**
Indication				
Treatment	4,500	22.6 [22.0–23.3]	6,365	66.4
Intended for community infection	2,919	14.7 [14.1–15.2]	3,957	41.3
Intended for hospital infection	1,539	7.7 [7.–-8.1]	2,300	24.0
Intended for other healthcare-associated infection	94	0.5 [0.4–0.6]	108	1.1
Surgical prophylaxis	1,396	7.0 [6.7–7.4]	1,654	17.3
Single dose	336	1.7 [1.5–1.9]	357	3.7
One day	265	1.3 [1.2–1.5]	293	3.1
More than one day	810	4.1 [3.8–4.4]	1,004	10.5
Medical prophylaxis	979	4.9 [4.6–5.2]	1,293	13.5
Unknown indication	211	1.1 [0.9–1.2]	276	2.9
Route of administration				
Parenteral	5,098	25.6 [24.9–26.3]	6,891	71.9
Oral	2,218	11.2 [10.7–11.6]	2,648	27.6
Other/unknown	49	0.2 [0.2–0.3]	49	0.5
Reason in patient charts/notes				
Yes	4,819	24.2 [23.6–24.9]	6,647	69.3
No	2,171	10.9 [10.5–11.4]	2,939	30.7
Unknown	2	0.0 [0.0–0.0]	2	0.0

CI: confidence interval; HAI: healthcare-associated infection.

[a] Patients receiving a least one antimicrobial agent.
[b] Prevalence of antimicrobial use in each category.
[c] Percentage of total number of antimicrobials (relative frequency).

Table 6

Prevalence of healthcare-associated infections and antimicrobial use, by patient risk factors (standard patient-based protocol only, 50 hospitals), ECDC pilot point prevalence survey, 2010 (n=14,329)

	Surveyed patients		Patients with HAIs[a]		Patients with antimicrobial use[b]	
	n[c]	%[d]	n	%[e]	n	%[e]
All patients	**14,329**	**100**	**1,072**	**7.5**	**5,201**	**36.3**
Age group (years)						
<1	746	5.2	58	7.8	181	24.3
1–4	267	1.9	18	6.7	135	50.6
5–14	393	2.7	12	3.1	148	37.7
15–24	699	4.9	30	4.3	228	32.6
25–34	1,224	8.5	34	2.8	313	25.6
35–44	1,160	8.1	75	6.5	385	33.2
45–54	1,527	10.7	106	6.9	570	37.3
55–64	2,325	16.2	212	9.1	939	40.4
65–74	2,582	18.0	241	9.3	1,012	39.2
75–84	2,481	17.3	202	8.1	903	36.4
≥85	925	6.5	84	9.1	387	41.8
Sex						
Female	7,267	50.7	456	6.3	2,364	32.5
Male	7,062	49.3	616	8.7	2,837	40.2
Length of stay (days)[f]						
1–3	4,622	32.3	104	2.3	1,352	29.3
4–7	3,916	27.3	300	7.7	1,608	41.1
8–14	2,824	19.7	272	9.6	1,137	40.3
>14	2,966	20.7	396	13.4	1,104	37.2
Surgical intervention since hospital admission						
No	10,089	70.4	569	5.6	3,163	31.4
Yes	4,240	29.6	503	11.9	2,038	48.1
McCabe score						
Non-fatal	9,705	67.7	491	5.1	3,088	31.8
Ultimately fatal	3,666	25.6	430	11.7	1,645	44.9
Rapidly fatal	791	5.5	143	18.1	419	53.0
Missing/unknown	167	1.2	8	4.8	49	29.3
Central vascular catheter						
No	12,621	88.1	651	5.2	4,033	32.0
Yes	1,594	11.1	411	25.8	1,117	70.1
Missing/unknown	114	0.8	10	8.8	51	44.7
Peripheral vascular catheter						
No	7,455	52.0	389	5.2	1,565	21.0
Yes	6,763	47.2	674	10.0	3,592	53.1
Missing/unknown	111	0.8	9	8.1	44	39.6
Urinary catheter						
No	11,702	81.7	612	5.2	3,594	30.7
Yes	2,512	17.5	452	18.0	1,558	62.0
Missing/unknown	115	0.8	8	7.0	49	42.6
Intubation						
No	13,734	95.8	888	6.5	4,775	34.8
Yes	486	3.4	173	35.6	369	75.9
Missing/unknown	109	0.8	11	10.1	57	52.3

ECDC: European Centre for Disease Prevention and Control; HAI: healthcare-associated infection.

[a] Patients with a least one HAI.
[b] Patients receiving at least one antimicrobial agent.
[c] Number of patients in category.
[d] Percentage of total (column percent).
[e] Percentage within category (category percent).
[f] Length of stay until onset of HAI in case of HAI during current hospitalisation.

data collection and 1.3 for data entry. Eighteen hospitals were surveyed by an external team (either national or regional coordination staff) (Table 7).

A large variation among responding countries was identified in the workload associated with the PPS. The calculation of workload included preparation and training before the actual PPS, as well as data collection and data entry. National PPS coordinators provided on average 12.4 hours (median: 6 hours) of training to the hospital staff and spent on average an additional 6.5 hours (median: 4 hours) on answering questions during the survey. The time needed for collection and entry of data for 100 patients, was estimated at about four working days (ca. 32 hours) with the patient-based protocol and about 2.5 working days (ca. 20 hours) with the unit-based protocol. This means that performing the survey with the unit-based protocol took about 37.5% less time than with the patient-based protocol.
The feasibility of the data collection was also evaluated by the analysis of missing data in the database. At the national level, 11 of 23 countries were unable to provide national hospital denominator data by hospital type as defined in the protocol. At hospital level however, the hospital type was always available and the number of beds was only missing for one hospital. Ward level data were complete because all fields were mandatory in the software. Similarly, some patient level data (age, sex, hospital admission date and medical specialty of the patient's disease or the consultant), infection data and antimicrobial use data were mandatory in the software. For the other, non-mandatory variables of the patient-based protocol (n=14,329 patients), the percentage of missing values ranged from less than 1% for the presence of invasive devices, 1.2% for McCabe score, and 1.9% for surgery since admission, to 7.6% for surgery in the previous 30 days.

Discussion

The ECDC pilot PPS of HAIs and antimicrobial use was successfully performed from May to October 2010 in 66 acute care hospitals from 23 countries. In total, 19,888 patients were surveyed. The number of participating hospitals was higher than the anticipated minimum of 25 hospitals. The collected data allowed for the estimation of the prevalence of HAIs and antimicrobial use, which was the primary objective set by ECDC. Both the patient-based protocol, preferred by the majority (76%) of hospitals, and the unit-based protocol (applied by 24% of hospitals) provided the necessary data.

Main study limitations

An important limitation of our study is that the hospitals participating in this ECDC pilot PPS were not representative of the total hospital patient population in the EU. Hospitals were not randomly selected, and

Table 7

Type of healthcare workers involved in data collection and data entry for the ECDC pilot point prevalence survey, 2010 (n=50 hospitals)

Type of healthcare worker	Hospitals where this type of healthcare worker was involved		Involved in data collection		Involved in data entry	
	n	%[a]	n	%[b]	n	%[b]
Infection control nurse	25	50	25	100	9	36
Infection control physician or equivalent	31	62	31	100	12	39
Ward nurse	18	36	18	100	0	0
Ward physician	15	30	15	100	0	0
Infectious disease physician	12	24	12	100	3	25
Hospital microbiologist	6	12	6	100	3	50
Medical specialist trainee	10	20	10	100	2	20
Hospital pharmacist	6	12	6	100	1	17
Infection control link nurse	5	10	5	100	1	20
Data nurse	4	8	3	75	2	50
Nurse aid	1	2	0	0	1	100
Medical student	1	2	1	100	0	0
Other hospital staff	10	20	6	60	6	60
National PPS coordination staff	13	26	12	92	6	46
Regional PPS coordination staff	5	10	5	100	2	40
Other	6	12	4	67	3	50

ECDC: European Centre for Disease Prevention and Control; PPS: point prevalence survey.

[a] Percentage of total number of responding hospitals (n=50).
[b] Percentage of number of healthcare workers in category.

tertiary or teaching hospitals were overrepresented in the study sample (52.3% instead of less than 10%, according to available national hospital statistics). This selection had consequences both for the results of the feasibility test of the protocol and for the interpretation of the epidemiological results of the study (see below).

In addition, since inference from the epidemiological study results to the total acute care hospital population in Europe was not an objective of the pilot study, we did not apply any statistical methods that could take into account the effects of the hierarchical design of the study (e.g. regions within countries, hospitals within regions, wards within hospitals, and types of patients within wards). Methods such as multilevel modelling for risk factor analysis and complex survey analysis to adjust confidence intervals for the prevalence estimates at the national and EU level will be used to analyse the EU-wide PPS of HAIs and antimicrobial use that was conducted in 2011–12. The pilot study database was also used to estimate the expected design effect (DEFF) for different average sizes of hospitals (patient clusters) in order to estimate the required sample size for each country in the EU-wide PPS [14]. The overall DEFF in the pilot PPS was 5.3 for the prevalence of HAIs and 22.7 for the prevalence of antimicrobial use, indicating indeed that the sample design for representative samples at the national level should be adjusted for the important clustering of the main survey outcomes within the hospitals.

Feasibility study

A minority of respondents to the feasibility questionnaire mentioned that the participating included hospitals in their country had had experience in performing PPSs and that it is unlikely that randomly selected hospitals would be able to participate in an ECDC EU-wide PPS. ECDC therefore provided training material to help national contact points improve the skills of hospital staff during preparation of the future EU-wide PPS. Part of this training material was already available before the pilot PPS and was used to organise the training of the hospital contact points in the current study.

Training is also of key importance for the standardisation of data collection in participating hospitals, including interpretation of the case definitions. The large variation in the number and type of HCWs involved in data collection for this pilot PPS (Table 7) illustrates the challenge of standardising data collection for an EU-wide PPS. For example, failure to consult the clinical team in charge of patient care during data collection, as recommended in the protocol, may impact on the ascertainment of variables such as the medical specialty of the patient's disease or of the consultant in charge of the patient (patient/consultant specialty), the McCabe score, the physician's motive for prescribing antimicrobials, or even the signs and symptoms of a suspected HAI. The fact that ward staff was not involved in the data collection in more than half of the hospitals may indeed indicate that physicians were not sufficiently consulted. Also, the fact that in 18 of the 66 hospitals the survey was performed by an external team may indicate that the pilot PPS was not always performed in real-life conditions since this scenario is unlikely to be a feasible option for the ECDC EU-wide PPS or a full-scale national PPS.

Another frequently mentioned feasibility issue was the difficulty to categorise hospitals at the national level according to the hospital types defined in the protocol (primary, secondary, tertiary and specialised). Information on hospital categories used in the different countries are needed for the future EU-wide PPS to ensure that all categories are represented proportionally in the national representative sample. In addition, national denominator data (e.g. number of hospitals and discharges per year) by hospital type would be needed (i) to extrapolate the PPS results by hospital type (category-specific burden estimates), and (ii) to adjust the national and EU burden estimates in case hospital types are not proportionally represented in the national samples. Only 13 of 23 countries were able to provide some categorisation of their national list of hospitals according to the categories of the protocol, using the national hospital type categories.

Therefore, for the purpose of drawing a representative systematic sample of hospitals for the EU-wide PPS, the standardised EU types of hospitals were replaced by the national hospital categories in the final protocol of the ECDC EU-wide PPS. This means that, for the analysis of the data collected in the ECDC EU-wide PPS, it will not be possible to stratify or adjust the estimates of the burden of HAIs and antimicrobial use (based on extrapolation to the total national denominator data) according to types of hospitals.

Patient-based versus unit-based protocol

Despite a higher workload, the patient-based protocol was used more often than the unit-based protocol, thus allowing a better description of patients and invasive procedures. During an expert meeting held in Brussels in November 2010, it was recommended that PPSs of HAIs and antimicrobial use should be carried out at least once every five years, and the patient-based protocol was selected as the preferred methodology for future PPSs [43]. This expert recommendation is anticipating the fact that, because of hospital changes and medical advances, a patient-based protocol would be required to allow for detailed adjustment for patient case-mix. The patient-based protocol allows for assessment of the prevalence of HAIs and antimicrobial use according to the presence or absence of various risk factors and enables categorisation of hospitals by patient case-mix at national and/or European level. Indeed, adjustment for patient case-mix has been used in other studies, including for outcomes in intensive care [44,45] and surgical patients [46], and for comparing HAI rates [47]. Patient-based PPSs can also be used to identify patient-related factors that influence

the prevalence of HAIs and thus help focus surveillance and infection prevention initiatives [48].

The unit-based protocol, however, will be kept, to offer a less labour-intensive option for countries and hospitals where human resources are limited. This protocol might also be more appropriate for very large hospitals and in situations that require repeated PPSs at short intervals. A limitation is that its only denominator variable is the number of patients per ward, for the total ward and for the specialty of each patient's disease within each ward. This only allows an estimation of the prevalence of HAIs and antimicrobial use by ward or patient's disease specialty.

The ECDC pilot PPS also aimed at identifying any issue with the methodology that required modification, e.g. availability of data for any of the collected variables, or applicability of the case definitions for HAIs, before finalising the patient-based and unit-based protocols for the ECDC EU-wide PPS that was started in May 2011. Denominator data in the unit-based protocol did not require any modification whereas, for the patient-based protocol, the only variable that was difficult to obtain was 'surgery in the previous 30 days'. This variable also overlapped with 'surgery since admission' which was less difficult to determine. It was therefore decided that, for the ECDC EU-wide PPS, the data for the variable 'surgery in previous 30 days' would eventually not be collected [14]. With respect to case definitions for HAIs, a major change was the decision to add the case definition of clinical sepsis in adults, because possible bloodstream infections for which microbiological results were not yet available at the time of the PPS would otherwise remain unreported.

Epidemiological results

The two sections of the ECDC pilot PPS, i.e. HAIs and antimicrobial use, were independent of each other and did not follow the same definitions: data on HAIs were recorded following standardised epidemiological case definitions, whilst the indication for antimicrobial use was based on clinical judgment by the treating physician. For example, a patient could have been registered in the antimicrobial use section as receiving antimicrobials with the intention to treat a hospital infection, but the same patient did not fulfil the case definition for HAI and therefore was not included as having a HAI in the HAI section. Conversely, a patient may have presented the symptoms and signs of a HAI, but not have been treated with an antimicrobial. Hence, among other things, the different proportions for hospital-acquired pneumonia in Table 2.

While the protocol for the EU-wide PPS foresees a representative systematic random sample of hospitals in the participating countries [14], the data collected through this ECDC pilot PPS were not representative of the epidemiology of HAIs in the EU and the results must be interpreted with caution. The HAI prevalence of 7.1% (inter-quartile range: 4.2–9.4%) observed in our study is likely to be slightly overestimated because of the overrepresentation of tertiary hospitals which had a higher prevalence of HAIs (7.4%) than secondary and primary hospitals. Nevertheless, the overall HAI prevalence in this pilot PPS is comparable to that reported in other European studies [9,11,12] and to the European prevalence of HAIs of 7.1%, estimated by ECDC based on a review of 30 national or multicentre PPSs in 19 countries in its Annual Epidemiological Report for 2008 [1]. The range of reported prevalence results in studies that used CDC definitions for HAIs in non-EU countries, ranged from 4.9% in Mauritius in 1992 to 19.1% in Malaysia in 2001 [30]. Such a wide range in the prevalence of HAIs could be explained by differences in methodology and patient case-mix, and should not immediately be interpreted as an indication of variations in performance.

The distribution of isolated microorganisms in patients with HAI in this pilot PPS was also similar to that previously reported in the review of national or multicentre point prevalence surveys, with *E. coli* being most frequent [1]. The fact that only 59.1% of the HAIs were documented by microbiological results was also in line with previous findings [9,49,50] and was expected because, with few exceptions, case definitions of HAIs are primarily based on clinical criteria.

With respect to antimicrobial use, the ECDC pilot PPS showed a prevalence about 5% higher than shown by previous ESAC hospital PPSs using an identical methodology [23,25,26]. Nevertheless, the ranking order of the most used antimicrobials was comparable to that observed in ESAC hospital PPSs, with the various beta-lactams (penicillins, cephalosporins and carbapenems) accounting for more than half of all antimicrobials used. Other PPSs have reported a wide range of prevalence of antimicrobial use in acute care hospitals due to varying inclusion criteria [23].

A final aspect that should be considered for the interpretation of the epidemiological results of this and future surveys is the fact that the ECDC pilot PPS was not performed on a single day. For feasibility reasons, hospitals were allowed to organise the PPS within a period of three weeks, with the only restriction being that a ward had to be surveyed on a single day. In practice, hospitals and countries performed the pilot PPS survey from May until October 2010. For the EU-wide PPS, ECDC agreed with the national PPS coordinating centres in November 2010 on three possible periods to organise the first national PPS using the ECDC methodology [43]. These periods (May–June 2011, September–October 2011 and May–June 2012) were selected to avoid the winter period because of the higher incidence of respiratory tract infections and the summer holiday period because shortage of staff and lower activity in the hospital during this period could influence the practical organisation as well as the main outcomes of the survey. Despite these considerations, the potentially long time span between the different

surveys may influence comparability of the results between hospitals, regions or countries, e.g. because of rapidly changing incidences of HAIs with epidemic pathogens or the implementation of local or national infection control measures.

In conclusion, the ECDC pilot PPS methodology was successfully implemented by the national contact points, the hospital contact points and the HCWs involved in data collection and entry in the participating hospitals, without any major feasibility issues that could have led hospitals to cancel their participation. The pilot PPS showed that the aim of estimating the burden of HAIs and antimicrobial use in European acute care hospitals was realistic, irrespective of the protocol used. The patient-based protocol, even if more resource-intensive, was used more widely and provided more detailed and valuable data than the unit-based protocol. It was therefore selected as the preferred option for the ECDC EU-wide PPS of HAIs and antimicrobial use.

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References

1. European Centre for Disease Prevention and Control (ECDC). Annual epidemiological report on communicable diseases in Europe 2008. Stockholm: ECDC; 2008. Available from: http://ecdc.europa.eu/en/publications/Publications/0812_SUR_Annual_Epidemiological_Report_2008.pdf
2. Agodi A, Auxilia F, Barchitta M, Brusaferro S, D'Alessandro D, Montagna MT, et al. Building a benchmark through active surveillance of intensive care unit-acquired infections: the Italian network SPIN-UTI. J Hosp Infect. 2010;74(3):258-65.
3. Lambert M, Suetens C, Savey A, Palomar M, Hiesmayr M, Morales I, et al. Clinical outcomes of healthcare-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. Lancet Infect Dis. 2011;11(1):30-8.
4. Wilson J, Ramboer I, Suetens C. Hospitals in Europe link for infection control through surveillance (HELICS). Inter-country comparison of rates of surgical site infection--opportunities and limitations. J Hosp Infect. 2007;65(Suppl 2):165-70.
5. Suetens C, Morales I, Savey A, Palomar M, Hiesmayr M, Lepape A, et al. European surveillance of ICU-acquired infections (HELICS-ICU): Methods and main results. J Hosp Infect. 2007;65 (Suppl 2):171-3.
6. Suetens C, Savey A, Labeeuw J, Morales I. The ICU-HELICS programme: Towards European surveillance of hospital-acquired infections in intensive care units. Euro Surveill. 2002;7(9):pii=359. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=359
7. Improving Patient Safety in Europe (IPSE). The IPSE report 2005-2008. Lyon: Université Claude Bernard Lyon1; November 2009]. Available from http://www.ecdc.europa.eu/en/activities/surveillance/HAI/Documents/0811_IPSE_Technical_Implementation_Report.pdf
8. Gravel D, Taylor G, Ofner M, Johnston L, Loeb M, Roth VR, et al. Point prevalence survey for healthcare-associated infections within Canadian adult acute-care hospitals. J Hosp Infect. 2007;66(3):243-8.
9. Lanini S, Jarvis WR, Nicastri E, Privitera G, Gesu G, Marchetti F, et al. Healthcare-associated infection in Italy: Annual point-prevalence surveys, 2002-2004. Infect Control Hosp Epidemiol. 2009;30(7):659-65.
10. Struwe J, Dumpis U, Gulbinovic J, Lagergren Å, Bergman U. Healthcare associated infections in university hospitals in Latvia, Lithuania and Sweden: a simple protocol for quality assessment. Euro Surveill. 2006;11(7):pii=640. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=640
11. The RAISIN Working Group. "RAISIN" – a national programme for early warning, investigation and surveillance of healthcare-associated infection in France. Euro Surveill. 2009;14(46):pii=19408. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19408
12. Reilly J, Stewart S, Allardice GA, Noone A, Robertson C, Walker A, Coubrough S. Results from the Scottish national HAI prevalence survey. J Hosp Infect. 2008;69:62-8.
13. Suetens C, Ammon A, Weist K, Sodano L, Monnet DL. Review of methods of national prevalence surveys of healthcare-associated infections in 17 European countries. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID); 16-19 May 2009; Helsinki, Finland. Clin Microbiol Infect. 2009;15(s4):P.624.
14. European Centre for Disease Prevention and Control (ECDC). Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals. Protocol version 4.3. Full scale survey and codebook. Stockholm: ECDC: 2012. Available from: http://www.ecdc.europa.eu/en/activities/surveillance/HAI/about_HAI-Net/Pages/PPS.aspx
15. Müller-Pebody B, Muscat M, Pelle B, Klein BM, Brandt CT, Monnet DL. Increase and change in pattern of hospital antimicrobial use, Denmark, 1997-2001. J Antimicrob Chemother. 2004;54(6):1122-6.
16. Vander Stichele RH, Elseviers MM, Ferech M, Blot S, Goossens H. Hospital consumption of antibiotics in 15 European countries: results of the ESAC retrospective data collection (1997-2002). J Antimicrob Chemother. 2006;58(1):159-67.
17. de Man P, Verhoeven BA, Verbrugh HA, Vos MC, van den Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. Lancet. 2000;355(9208):973-8.
18. Cooke DM, Salter AJ, Phillips I. The impact of antibiotic policy on prescribing in a London teaching hospital. a one-day prevalence survey as an indicator of antibiotic use. J Antimicrob Chemother. 1983;11(5):447-53.
19. Berild D, Ringertz SH, Lelek M. Appropriate antibiotic use according to diagnoses and bacteriological findings: Report of 12 point-prevalence studies on antibiotic use in a University hospital. Scand J Infect Dis. 2002;34(1):56-60.
20. Ufer M, Radosević N, Vogt A, Palcevski G, Francetić I, Reinalter SC, et al. Antimicrobial drug use in hospitalised paediatric patients: a cross-national comparison between Germany and Croatia. Pharmacoepidemiol Drug Saf. 2005;14(10): 735-9.
21. Usluer G, Ozgunes I, Leblebicioglu H. A multicenter point-prevalence study: antimicrobial prescription frequencies in hospitalized patients in Turkey. Ann Clin Microbiol Antimicrob. 2005;4:16.
22. Ciofi Degli Atti ML, Raponi M, Tozzi AE, Ciliento G, Ceradini J, Langiano T. Point prevalence study of antibiotic use in a paediatric hospital in Italy. Euro Surveill. 2008;13(41):pii=19003. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19003
23. Ansari F, Erntell M, Goossens H, Davey P. The European surveillance of antimicrobial consumption (ESAC) point-prevalence survey of antibacterial use in 20 European hospitals in 2006. Clin Infect Dis. 2009;49(10):1496-504.
24. Amadeo B, Zarb P, Muller A, Drapier N, Vankerckhoven V, Rogues A, et al. European surveillance of antibiotic consumption (ESAC) point prevalence survey 2008: Paediatric antimicrobial prescribing in 32 hospitals of 21 European countries. J Antimicrob Chemother. 2010;6(10)5:2247-52.
25. Zarb P, Amadeo B, Muller A, Drapier N, Vankerckhoven V, Davey P, et al. Identification of targets for quality improvement in antimicrobial prescribing: The web-based ESAC point prevalence survey 2009. J Antimicrob Chemother. 2011;66(2):443-9.
26. Zarb P, Goossens H. European surveillance of antimicrobial consumption (ESAC): Value of a point-prevalence survey of antimicrobial use across Europe. Drugs. 2011;71(6):745-55.
27. Zarb P, Ansari F, Muller A, Vankerckhoven V, Davey PG, Goossens H. Drug utilization 75% (DU75%) in 17 European hospitals (2000-2005): Results from the ESAC-2 hospital care sub project. Curr Clin Pharmacol. 2011;6(1):62-70.
28. Ang L, Laskar R, Gray JW. A point prevalence study of infection and antimicrobial use at a UK children's hospital. J Hosp Infect. 2008;68(4):372-4.
29. O'Neill E, Morris-Downes M, Rajan L, Fitzpatrick F, Humphreys H, Smyth E. Combined audit of hospital antibiotic use and a prevalence survey of healthcare-associated infection. Clin Microbiol Infect. 2010;16(5):513-5.
30. Ider BE, Clements A, Adams J, Whitby M, Muugolog T. Prevalence of hospital-acquired infections and antibiotic use in two tertiary Mongolian hospitals. J Hosp Infect. 2010;75(3):214-9.
31. Hajdu A, Samodova OV, Carlsson TR, Voinova LV, Nazarenko SJ, Tjurikov AV, et al. A point prevalence survey of hospital-acquired infections and antimicrobial use in a paediatric hospital in north-western Russia. J Hosp Infect. 2007;66(4):378-84.
32. Maugat S, Thiolet J, L'Hériteau F, Gautier C, Tronel H, Metzger M, et al. Prévalence des traitements antibiotiques dans les établissements de santé, France 2006 [Prevalence of antibiotic treatments in healthcare facilities, France, 2006]. Bull Epidemiol Hebd. 2007;51-52:432-7. French. Available from: http://opac.invs.sante.fr/doc_num.php?explnum_id=3474
33. 33.Council of the European Union. Council Recommendation of 9 June 2009 on patient safety, including the prevention and control of healthcare associated infections (2009/C 151/01). Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2009:151:0001:0006:EN:PDF
34. Council of the European Union. Council Recommendation of 15 November 2001 on the prudent use of antimicrobial agents in human medicine (2002/77/EC). Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:034:0013:0016:EN:PDF
35. Hospital in Europe Link for Infection Control through Surveillance (HELICS). Surveillance of surgical site infections. Protocol version 9.1. Lyon: HELICS; Sept 2004. Available from: http://www.ecdc.europa.eu/en/activities/surveillance/HAI/Documents/0409_IPSE_SSI_protocol.pdf
36. Kuijper EJ, Coignard B, Tüll P, ESCMID Study Group for Clostridium difficile; EU Member States; European Centre for Disease Prevention and Control. Emergence of Clostridium difficile-associated disease in North America and Europe. Clin Microbiol Infect. 2006;12(Suppl 6): 2-18.
37. Nosocomial infection surveillance system for preterm infants on neonatology departments and intensive care units (Neo-KISS). Protokoll.Surveillance nosokomialer Infektionen bei Frühgeborenen mit einem Geburtsgewicht <1.500g. [Protocol. Surveillance of nosocomial infections in preterm infants with a birth weight <1,500 g]. Berlin: Institut für Hygiene und Umweltmedizin, Charité; Dec 2009. German. Available

from: http://www.nrz-hygiene.de/fileadmin/nrz/download/NEOKISSProtokoll221209.pdf

38. Hospital in Europe Link for Infection Control through Surveillance (HELICS). Surveillance of nosocomial infections in intensive care units. Protocol version 6.1. Lyon: HELICS; Sept 2004. Available from:http://www.ecdc.europa.eu/en/activities/surveillance/HAI/Documents/0409_IPSE_ICU_protocol.pdf
39. Geffers C, Baerwolff S, Schwab F, Gastmeier P. Incidence of healthcare-associated infections in high-risk neonates: results from the German surveillance system for very-low-birthweight infants. J Hosp Infect. 2008;68(3):214-21.
40. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
41. World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology. The ATC/DDD system: International language for drug utilization research. Oslo: WHO Collaborating Centre for Drug Statistics Methodology; Oct 2007. Available from: http://www.fhi.no/dav/a0fb3024e7.pdf
42. McCabe WR, Jackson GG. Gram-negative bacteremia: I. Etiology and ecology. Arch Intern Med. 1962;110:847-53.
43. Goossens H. Expert-proposed European strategies to monitor and control infection, antibiotic use, and resistance in health-care facilities. Lancet Infect Dis. 2011;11(5):338-40.
44. Pappachan JV, Millar B, Bennett ED, Smith GB. Comparison of outcome from intensive care admission after adjustment for case mix by the APACHE III prognostic system. Chest. 1999;115(3):802-10.
45. Rowan KM, Kerr JH, Major E, McPherson K, Short A, Vessey MP. Intensive Care Society's APACHE II study in Britain and Ireland--II: outcome comparisons of intensive care units after adjustment for case mix by the American APACHE II method. BMJ. 1993;307(6910):977-81.
46. McArdle CS, Hole DJ. Outcome following surgery for colorectal cancer: analysis by hospital after adjustment for case-mix and deprivation. Br J Cancer. 2002;86(3):331-5.
47. Sax H, Pittet D. Interhospital differences in nosocomial infection rates: importance of case-mix adjustment. Arch Intern Med. 2002;162(21):2437-42.
48. Reilly J, Stewart S, Allardice G, Cairns S, Ritchie L, Bruce J. Evidence-based infection control planning based on national healthcare-associated infection prevalence data. Infect Control Hosp Epidemiol. 2009;30(2):187-9.
49. Valintiliene R, Gailiene G, Berzanskyte A. Prevalence of healthcare-associated infections in Lithuania. J Hosp Infect. 2012;80(1):25-30.
50. Lyytikäinen O, Kanerva M, Agthe N, Möttönen T, Ruutu P; Finnish Prevalence Survey Study Group. Healthcare-associated infections in Finnish acute care hospitals: a national prevalence survey, 2005. J Hosp Infect. 2008:69(3):288-94.

Haemophilus influenzae serotype B (Hib) seroprevalence in England and Wales in 2009

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A national seroprevalence study was performed to determine the prevalence of *Haemophilus influenzae* type b (Hib) antibodies in England and Wales in 2009, when Hib disease incidence was the lowest ever recorded. A total of 2,693 anonymised residual sera from routine diagnostic testing submitted by participating National Health Service hospital laboratories were tested for Hib anti-polyribosyl-ribitol phosphate (PRP) IgG antibodies using a fluorescent bead assay. Median anti-PRP IgG concentrations were highest in toddlers aged 1–4 years (2.65 µg/ml), followed by children aged 5–9 years (1.95 µg/ml). Antibody concentrations were significantly lower after this age, but were still significantly higher among 10–19 year-olds (0.54 µg/ml) compared with adults aged >20 years (0.16 µg/ml; p<0.0001). Half of the adults (51%) did not have Hib antibody concentrations ≥0.15 µg/ml, the level considered to confer short-term protection. Thus, the current excellent Hib control appears to be the result of high anti-PRP antibody concentrations in children aged up to 10 years, achieved through the various childhood vaccination campaigns offering booster immunisation. The lack of seroprotection in adults emphasises the importance of maintaining control of the disease and, most probably carriage, in children, therefore raising the question as to whether long-term routine boosting of either pre-school children or adolescents may be required.

Introduction

The incidence of invasive *Haemophilus influenzae* type b (Hib) disease in England and Wales is currently the lowest ever recorded in both children and adults [1]. Conjugate vaccines against Hib are highly effective in preventing invasive disease [2]. The United Kingdom (UK) introduced the Hib conjugate vaccine into the national childhood immunisation programme in October 1992. Unlike most other countries, the UK initially opted for a three dose infant schedule at two, three and four months of age without a booster in the second year of life. Instead, a catch-up campaign offering two doses of the vaccine to infants aged 8–11 months and one dose to toddlers aged up to four years was performed during 1992–93 [1]. Hib cases declined rapidly such that, in children aged <5 years, the annual number of reported cases in England and Wales fell from almost 500 in the pre-vaccine era to around 20 within two years of vaccine implementation [1]. In 1998, the vaccine failure rate, derived from the observed number of true vaccine failures and the vaccine coverage in the eligible cohorts, was estimated at 2.2 per 100,000 vaccinees (95% confidence interval: 1.8–2.7 per 100,000) [3]. In addition, because conjugate vaccines also reduce the acquisition of carriage and young children have the highest carriage rates [1], cases in older children and adults also declined through indirect (herd) protection [4].

From 1999, however, the number of invasive Hib cases started to increase in all age groups, but particularly in toddlers aged 1–4 years [1]. Reasons for this increase included a decline in indirect protection offered by the 1992 catch-up campaign over time [4], lower than predicted long-term protection in children who were vaccinated in infancy [5] and a temporary change to a less immunogenic acellular pertussis-containing combination Hib conjugate vaccine (DTaP-Hib) used during 2000–01 [6].

In response to this increase, the DTaP-Hib preparation was replaced with a more immunogenic whole-cell pertussis-containing combination Hib conjugate vaccine (DTwP-Hib) from 2002, and subsequently with a DTaP-Hib-IPV vaccine with better Hib immunogenicity from 2004. A booster campaign was subsequently undertaken in 2003 to provide an extra dose of Hib vaccine to the cohort of children (born between April 1999 and March 2003) who may have received the less immunogenic DTaP-Hib conjugate vaccine in infancy. This was followed in 2006 by the introduction of a routine 12-month Hib-Meningococcal serogroup C (Hib-MenC) combination booster into the national childhood immunisation schedule. Additionally, in 2007, following an increase in cases in certain birth cohorts during 2005 and 2006, a pre-school booster dose of a Hib containing vaccine was offered to those born between March

2003 and September 2005. This cohort would have been too young for the 2003 booster campaign and too old for the routine 12-month booster. Together, these measures have, once again, resulted in a decline in the incidence of invasive Hib disease among both children and adults such that there were only 37 reported cases in 2009, mainly in adults (26 cases) [7].

In 2010, the Health Protection Agency (HPA) undertook a seroprevalence study to assess population immunity against Hib in 2009 by measuring antibodies against the Hib polysaccharide capsule across age groups in order to help explain the excellent control of invasive Hib disease, to identify potential susceptible cohorts and to help guide future national Hib vaccination policies.

Methods

Serum samples

Participating laboratories submit residual sera from routine diagnostic testing to the HPA Sero-epidemiology Unit (SEU). All samples are anonymised, a unique identity number is assigned and details of age, sex, date of sample collection and geographic location are collated on a database.

For this study, a total of 2,693 sera were selected for infants aged 6–11 months (n=104), toddlers aged 1–4 years (n=653), 5–14 year-olds (n=990), 15–24 year-olds (n=343), 25–44 year-olds (n=301), 45–64 year-olds (n=121) and ≥65 year-olds (n=181) from the HPA SEU as described by Osborne et al. [8]. Antibody concentrations (IgG) against the Hib capsular polysaccharide (polyribosyl-ribitol phosphate (PRP)) were quantified using a fluorescent bead assay as described previously [9]. Briefly, PRP was conjugated to carboxylated microspheres (Luminex Corporation; Texas, United States) following bead activation (via a two-step carbodiimide reaction). Serum was diluted 1:100 and a standard curve prepared using the World Health Organization (WHO) international standard TE-3. Diluted preparations were added to a filter plate (Millipore, Watford, UK) and mixed with conjugated beads. Following incubation, the plate was washed and anti-human IgG-R-Phycoerythrin (RPE) added to each well. Following incubation and washing, the plate was read on a BioPlex workstation (BioRad, Hertfordshire, UK) and analysis undertaken using Bioplex manager software, with a four parameter logistic fit model.

Definitions

The thresholds for short-term and long-term protection against invasive Hib disease are based on previous animal experiments, studies on natural immunity, passive immunisation and the original clinical trials of Hib-PRP polysaccharide vaccines, which suggested that minimum anti-PRP IgG concentrations of 0.05–0.15 µg/ml at the time of exposure to the organism were required to protect against invasive disease [10,11]. Therefore, anti-PRP antibody concentrations ≥0.15 µg/ml were considered to confer short-term protection [12,13], while concentrations ≥1 µg/ml would ensure a minimum concentration of 0.1 µg/ml after 12 months, thereby conferring long-term protection [10]. Anti-PRP IgG concentrations ≥5.0 µg/ml were considered to confer protection against acquisition of Hib carriage [14,15].

Data analysis

Data for anti-PRP IgG concentrations were initially analysed by age group of individuals at the time of serum collection in 2009 and compared with raw data from previous seroprevalence studies which utilised the same source of samples [4,16]. The data from these previous seroprevalence studies, which analysed sera from 1993 to 2001, were grouped into two time periods: (i) 1993–94, when the Hib conjugate vaccine had recently been introduced into the national childhood immunisation programme; and (ii) 1995–2001, when routine infant Hib conjugate vaccination was in place.

Subsequently, data from individuals aged <25 years in this 2009 seroprevalence study were categorised and analysed by birth cohorts eligible for different Hib vaccination, catch-up and booster programmes. Anti-PRP IgG concentrations for adults aged ≥25 years, who would not have been eligible for Hib conjugate vaccination at any time, were included for comparison. As anti-PRP IgG results were highly skewed and not normally distributed even when log-transformed, median values with interquartile ranges (IQR) are reported and compared using the Mann-Whitney U test. Categorical variables are expressed as proportions and compared using the chi-squared test. Trends in median anti-PRP concentrations and proportion achieving Hib antibody concentrations above specified thresholds over the three surveillance studies were assessed using the non-parametric test for trend and the chi-squared test for trend, respectively.

Ethical approval

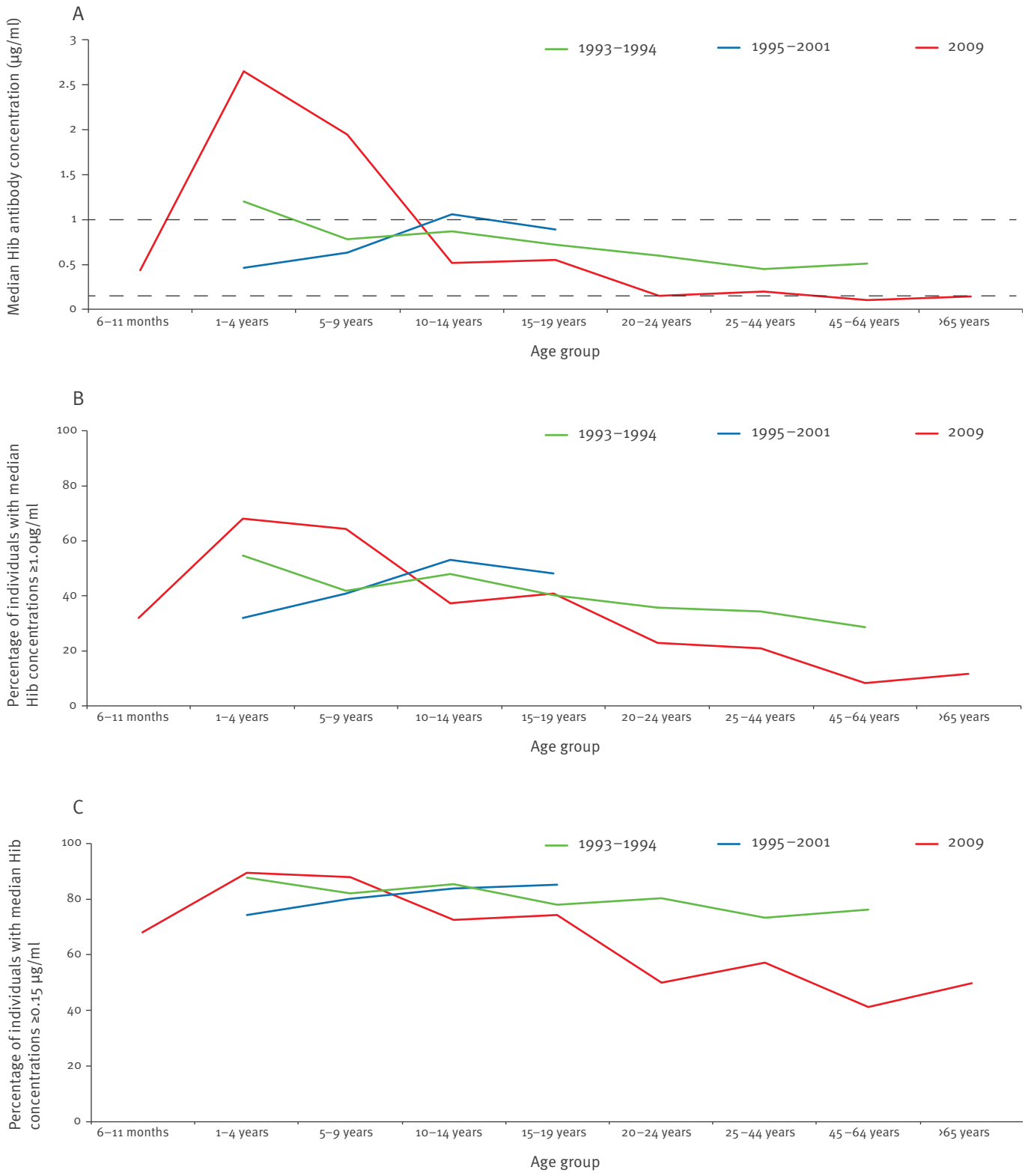
National Research Ethics Service (NRES) approval for the sero-epidemiological surveillance of the National Immunisation programme of England and Wales (Research Ethics Committee number 05/Q0505/45) was granted by the Joint University College London/University College London Hospital (UCL/UCLH) Committees on the Ethics of Human Research.

Results

In 2009, median anti-PRP IgG concentrations in children were highest among toddlers aged 1–4 years (2.65 µg/ml; IQR: 0.68–9.39), and significantly higher than in children aged 5–9 years (1.95 µg/ml; IQR: 0.49–6.25; p=0.0063) (Figure 1). Antibody concentrations in both age groups were significantly ($p<0.01$) higher in 2009 compared with the 1993–94 and 1995–2001 seroprevalence periods. After this age, the 2009 seroprevalence study shows that antibody concentrations declined, but were still significantly higher among adolescents of 10–19 years-old (who would have been vaccinated

Figure 1

Comparison by age group of the results of three *Haemophilus influenzae* serotype B seroprevalence studies, England and Wales, 1993–2009



Hib: *Haemophilus influenzae* serotype B.

Hib antibody refers to Hib anti-polyribosyl-ribitol phosphate (PRP) IgG antibody. Hib antibody concentrations ≥0.15 µg/ml and ≥1.0 µg/ml refer to the putative levels considered as respectively providing short-term and long-term protection against invasive Hib disease.

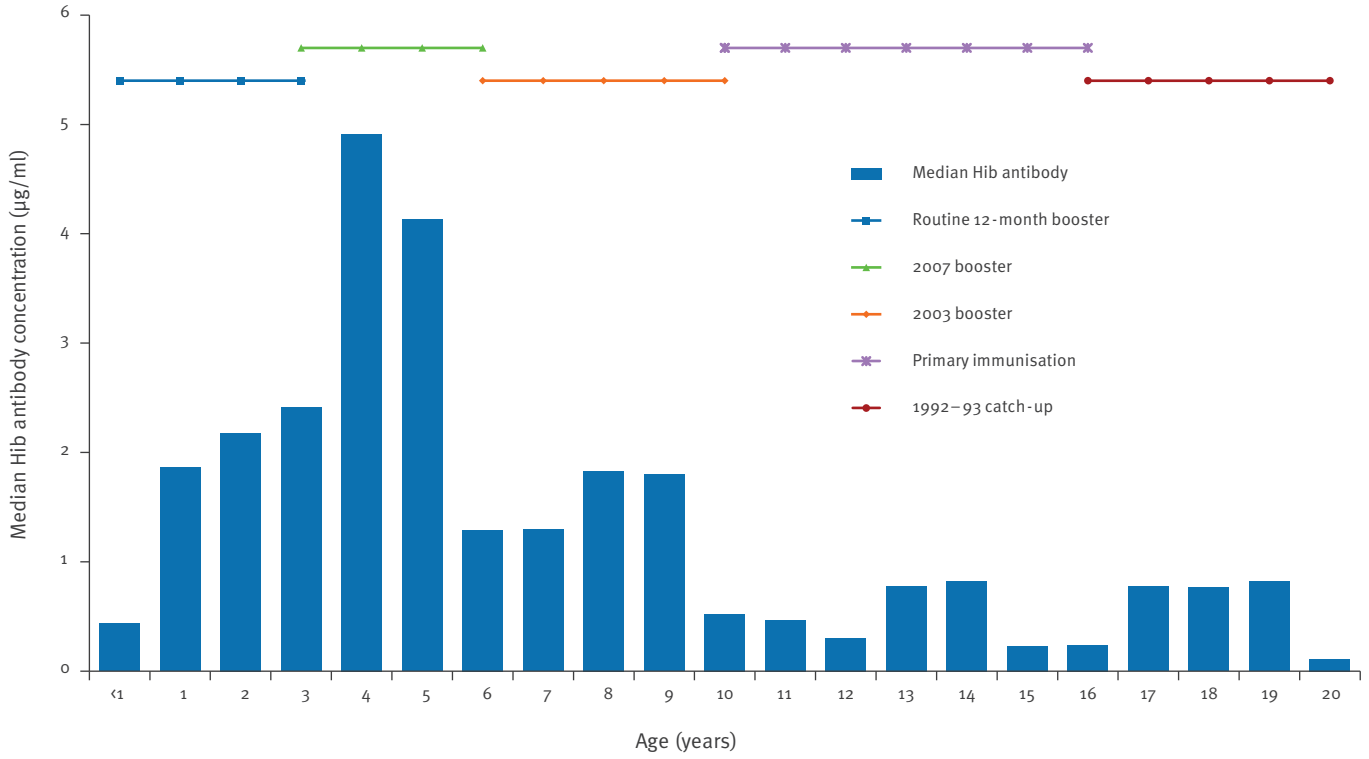
A. Median Hib antibody serum concentration by age group according to each seroprevalence study.

B. Percentage of individuals with Hib antibody concentrations ≥1.0 µg/ml, by age group according to each seroprevalence study.

C. Percentage of individuals with Hib antibody concentrations ≥0.15 µg/ml, by age group according to each seroprevalence study.

Figure 2

Median *Haemophilus influenzae* serotype B antibody concentrations in the vaccinated cohort up to 20 years of age, seroprevalence study, England and Wales, 2009



Hib: *Haemophilus influenzae* serotype B.

Hib antibody refers to Hib anti-polyribosyl-ribitol phosphate (PRP) IgG antibody. The horizontal bars denote birth cohorts receiving different vaccination schedules (Table 1).

against Hib in early childhood) compared with older, mostly unvaccinated adults (median: 0.54 µg/ml; IQR: 0.13–2.58 vs. 0.16 µg/ml; IQR: 0.05–0.55; p<0.0001). Anti-PRP IgG concentrations among 10–19 year-olds in 2009 were comparable to the 1993–94 and 1995–2001 cohorts, with >70% and >40% in the three surveillance periods having antibody concentrations consistent with short-term protection (≥0.15 µg/ml) and long-term protection (≥1.0 µg/ml), respectively. This compares with 89% (542/606) and 68% (413/606) of toddlers having anti-PRP concentrations ≥0.15 µg/ml and ≥1.0 µg/ml, respectively, in 2009.

Subsequent analysis of anti-PRP IgG concentrations in 2009 by birth cohort showed the highest anti-PRP IgG concentrations in those eligible for the 2007 pre-school booster campaign targeting children who had been too young for the 2003 booster campaign and too old for the routine 12 month booster introduced in September 2006 (Figure 2, Table 1). The 1992 catch-up cohort that was eligible for the Hib conjugate vaccine as toddlers in 1992–93 had median anti-PRP IgG concentrations of 1.54 µg/ml (IQR: 0.61–7.28, n=102 tested) at three years of age and 1.36 µg/ml (IQR: 0.34–4.38, n=108 tested) at four years of age in the 1993–94 seroprevalence study. This compared with 4.49 µg/ml (IQR: 1.31–18.5, n=34 tested) and 4.92 µg/ml (IQR: 0.87–18.9, n=134 tested) for the same ages, respectively, who had been eligible for the 2007 booster campaign and were tested in the 2009 seroprevalence study.

In two cohorts eligible for the same immunisation schedule (those eligible for primary immunisation in 1992–93 and toddlers who were part of the 1992 catch-up campaign) median antibody concentrations during all three surveillance periods were compared and showed significant declining trends (Table 2). Waning of antibodies with age was also demonstrated for children eligible for the same immunisation schedule. In children aged 1–5 years who were born during 1995–99 and, therefore, eligible for the primary immunisation schedule and tested in 2000 (n=414 tested), median Hib antibody concentrations fell from 0.88 µg/ml (IQR: 0.30–3.24) among one year-olds to 0.40 µg/ml (IQR: 0.16–0.95) among five year-olds (p=0.02). The proportion with anti-PRP antibody concentrations ≥1.0 µg/ml declined similarly from 47% (41/88 cases) to 24% (9/37 cases), respectively (p=0.015).

Table 1

Hib antibody concentrations and proportion of individuals with distinct protective antibody concentration levels in unvaccinated age groups and in birth cohorts eligible for different Hib immunisation programmes respectively, England and Wales, 2009 (n=2,411 samples)

Group description	Birth dates[a] (age range of serum donors in years[b])	Description	Number of samples tested	Median Hib antibody concentration in µg/ml (IQR)	Number of samples n (%) with Hib antibody concentrations		
					≥0.15 µg/ml	≥1.0 µg/ml	≥5.0 µg/ml
Cohort having primary immunisation with no booster	01 August 1992–01 April 1999 (9.9–16.3)	Hib conjugate vaccine was first offered to all UK infants on 01 October 1992 at a two, three and four month schedule	319	0.42 (0.11–2.45)	226 (71)	112 (35)	44 (14)
Cohort with 1992–93 catch-up	01 August 1988–31 July 1992 (16.3–20.5)	With the introduction of infant Hib vaccination in 1992, a 12-month catch-up campaign targeting all children up to four years-old was initiated	146	0.59 (0.13–2.40)	106 (73)	60 (41)	24 (16)
Cohort with 2003 booster	02 April 1999–12 March 2003 (6.0–10.3)	Between May and September 2003, the birth cohort most likely to have received the less immunogenic DTaP-Hib vaccine in infancy was offered an extra dose of Hib vaccine	531	1.62 (0.46–5.30)[c]	464 (87)[c]	328 (62)[c]	141 (27)[c]
Cohort with 2007 pre-school booster	13 March 2003–03 September 2005 (3.4–6.5)	Children who were too young to be eligible for the 2003 booster campaign and too old for the 12-month routine booster were offered an extra dose of Hib vaccine as part of their pre-school vaccination	375	3.80 (0.81–12.8)[c]	333 (89)[c]	271 (72)[c]	165 (44)[c]
Cohort with 2006 routine booster[d]	04 September 2005–31 December 2009 (0.5–4.1 years)	Since 04 September 2006, all UK infants are offered a 12-month routine Hib booster	437	1.94 (0.66–8.00)[c]	390 (89)[c]	289 (66)[c]	141 (32)[c]
Unvaccinated 25–44 year-olds	NA (25–44.9)	Unvaccinated population	301	0.20 (0.07–0.80)[c]	172 (57)[c]	63 (21)[c]	15 (5)[c]
Unvaccinated 45–64 year-olds	NA (45.0–64.7)	Unvaccinated population	121	0.10 (0.02–0.38)[c]	50 (41)[c]	10 (8)[c]	3 (2)[c]
Unvaccinated ≥65 year-old	NA (65.0–85.0)	Unvaccinated population	181	0.15 (0.02–0.39)[c]	90 (50)[c]	21 (12)[c]	4 (2)[c]

DTaP-Hib: acellular pertussis-containing combination Hib conjugate vaccine; Hib: *Haemophilus influenzae* serotype B; IQR: interquartile range; NA: not applicable; UK: United Kingdom.

Hib antibody refers to Hib anti-polyribosyl-ribitol phosphate (PRP) IgG antibody. Hib antibody concentrations ≥0.15, ≥1.0 and ≥5.0 µg/ml refer respectively to the putative levels considered to confer short-term protection, long-term protection and protection against carriage.

[a] Birth dates define the cohorts eligible for the different Hib immunisation programmes and are, therefore, not applicable to unvaccinated adults.

[b] The serum donor age is the age of the donor at the time serum is collected. The age range of serum donors for the different cohorts may overlap because sera were obtained throughout the 2009 calendar year.

[c] $p<0.001$ when compared to respective values from individuals who only received primary immunisation as infants with no booster.

[d] Only data from children aged ≥12 months were presented for this cohort in order to compare median Hib antibody concentrations and respective proportions of individuals with defined antibody concentrations to the values for individuals who only received primary immunisation with no booster.

In the 2009 seroprevalence study, the cohort eligible for the 2007 pre-school booster was more likely to have very high anti-PRP IgG concentrations than the cohort eligible for the booster in 2003 (given between 6 months and 4 years of age) and those eligible for a booster at 12 months of age after 2006. Forty four per cent (165/375) of children in the pre-school cohort achieved concentrations ≥5 µg/ml (the putative level considered to prevent acquisition of Hib carriage), compared with 27% (141/531; p<0.001) and 32% (141/437; p=0.001) of the 2003 booster cohorts and the 2006 routine 12-month booster cohort, respectively (Table 1). These findings were similar when comparing median anti-PRP IgG antibody concentrations (Figure 2, Table 1). Among children aged ≥12 months in the cohort eligible for a routine 12-month Hib booster, the median concentration was 1.94 µg/ml (IQR: 0.66–8.00), with 89% (309/437) and 66% (289/437) achieving concentrations ≥0.15 µg/ml and ≥1.0 µg/ml, respectively. This cohort would have been vaccinated more recently compared with those vaccinated during 2003 and 2007 booster campaigns. Older children and young adults who would have been eligible for the 1992 primary immunisation and catch-up campaign had substantially lower anti-PRP IgG concentrations in 2009, but these were still higher than older, unvaccinated adults (≥25 years of age) (Table 1).

In adults, anti-PRP IgG concentrations in 2009 are the lowest since routine Hib immunisation was introduced (Figure 1). The median antibody concentration among adults aged ≥20 years was 0.16 µg/ml (IQR: 0.05–0.55), with only 51% (388/755) and 17% (129/755) achieving concentrations ≥0.15 and ≥1.0 µg/ml, respectively. These values are significantly lower than for the same age group in 1993–94, where the median anti-PRP IgG concentration was 0.57 µg/ml (IQR: 0.18–1.46; p<0.0001), and 77% (150/195) (p<0.0001) and 35% (68/195) (p<0.0001) achieved concentrations ≥0.15 µg/ml and ≥1.0 µg/ml, respectively. Among adults aged 30–39 years, the median anti-PRP IgG concentration in 151 samples tested was 0.24 µg/ml (IQR: 0.06–0.96), with 59% (89/151) and 25% (37/151) achieving concentrations ≥0.15 and ≥1.0 µg/ml, respectively. This compares with median anti-PRP IgG antibody concentrations (µg/ml) in the same age group of 1.29 in 1991 (pre-vaccine), 0.70 in 1994 (2 years after vaccine introduction), 0.53 in 1997 (time of excellent Hib control), 0.69 in 2000 (beginning of Hib resurgence) and 0.77 in 2002 (middle of Hib resurgence) [4].

Table 2

Demonstration of waning of Hib antibody over time in two birth cohorts for which seroprevalence was assessed over 1993–94, 1995–2001 and 2009 periods, England and Wales (n=956 samples)

		Seroprevalence periods			Significance[a]
		1993–94	1995–2001	2009	
Primary immunisation schedule (birth cohort: 01 August 1992–31 July 1993)	Median Hib antibody concentration in µg/ml (IQR)	0.65 (0.33–3.3)	0.63 (0.19–2.0)	0.23 (0.05–3.6)	p=0.006
	Proportion of samples tested (%) with Hib antibody concentrations ≥0.15 µg/ml	87/99 (88)	133/166 (80)	30/44 (68)	p=0.006
	Proportion of samples tested with Hib antibody concentrations ≥1.0 µg/mL	44/99 (44)	62/166 (37)	16/44 (36)	p=0.27
1992 catch-up campaign (birth cohort: 01 August 1988–31 July 1992)	Median Hib antibody concentration in µg/ml (IQR)	1.40 (0.38–4.5)	0.68 (0.25–1.7)	0.59 (0.13–2.4)	p<0.001
	Proportion of samples tested (%) with Hib antibody concentrations ≥0.15µg/ml	260/294 (88)	166/207 (80)	106/146 (73)	p<0.001
	Proportion of samples tested (%) with Hib antibody concentrations≥1.0 µg/ml	168/294 (57)	84/207 (41)	60/146 (41)	p<0.001

Hib: *Haemophilus influenzae* serotype B.

Hib antibody refers to Hib anti-polyribosyl-ribitol phosphate (PRP) IgG antibody. Hib antibody concentrations ≥0.15, ≥1.0 µg/ml refer to the putative levels considered to confer short and long-term protection, respectively.

[a] Medians were compared using the non-parametric test for trend and proportions were compared using the chi-squared test for trend.

Discussion

High post-immunisation antibody levels are considered the most important factor in preventing invasive Hib disease both at an individual and at the population level [17]. In England and Wales, Hib disease control is currently excellent [7] and vaccine coverage for both primary Hib immunisation (95–96%) and the 12-month booster (92–94%) remains very high [18]. In 2009, anti-PRP IgG concentrations in children aged up to 10 years of age in England and Wales were the highest that have ever been observed. In this age group, the various booster campaigns have provided excellent antibody levels and it is likely that these children will remain protected for some time.

Unlike the plain polysaccharide vaccine, conjugate vaccines, which have been used in the UK since Hib conjugate vaccine was introduced into the national childhood immunisation programme, induce a T cell-dependent immune response that results in development of immunological memory [19] and, subsequently, production of high avidity antibodies on re-exposure to the antigen [20]. Mathematical modelling of the impact of the Hib conjugate vaccine in the UK, however, cautioned against over-reliance on immunological memory for protection. The lower than expected protection offered by immunological memory probably occurs because, even in those primed with conjugate vaccination, it can take several days to observe a detectable increase in antibody concentrations following infection or vaccination [21]. This delay is too late to protect against invasive infection following exposure to the pathogen which is thought to occur over a period of a few hours. On the other hand, the mathematical model emphasised the importance of maintaining high post-immunisation antibody titres [17], which would not only protect children against invasive disease but also help reduce carriage acquisition [11,22] and, therefore, transmission to unvaccinated cohorts, particularly adults.

In 2009, median anti-PRP IgG concentrations in 10–19 year-olds were substantially lower than in younger children, although the vast majority had protective antibody concentrations (≥0.15 µg/ml). This age group would have been eligible for either the primary immunisation schedule recommended from 1992 onwards or the 1992–93 catch-up campaign. Although some of these adolescents would have been vaccinated almost two decades ago and there was evidence of waning of immunity with time, many of them still had detectable IgG concentrations which appear to be protective, since invasive Hib disease is rare in this cohort compared with older, unvaccinated adults.

Outside the vaccinated cohort, however, over half the adults did not have anti-PRP IgG concentrations considered to confer short-term protection against invasive Hib disease. The validity of these thresholds in unimmunised adults, however, has not been established. It is speculated that natural immunity is acquired following exposure through carriage, for example [17], which is likely to confer much broader protection through development of protective antibodies not only against the polysaccharide capsule (as would occur following vaccination) but also against other Hib surface proteins and antigens. As a consequence, it is possible that individuals may be protected against disease even if they have undetectable anti-PRP antibodies and functional antibody assays to measure bactericidal activity, might be more informative.

The Hib polysaccharide capsule, however, is considered to be the primary activator of the immune response against this pathogen, with evidence dating as far back as the 1930's of an inverse correlation between anti-PRP IgG levels and risk of invasive Hib disease [1]. Such antibodies have been shown to be bactericidal both in vitro and in vivo [23], as have antibodies developed after Hib polysaccharide vaccination [24]. Prior to routine Hib vaccination, too, the epidemiology of invasive Hib disease in infants and toddlers closely correlated with the level of transplacentally-acquired protective maternal IgG antibodies [1]. In adults, the importance of the relationship between anti-PRP antibody and exposure to Hib is suggested by the secular change in antibody levels associated with the initial decline in childhood Hib disease after 1992 followed by the resurgence after 1999 in the UK [4]. The increase in adult Hib cases, with annual number of cases reaching levels similar to that observed in the pre-vaccine era was associated with a decline in anti-PRP IgG among English adults aged 30–39 years following the introduction of routine Hib vaccination [4]. The most plausible explanation is that initial reduction in adult Hib cases after 1992 reduced carriage of the organism among vaccinated children and, therefore, reduced transmission to susceptible individuals, including adults. One consequence of this phenomenon, however, was that adults were less likely to be exposed to Hib and, therefore, had reduced opportunities for natural acquisition or boosting of immunity. This was subsequently predicted by mathematical modelling of Hib transmission in the UK [25]. In addition, the model did not support a role for natural antibodies derived from other colonising encapsulated bacteria that might cross-react against the Hib capsule and, therefore, serve to boost natural immunity against this organism [17].

The rise in adult cases following the Hib resurgence in children suggests that disease control relies strongly on induction of high antibody levels in children not only to provide long-term protection for the vaccinated children but also to reduce carriage and, therefore, transmission to others [4]. This observation is supported by the impact of the two childhood booster campaigns in 2003 and 2007, which resulted in re-establishment of disease control among adults, who were not subjected to any intervention [26]. The finding of such low anti-PRP IgG levels among adults is, therefore, concerning. In 2009 and 2010, 26/37 and 23/30 of invasive Hib cases occurred in adults, respectively, with a relatively even distribution of cases among 25–44, 45–64

and ≥65 year-olds [7]. It is, therefore, imperative that Hib control through adequate childhood immunisation is maintained in order to protect the adult population who are currently more vulnerable than at any other time in the past two decades.

In addition to the 2003 and 2007 booster campaigns, the introduction of a routine 12-month Hib booster appears to have a positive impact on maintaining high anti-PRP IgG levels after one year of age. That median anti-PRP IgG concentrations are not as high when compared with the 2007 pre-school booster campaign suggests that receiving a Hib conjugate vaccine at an older age may provide more sustained protection against invasive Hib disease and, perhaps carriage, too. Whether the current routine 12-month Hib booster will be sufficient to maintain disease control in the long-term is difficult to assess given the added protection currently offered by the other two booster campaigns. Hib antibody responses to DTaP-Hib combination vaccines are known to be substantially lower than either DTwP-Hib vaccines [27] or Hib conjugate vaccines administered alone [28]. In the UK, infants now routinely receive Pediacel (DTa_5P-IPV-Hib), which replaced the DTwP-Hib vaccine in September 2004 because it is less reactogenic and, as it contains inactivated polio virus, removes the risk of vaccine-associated paralytic poliomyelitis with oral polio vaccine [26]. This vaccine contains a different acellular pertussis component to that implicated in the increase in invasive Hib disease after 1999 [29]. Given that Hib antibodies decline at a relatively constant rate in infants and young children [30] and that, currently, opportunities for natural boosting of immunity through Hib exposure are likely to be rare (as suggested by the low anti-PRP IgG seroprevalence in adults), long-term protection essentially relies on the peak antibody response achieved after the 12-month routine booster.

Seroprevalence studies have an important role in describing immunity at a population level. Like all seroprevalence studies, the results must be interpreted with the knowledge of the source of the samples and the tests performed. In our study, anonymised serum samples were acquired from a national serosurvey resource that collects residual sera from participating National Health Service (NHS) hospital laboratories and, therefore, may not be representative of the general population. However, the large number of samples does allow comparison of seroprotection across age groups, including different birth cohorts eligible for specific immunisation schedules. Moreover, results can be compared with previously published seroprevalence studies, which had used the same similar sample sources.

In conclusion, the current excellent control of invasive Hib disease in the UK appears to be the result of high IgG levels in children up to 10 years, who were eligible for the 2003 and 2007 booster campaigns or the routine 12-month booster, although antibody levels in the latter cohort were not as high. As the cohorts protected by the two booster campaigns age, disease control will rely mainly on children receiving the routine 12-month booster. This cohort will, therefore, require close monitoring to ensure that they sustain sufficiently high antibody levels not only to protect themselves but also to protect others through reduced transmission. The lack of seroprotection in adults emphasises the importance of maintaining good control in children. In the absence of natural boosting, this study raises the question as to whether long-term control across all ages may require routine boosting of either pre-school children or adolescents. Given the current excellent control of invasive Hib disease in children, further studies should also focus on risk factors for and strategies to prevent invasive Hib disease in adults.

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Conflict of interest statement

SNL has performed contract research for the HPA and St. George's University of London on behalf of Pfizer, GSK and Novartis Vaccines. RB and JF have performed contract research work for the HPA on behalf of Pfizer, GSK, Sanofi Pasteur MSD, Novartis Vaccines and Merck. SNL, MPS, JSF and JF have received assistance for attending conferences from Pfizer and GSK. All other authors: no conflict of interest.

References

1. Ladhani SN. Two decades of experience with the Haemophilus influenzae serotype b conjugate vaccine in the United Kingdom. Clin Ther. 2012;34(2):385-99.
2. Swingler G, Fransman D, Hussey G. Conjugate vaccines for preventing Haemophilus influenzae type B infections. Cochrane Database Syst Rev. 2007;(2):CD001729.
3. Heath PT, Booy R, Azzopardi HJ, Slack MP, Bowen-Morris J, Griffiths H et al. Antibody concentration and clinical protection after Hib conjugate vaccination in the United Kingdom. JAMA. 2000;284(18):2334-40.
4. McVernon J, Trotter CL, Slack MP, Ramsay ME. Trends in Haemophilus influenzae type b infections in adults in England and Wales: surveillance study. BMJ. 2004;329(7467):655-8.
5. Ramsay ME, McVernon J, Andrews NJ, Heath PT, Slack MP. Estimating Haemophilus influenzae type b vaccine effectiveness in England and Wales by use of the screening method. J Infect Dis. 2003;188(4):481-5.
6. McVernon J, Andrews N, Slack MP, Ramsay ME. Risk of vaccine failure after Haemophilus influenzae type b (Hib) combination vaccines with acellular pertussis. Lancet. 2003;361(9368):1521-3.
7. Anonymous. Laboratory reports of Haemophilus influenzae by age group and serotype, England and Wales, fourth quarter, 2010 (and 2009). Health Protection Reports 2011; 5: 6-7.
8. Osborne K, Gay N, Hesketh L, Morgan-Capner P, Miller E. Ten years of serological surveillance in England and Wales: methods, results, implications and action. Int J Epidemiol. 2000;29(2):362-8.
9. Pickering JW, Martins TB, Schroder MC, Hill HR. Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for auantitation of antibodies to tetanus, diphtheria, and Haemophilus influenzae Type b. Clin Diagn Lab Immunol. 2002;9(4): 872-6.
10. Anderson P. The protective level of serum antibodies to the capsular polysaccharide of Haemophilus influenzae type b. J Infect Dis. 1984;149(6):1034-5.
11. Eskola J, Ward J, Dagan R, Goldblatt D, Zepp F, Siegrist CA. Combined vaccination of Haemophilus influenzae type b conjugate and diphtheria-tetanus-pertussis containing acellular pertussis. Lancet. 1999;354 (9195):2063-8.
12. Kayhty H, Peltola H, Karanko V, Makela PH. The protective level of serum antibodies to the capsular polysaccharide of Haemophilus influenzae type b. J Infect Dis. 1983;147(6): 1100.
13. Kayhty H. Difficulties in establishing a serological correlate of protection after immunization with Haemophilus influenzae conjugate vaccines. Biologicals. 1994;22(4):397-402.
14. Fernandez J, Levine OS, Sanchez J, Balter S, LaClaire L, Feris J et al. Prevention of Haemophilus influenzae type b colonization by vaccination: correlation with serum anti-capsular IgG concentration. J Infect Dis. 2000;182(5):1553-6.
15. Goldblatt D, Hussain M, Andrews N, Ashton L, Virta C, Melegaro A et al. Antibody responses to nasopharyngeal carriage of Streptococcus pneumoniae in adults: a longitudinal household study. J Infect Dis. 2005;192(3):387-93.
16. Trotter CL, McVernon J, Andrews NJ, Burrage M, Ramsay ME. Antibody to Haemophilus influenzae type b after routine and catch-up vaccination. Lancet 2003;361 (9368):1523-4.
17. McVernon J, Ramsay ME, McLean AR. Understanding the impact of Hib conjugate vaccine on transmission, immunity and disease in the United Kingdom. Epidemiol Infect. 2008;136(6):800-12.
18. Anonymous. Quarterly vaccination coverage statistics for children aged up to five years in the UK (COVER programme): January to March 2011. Health Protection Reports 2011; 5: 9-13.
19. Avery OT, Goebel WF. Chemo-immunological studies on the soluble specific substance of pneumococcus : I. The isolation and properties of the acetyl polysaccharide of pneumococcus type I. J Exp Med. 1933;58(6):731-55.
20. Schlesinger Y, Granoff DM. Avidity and bactericidal activity of antibody elicited by different Haemophilus influenzae type b conjugate vaccines. The Vaccine Study Group. JAMA. 1992;267(11): 1489-94.
21. Snape MD, Kelly DF, Salt P, Green S, Snowden C, Diggle L et al. Serogroup C meningococcal glycoconjugate vaccine in adolescents: persistence of bactericidal antibodies and kinetics of the immune response to a booster vaccine more than 3 years after immunization. Clin Infect Dis. 2006; 43(11):1387-94.
22. Kauppi M, Saarinen L, Kayhty H. Anti-capsular polysaccharide antibodies reduce nasopharyngeal colonization by Haemophilus influenzae type b in infant rats. J Infect Dis. 1993;167(2):365-71.
23. Anderson P, Johnston RB Jr, Smith DH. Human serum activities against Hemophilus influenzae, type b. J Clin Invest. 1972;51(1):31-8.
24. Anderson P, Pichichero ME, Insel RA. Immunogens consisting of oligosaccharides from the capsule of Haemophilus influenzae type b coupled to diphtheria toxoid or the toxin protein CRM197. J Clin Invest. 1985;76(1):52-9.
25. Leino T, Auranen K, Makela PH, Kayhty H, Takala AK. Dynamics of natural immunity caused by subclinical infections, case study on Haemophilus influenzae type b (Hib). Epidemiol Infect. 2000; 125(3):583-91.
26. Ladhani S, Slack MP, Heys M, White J, Ramsay ME. Fall in Haemophilus influenzae serotype b (Hib) disease following implementation of a booster campaign. Arch Dis Child. 2008;93(8):665-9.
27. Bar-On ES, Goldberg E, Fraser A, Vidal L, Hellmann S, Leibovici L. Combined DTP-HBV-HIB vaccine versus separately administered DTP-HBV and HIB vaccines for primary prevention of diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae B (HIB). Cochrane Database Syst Rev. 2009;(3):CD005530.
28. Schmitt HJ, Zepp F, Muschenborn S, Sumenicht G, Schuind A, Beutel K et al. Immunogenicity and reactogenicity of a Haemophilus influenzae type b tetanus conjugate vaccine when administered separately or mixed with concomitant diphtheria-tetanus-toxoid and acellular pertussis vaccine for primary and for booster immunizations. Eur J Pediatr. 1998;157(3):208-14.
29. Kitchin NR, Southern J, Morris R, Hemme F, Thomas S, Watson MW et al. Evaluation of a diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae type b vaccine given concurrently with meningococcal group C conjugate vaccine at 2, 3 and 4 months of age. Arch Dis Child. 2007;92(1):11-6.
30. Borrow R, Andrews N, Findlow H, Waight P, Southern J, Crowley-Luke A et al. Kinetics of antibody persistence following administration of a combination meningococcal serogroup C and haemophilus influenzae type b conjugate vaccine in healthy infants in the United Kingdom primed with a monovalent meningococcal serogroup C vaccine. Clin Vaccine Immunol. 2010;17(1):154-9.

Two geographically separated food-borne outbreaks in Sweden linked by an unusual *Cryptosporidium parvum* subtype, October 2010

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The number of sporadic cases of *Cryptosporidium* identified in the Stockholm county area increased above the expected limit during October 2010. Additionally, two food-borne outbreaks of cryptosporidiosis occurred in two other Swedish cities: Umeå (4 October) and Örebro (9 October). The outbreak investigations did not reveal any responsible food item, however fresh herbs were suspected. Thirty stool samples, originating from all three events, tested positive for *Cryptosporidium* oocysts. Polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (RFLP) revealed that 27 individuals were infected with *C. parvum*, two with *C. hominis*, and one with *C. felis*. Using sequence analysis of the GP60 glycoprotein gene, a polymorphic marker with high intra-species diversity, we identified the same *C. parvum* subtype IIdA24G1 in samples from both the Umeå outbreak and the Stockholm area cases, thus indicating a possible outbreak in the Stockholm area and establishing a link between these two events. *C. parvum* IIdA24G1 has not previously been described in connection with a food-borne outbreak. For the outbreak in Örebro, another subtype was identified: *C. parvum* IIdA20G1e. These findings demonstrate that subtyping *C. parvum* isolates using GP60 gene amplification can be used to link cases in an outbreak investigation and we recommend its use in future similar events.

Introduction

Cryptosporidiosis is a diarrhoeal disease caused by protozoa of the genus *Cryptosporidium*. Human infection is predominantly caused by the species *C. hominis* and *C. parvum* [1]. While *C. hominis* infection affects only humans [2,3], *C. parvum* can infect a wider range of mammals. The transmission route is faecal-oral and may be caused by direct contact with infected persons or animals, or indirectly by either ingesting contaminated drinking water or water during aquatic recreational activities or consuming contaminated food. Watery diarrhoea with sudden onset is the most common symptom but abdominal pain, low-grade fever, nausea, dehydration, and weight loss also occur. The incubation period may vary between two and 12 days and symptoms can last up to two weeks [1]. Infections are usually self-limited in individuals without underlying conditions but for the immunocompromised diarrhoea can be prolonged, severe, and life-threatening [4].

Worldwide, cases of cryptosporidiosis may be detected sporadically or as part of water-borne or food-borne outbreaks. Food-borne outbreaks are less often detected and described than water-borne outbreaks. A recent review article found that, in the last decade, only 15 of 71 worldwide *Cryptosporidium*-linked outbreaks appeared to be correlated to food-borne transmission [2]. Consumption of fresh vegetables has been associated with cryptosporidiosis in Finland, Denmark and Sweden [1,5,6]. In the United States (US) insufficiently ozonated apple cider was described as a source of infection for a cryptosporidiosis outbreak [7], while one report documented contamination of food by a food handler [8].

In Sweden, cryptosporidiosis has been a notifiable disease since 2004. Overall, the incidence of cryptosporidiosis in Sweden has increased from 0.76/100,000 in 2005 to 1.7/100,000 in 2009 [9], with a seasonal peak during late summer months and autumn. However, the reported data are likely to underestimate the cryptosporidiosis burden since most laboratories do not test for *Cryptosporidium* unless specifically requested [6].

Several fingerprinting tools have been developed to examine the population structure and transmission dynamics of *C. parvum* and *C. hominis*, including sequencing of the 60-kDa glycoprotein (GP60) gene [10]. The GP60 gene is the most polymorphic gene

identified in *Cryptosporidium* spp. to date, and has been used to further classify *C. parvum* and *C. hominis* into different allele families and subtypes [11]. Sequencing of the GP60 gene, including the microsatellite region, has provided a clearer understanding of the host specificity of *C. parvum* [12,13] and has also proved to be a useful tool in investigations of *Cryptosporidium* outbreaks [10,14]. In Sweden, in addition to being based on information from the GP15 sequence within the GP60 gene, subtyping also relies on analysis of the mini- and microsatellite loci MS1 and TP14. This allowed to identify two different sources of a *C. parvum* outbreak in relation to exposure to outdoor swimming-pool water [15].

In Stockholm county the incidence of cryptosporidiosis increased two fold in October 2010 (1.26/100.000), compared to October 2009 (0.64/100.000). The number of cases diagnosed in October 2010 was 26, more than three times higher than the average number of cases (8 cases; range: 1–26 cases) reported for the same month in Stockholm in the past six years.

The preliminary investigation initially revealed that three of the cases had attended a national conference organised in Umeå (Västerbotten county, northern Sweden) between 4 and 5 October 2010 where a *Cryptosporidium* outbreak occurring in parallel was revealed. Additionally during October 2010, *Cryptosporidium* cases were reported among participants at a private party in the city of Örebro (Örebro county, central Sweden). We investigated the increased number of cryptosporidiosis cases in the Stockholm area, as well as the outbreaks in Umeå and Örebro in order to assess the magnitude and to identify the potential sources and vehicles of the disease. Furthermore, for the first time in Sweden, we explored the possibility of a connection between the three events using a molecular subtyping method based on nested polymerase chain reaction (PCR) GP60 gene amplification in real time.

Methods

Epidemiological investigation

Cryptosporidium cases confirmed in the Stockholm area were interviewed by phone using a standardised questionnaire regarding possible exposures in the two weeks prior to onset of symptoms. Specifically, we enquired about possible places of infection, history of travelling abroad, visits to swimming pools, meals at restaurants and food history.

We investigated the outbreaks in Umeå and Örebro using a cohort study approach for each event. We formulated the hypothesis that people became ill with gastrointestinal symptoms after consuming certain food items contaminated with *Cryptosporidium* oocysts. We defined a probable case as a person who attended the conference (Umeå) or party (Örebro) and developed diarrhoea (more than 3 loose stools/day) in the following 2–12 day interval. A confirmed case was any person who fulfilled the probable case definition and had a positive stool sample for *Cryptosporidium* oocysts.

Complete participant lists with email addresses were available for both events. We used a web-based questionnaire (Artologik - Artisan Global Software, Sweden; www.artologik.com) to collect data from conference attendees and staff in Umeå, as well from party guests in Örebro. We enquired about personal data (age, sex, place of residence), illness (symptoms, day of onset, duration and severity), meals attended and food items consumed. We estimated food-specific attack rates (AR), relative risks (RR) and 95% confidence intervals (95% CI), for each meal served and each food item or beverage consumed. Bivariate analysis of the individual food items served during conference meals was restricted to persons who attended those meals. The risk ratios which were significant in the bivariate analysis ($p<0.05$) were adjusted using binomial regression. We performed data analysis using Microsoft Excel and STATA 10 (StataCorp, USA).

An environmental investigation was carried out at the Umeå conference centre in order to evaluate food safety procedures.

Laboratory investigation

Faecal specimens included in this study originated either from sporadic cases from the Stockholm/Uppsala area (67 km north of Stockholm) or from patients connected to the outbreaks in Umeå or Örebro. Stool samples were checked for the presence of *Cryptosporidium* at the regional laboratories. Identification of oocysts was performed using microscopy of acid-fast stained smears.

Sample collection and DNA extraction

Samples containing *Cryptosporidium* oocysts were submitted to the Swedish Institute for Communicable Disease Control (SMI) for further species and subtype identification. DNA was extracted directly from stool specimens using QIAampDNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Disruption of the oocysts using a MiniBeadBeater (Biospec Products Inc., Beatsville) was performed before the extraction procedure.

Species determination and subtyping

Species identification was determined by PCR and subsequent restriction fragment length polymorphism (RFLP) as described previously [16]. This technique amplifies 830–840 bp of the small sub-unit rRNA (SSU rRNA) gene by nested PCR and differentiates *Cryptosporidium* species by banding patterns using restriction analysis of the secondary PCR product with the enzymes SspI and VspI.

For subtype analysis, a nested PCR which amplifies the GP60 gene was used as described elsewhere [17,18].

Bidirectional sequencing was performed on all amplicons obtained. Subtypes within GP60 allele families were determined [17]. The sequences were compared with published sequences in the GenBank database using the basic local alignment search tool (BLAST) tool (http://www.ncbi.nlm.nih.gov/BLAST). Representative sequences were deposited in GenBank under the following accession numbers: JQ028865–JQ028868.

Results

Epidemiological investigation

Stockholm cases investigation

In total, 34 laboratory-confirmed *Cryptosporidium* cases were identified in Stockholm county between 11 October and 30 November 2010, of which 31 were interviewed. Two cases stated that they had travelled to the Canary Islands and Cuba, respectively, 14 days prior to onset of symptoms. For 24 cases, the most probable place of infection was considered to be the Stockholm/Uppsala area. Of these, 11 were female. The median age of the cases was 30 years (range: 6–54). None of the cases were hospitalised. Twenty individuals reported having lunch daily, two weeks prior to onset of symptoms, in restaurants close to their work offices in Stockholm or Uppsala. The interviews revealed no common food item consumed by the cases.

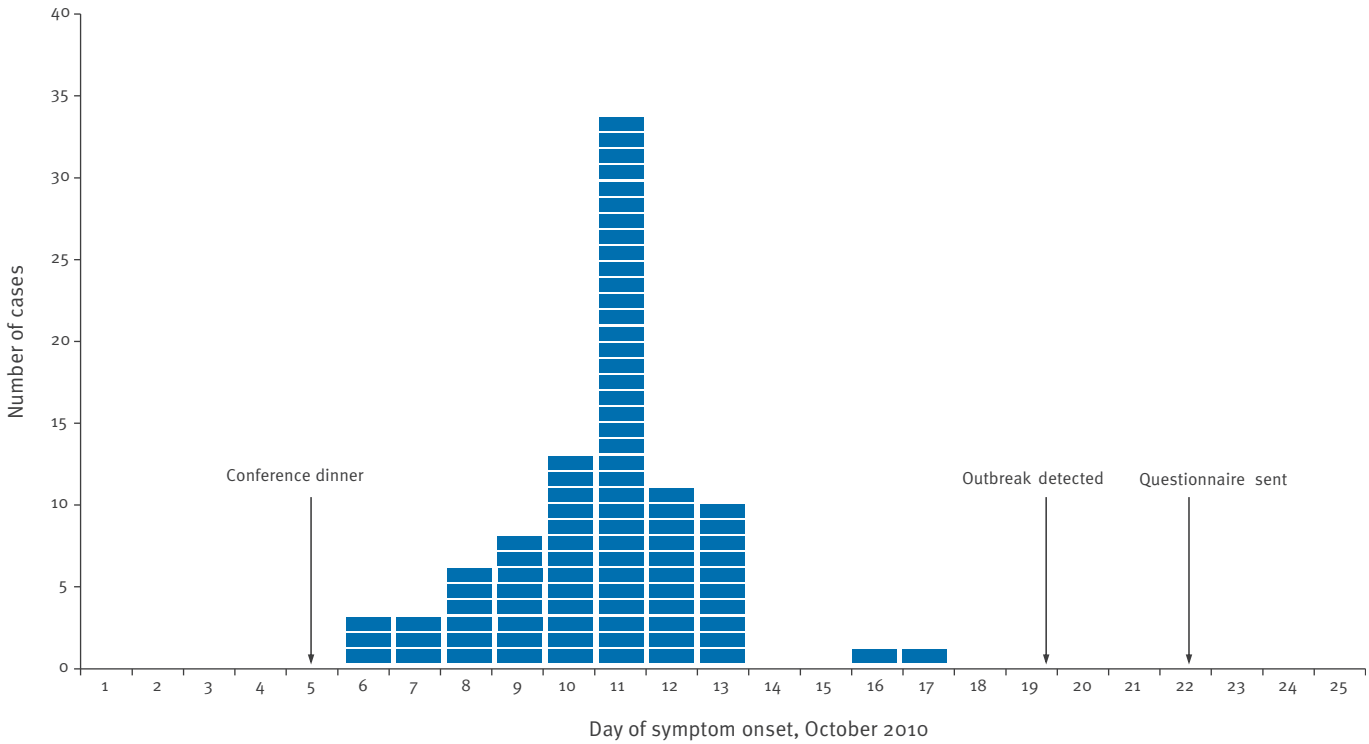
One case reported that she and nine work colleagues from Stockholm attended a conference in Umeå between 4 and 5 October. Five individuals, in this group fell ill with gastrointestinal symptoms after that event. This case proved to be the first laboratory-confirmed case of *Cryptosporidium* associated with the conference in Umeå. The four colleagues that showed similar symptoms were also confirmed with cryptosporidiosis.

Umeå outbreak

The national conference organised in Umeå, and attended by some of the cases diagnosed in Stockholm, had taken place between 4 and 5 October with 278 participants (240 attendees and 38 staff). Of 278 participants 203 replied to the questionnaire, of which eight were conference centre employees. Of the respondents, 118/203 (58%) were female and the respondents' median age was 46 years (range: 18–76). Aside from the cases who attended the conference and were identified in Stockholm, the web-based questionnaire allowed us to additionally detect 89 probable cases of whom nine were confirmed (44% attack rate among respondents). Most of the cases 58/89 (65%) were female and the cases median age was 46 years (range: 24–70). The main symptoms were diarrhoea for all cases (100%), bloating for 81/89 (91%) and abdominal pain for 69/89 (78%). None of the cases were hospitalised. The median incubation period was seven

Figure

Number of probable cases by date of symptom onset, *Cryptosporidium parvum* outbreak, Umeå, October 2010 (n=89)



days (range: 2–13) (Figure), while the median duration of symptoms was four days (range: 2–21). Additionally, five more confirmed *Cryptosporidium* cases attending the conference, were diagnosed elsewhere in Sweden and reported to the Swedish national surveillance system (SmiNet) as linked to the Umeå outbreak.

The attendees were served four meals during the conference. On 4 October there was an afternoon coffee-break and a two course dinner (main course and dessert), preceded by a drinks reception. On 5 October, only a morning coffee-break was organised. The only meal significantly associated with the cases was the main course of the dinner served on 4 October (RR: 24; 95% CI: 3.4–167; p=0.000). In the bivariate analysis of food items served during this main course, the chanterelle sauce had the highest risk ratio (RR: 4.2; 95% CI: 0.7–27; p=0.042). Also, people who ate at least one of the five herbs served as salad garnish had two times higher risk of becoming ill than people who did not (Table 1).

Besides food items included in the main course, we identified other theoretically possible sources of infection as: components of the dessert (mascarpone, cooked apples and hazelnuts), mixed nuts and water from a water dispenser (Table 1). In a binomial regression model we estimated an adjusted RR (aRR) of 11.3 (95% CI: 1.5–83.1) for the main course. Mixed nuts and drinking from the water dispenser were also significant with aRRs of 1.5 (95% CI: 1.2–2) and 1.6 (95% CI: 1.1–2.3) respectively. The dessert components had no association with disease: aRR 1.3 (95% CI: 0.7–2.3).

The environmental investigation performed at the Umeå conference centre revealed that none of the restaurant staff had gastrointestinal symptoms prior to the conference. Among the eight employees who replied, one was classified as a probable case – a woman who served at the dinner, ate the same meal as the guests, and became ill afterwards. None of the employees submitted a stool sample for *Cryptosporidium* oocyst identification. No violations of food safety procedures were discovered during the investigation. No food-leftovers were available for microbiological analysis.

Table 1

Relative risks for food items served at one conference dinner, 95% confidence intervals and p-values, *Cryptosporidium* outbreak, Umeå, Sweden, 4 October 2010

Exposure	Exposed			Unexposed			Risk ratio	95% confidence interval	p-value[a]	Percentage (%) of cases exposed in relation to the total number of cases (N=89)
	Total	Cases	Attack rate (%)	Total	Cases	Attack rate (%)				
Chanterelle sauce	138	83	60.1	7	1	14.3	4.2	0.7–27	0.042	93
Garnish[b]	101	62	61.4	13	4	30.8	2	0.8–4.6	0.042	69
Rocket salad	79	50	63.3	10	2	20	3.2	0.9–11.1	0.015	56
Parsley	69	43	62.3	10	2	20	3.1	0.9–10.1	0.016	48
Leek shoots	67	41	61.2	10	2	20	3.1	0.9–10.7	0.019	46
Green Salad	70	43	61.4	9	2	22.2	2.8	0.8–9.5	0.034	48
Pea shoots	72	42	58.3	10	3	30	1.9	0.7–5.1	0.173	47
Fillet of pork	147	85	57.8	9	3	33.3	1.7	0.7–4.4	0.179	96
Potatoes/ root vegetables	144	81	56.2	7	4	57.1	1.0	0.5–1.9	1	91
Hazelnuts[c]	93	58	62.4	24	7	29.2	2.1	1.1–4	0.005	65
Water (from water dispenser)[d]	79	44	55.7	84	24	28.6	1.9	1.3–2.8	0	49
Mixed nuts[d]	54	39	72.2	45	21	46.7	1.5	1–2.2	0.013	44

[a] The p-values were derived through Fisher's exact test.

[b] The variable 'garnish' was created in the analysis based on consumption of at least one of the five herbs served at dinner (rocket salad, parsley, leek shoots, green salad, pea shoots).

[c] Component of the dinner's dessert.

[d] [x]Both water from the water dispenser and mixed nuts were available for the entire duration of the conference on 4 and 5 October 2010.

Table 2

Relative risks for foods and beverages served at a party, 95% confidence intervals and p-values, *Cryptosporidium* outbreak, Örebro, Sweden, 9 October 2010

Exposure	Exposed			Unexposed			Risk ratio	95% confidence interval	p-value[a]	Percentage (%) of cases exposed in relation to the total number of cases (N=16)
	Total	Cases	Attack rate (%)	Total	Cases	Attack rate (%)				
Salad	18	15	83.3	3	0	0	NA	NA	0.015	94
Tart	21	16	76.2	2	0	0	NA	NA	0.083	100
Wine sauce	16	10	62.5	4	3	75	0.8	0.4–1.6	1	63
Whiskey sauce	17	12	70.6	2	2	100	0.7	0.5–0.9	1	75
Beef steak	24	16	66.7	0	0	0	NA	NA	NA	100
Mixed fruits	22	16	72.7	0	0	0	NA	NA	NA	100
Beer	10	4	40	10	9	90	0.4	0.2–0.9	0.057	25
Cider	3	3	100	14	7	50	2	1.2–3.4	0.228	19
Milk	6	5	83.3	12	6	50	1.7	0.8–3.2	0.316	31
Tea	2	2	100	15	8	53.3	1.9	1.1–3	0.485	13
Sparkling water	9	6	66.7	10	5	50	1.3	0.6–2.9	0.65	38
Wine	17	11	64.7	5	3	60	1.1	0.5–2.4	1	69

NA: not applicable.

[a] The p-value was derived through Fisher's exact test.

Örebro outbreak

Of 34 participants at the party in Örebro on 9 October, 24 replied to the questionnaire of which 14 (58%) were female. Sixteen individuals, including 12 (75%) female met the probable case definition, giving an attack rate of 67% among respondents. Only two laboratory-confirmed cases were indentified, whose samples were also submitted for genotyping. Being a female was a risk factor for becoming a case (RR: 2.4; p=0.02), and party guests aged under 30 were at higher risk of being a case than older people (RR: 2.5; p=0.007). Due to small population size (no cases unexposed), the RR and 95% CI could not be estimated for all the food and beverages served at the party (Table 2).

Laboratory investigation

In total, 31 samples positive for *Cryptosporidium* oocysts were sent to SMI for molecular investigation; 23 originated from cases from the Stockholm/Uppsala area (including the two cases with a history of travel abroad), six from the Umeå outbreak and two from the Örebro outbreak.

Species and subtype identification

Species determination and subtyping was successfully accomplished for 30 of 31 *Cryptosporidium* samples (Table 3). RFLP analysis of the amplified products of the SSU rRNA gene revealed *C. parvum* in 27 isolates, *C. hominis* in two and *C. felis* in one.

Subtyping based on sequencing of the GP60 gene amplicons identified five different *C. parvum* subtypes belonging to either allele family IIa or IId. The most frequent subtype identified, IIdA24G1, was found in 21 of the 27 *C. parvum* isolates, of which 15 originated from the Stockholm/Uppsala area and six from the outbreak linked to the Umeå conference. *C. parvum* subtype IIdA20G1e was identified in two isolates related to the Örebro outbreak. For the remaining six patients infected with *C. parvum*, four different subtypes were identified: IIdA20G1e (n=2), IIaA20R1 (n=1), IIaA15G2R1 (n=1) and IIaA16G1R1 (n=2). *C hominis* subtypes IbA10G and IdA15 were isolated from two patients who had travelled abroad.

Discussion

We investigated an increase in *Cryptosporidium* cases in the Stockholm/Uppsala area which subsequently led to the discovery of a *C. parvum* food-borne outbreak comprising 89 probable cases in the geographically distant city of Umeå in Northern Sweden. The laboratory results revealed the same *C. parvum* subtype (IIdA24G1) harboured by most of the cases from Stockholm/Uppsala area (15 of 20), thus confirming the outbreak in this region. The same subtype, *C. parvum* IIdA24G1 was also identified in all six isolates available from the Umeå cases.

Table 3

Provenance of clinical isolates with *Cryptosporidium* species characterised, *Cryptosporidium* outbreaks, Sweden, October–November 2010 (n=30)

Species and subtype[a]	Probable locations of infection				Total
	Stockholm /Uppsala	Umeå	Örebro	Others	
Cryptosporidium hominis					
IbA10G2	NA	NA	NA	Canary Islands	1
IdA15	NA	NA	NA	Cuba	1
Cryptosporidium parvum					
IIdA20G1e	NA	NA	2	NA	2
IIdA24G1	15	6	NA	NA	21
IIaA20R1	1	NA	NA	NA	1
IIaA15G2R1	1	NA	NA	NA	1
IIaA16G1R1	2	NA	NA	NA	2
Cryptosporidium felis					
ND	1		NA	NA	1
Total	**20**	**6**	**2**	**2**	**30**

NA: not applicable; ND: not determined.

[a] Unless otherwise specified.

Among the Umeå conference attendees, the distribution of cases over time suggested a food-borne point source outbreak with the most probable exposure being the dinner's main course. Bivariate analysis of food items indicated the chanterelle sauce and vegetables used as garnish as possible vehicles of transmission. These results could be explained by the fact that both food items were served on the same plate. Chanterelle sauce was prepared using high temperature cooking, while the salad garnish was the only food item served uncooked, thus increasing the possibility of harbouring the parasite. It has been previously documented that *Cryptosporidium* oocysts may enter and survive within the leaves of vegetables, thus increasing the likelihood of transmission if the vegetables are served without prior high temperature cooking [19]. Furthermore, simple washing may fail to remove all *Cryptosporidium* oocysts from contaminated vegetables [20]. In 2008, a *C. parvum* outbreak in Sweden was linked to chanterelle sauce with fresh parsley added after the preparation of the sauce [6], while in Finland a salad mixture was the suspected vehicle for a *C. parvum* outbreak [5]. Moreover, two other food-borne outbreaks were described recently in Sweden, in connection with parasite-contaminated vegetables. Sugar snap peas imported from Guatemala, harbouring *Cyclospora cayetanensis*, were the suspected vehicle for a cyclosporiasis outbreak in Stockholm 2009 [21]. The same year, the first reported food-borne outbreak associated with microsporidia (*Enterocytozoon bieneusi*) was described in connection with pre-washed, ready-to-eat cucumber [22]. These findings support the hypothesis that salad garnish could have been the vehicle for the Umeå outbreak.

We could not find an explanation for the association between disease and either the water dispenser or the mixed nuts. Bivariate analysis showed that 58 of 89 cases stated that they ate mixed nuts, while water from water dispenser could potentially explain only 44 cases. Since no water samples were available, the presence of oocysts in the water from the dispenser or tap could not be verified. There was no increase in numbers of gastrointestinal illness reported in Umeå during October, leading to the conclusion that the outbreak did not originate from the city's water-supply system.

We could not rule out the possibility that one of the conference centre staff could have been the source of the outbreak, as not all of the employees responded to the questionnaire and none provided a stool sample.

Eleven of 15 cases from the Stockholm/Uppsala area outbreak, infected with the same genotype (*C. parvum* IIdA24G1) reported having lunch frequently in restaurants and bars close to their offices in Stockholm and Uppsala, two weeks prior to symptom onset. The investigation revealed no common place of exposure for these cases, nor possible common food item eaten. Furthermore, we could not identify a common food item consumed by Umeå and Stockholm/Uppsala cases.

The outbreak in Örebro affected a smaller number of people compared to Umeå. Nevertheless, the attack

rate was high and the distribution of cases over time also indicated a point source food-borne outbreak. Only two stool samples were available from the participants, and the same *Cryptosporidium* subtype, IIdA20G1e, was identified in both. Due to small sample size, no significant association could be determined between the exposure to any food item and the disease.

Our epidemiological investigations had several limitations. Due to the long incubation period, the epidemiological data were obtained almost three weeks after the conference so recall bias may have led to misclassification of exposures. Since the food items of the main course were all served on the same plate, cross-contamination between food items was possible. We did not enquire about the amount of food items consumed and were therefore unable to calculate dose response relationships. For the interviewed cases in Stockholm, it is possible that some cases could not remember exactly if they ate the salad garnish. Poor recall of garnish items is common and was recently documented in Germany during a large outbreak of *Escherichia coli* O104:H4 associated with sprouts [23]. Moreover, because no food leftovers were available for microbiological analysis, we could not determine the presence of *Cryptosporidium* oocysts in any of the suspected vegetables. The origin of the vegetables could not be traced further than the wholesalers in Sweden and consequently, the mechanism of food contamination could not be determined. In the future, increasing the speed and ease of tracing suspected vegetables would be useful, especially since herbs appear to play an important role in outbreaks of gastrointestinal infections worldwide.

C. parvum subtype IIdA24G1 was identified in all samples available from Umeå and considered the probable aetiologic agent for this outbreak. All cases with this subtype from Umeå and Stockholm/Uppsala region had sequences that were 100% identical, suggesting a possible common vehicle for the two outbreaks. The presence of this subtype in humans is scarcely documented and has not previously been identified in Swedish patients [24]. Only one *C. parvum* IIdA24G1 sequence available in GenBank (accession number: HQ005751) and isolated from a sporadic *C. parvum* case in the United Kingdom was 100% identical with the one described in our report [13]. The subtype IIdA24G1 has also been described in human cases from Jordan and Australia [25,26] as well as in lambs and goat kids in Spain [27]. To our knowledge this is the first time that the *C. parvum* IIdA24G1 genotype has been linked to a food-borne outbreak.

Subtype *C. parvum* IIdA20G1e was isolated from the Örebro cases, suggesting that this event was not linked to the outbreaks in Stockholm and Umeå. Five different variants have been described for this subtype, IIdA20G1a-e [17,28]. Interestingly, the same variant as in our outbreak isolates, IIdA20G1e, was described in a Swedish calf, suggesting a possible zoonotic source for the Örebro outbreak [29].

The *C. parvum* IIaA20R1 subtype, isolated from a case in the Stockholm area, has not been reported previously. The other two *C. parvum* subtypes (IIaA15G2R1 and IIaA16G1R1) are commonly found worldwide, and recognised for their zoonotic potential [2,13,25,27,30,31].

The sequence of the *C. hominis* IbA10G2 isolated from the traveller to the Canary Islands was identical with the *C. hominis* subsequently isolated in Sweden from cryptosporidiosis cases during water-borne outbreaks in Östersund (November 2010) and Skellefteå (April 2011)[32]. This subtype was the most common in a study of Swedish patients with cryptosporidiosis [24] and it has also been identified as the most common *C. hominis* subtype worldwide [2,33]. The other *C. hominis* case with subtype IdA15 had visited Cuba, where this subtype was previously described in sporadic cases in children [34]. One patient was infected with *C. felis*, a *Cryptosporidium* species usually found in cats and rarely infecting humans [35].

The characterisation of *Cryptosporidium* isolates by the GP60 gene amplification method proved to be a useful tool in our investigation. The extra information supplied added important elements for the investigation of the three outbreaks in addition to providing valuable knowledge about cryptosporidiosis epidemiology in Sweden. Molecular characterisation of the isolates showed heterogeneity of subtypes among studied cases. There was a large variation in the GP60 gene with five different subtypes identified in 27 isolates. Subtypes isolated worldwide from sporadic or water-borne outbreaks, were also identified in Sweden by our study, as well as new or rare subtypes such as *C. parvum* IIdA24G1. Further studies are needed in order to improve the knowledge about cryptosporidiosis in Sweden.

It is important to bear in mind that we were only able to identify these cases as a result of our investigation into the cryptosporidiosis cases in Stockholm. We would therefore like to emphasise the importance both of testing for cryptosporidiosis in cases of diarrhoea (particularly domestic cases) and also of sending positive samples to the reference laboratory for genotyping. Molecular characterisation of isolates from cryptosporidiosis cases is not routinely performed in Sweden but we suggest that this method should be used in real time when investigating cryptosporidiosis cases that seem to cluster or belonging to an outbreak.

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References

1. Ethelberg S, Lisby M, Vestergaard LS, Enemark HL, Olsen KE, Stensvold CR, et al. A foodborne outbreak of Cryptosporidium hominis infection. Epidemiol Infect. 2009;137(3):348-56.
2. Putignani L, Menichella D. Global distribution, public health and clinical impact of the protozoan pathogen cryptosporidium. Interdiscip Perspect Infect Dis. 2010;2010.
3. Chappell CL, Okhuysen PC, Langer-Curry R, Widmer G, Akiyoshi DE, Tanriverdi S, et al. Cryptosporidium hominis: experimental challenge of healthy adults. Am J Trop Med Hyg. 2006;75(5):851-7.
4. Aragon TJ, Novotny S, Enanoria W, Vugia DJ, Khalakdina A, Katz MH. Endemic cryptosporidiosis and exposure to municipal tap water in persons with acquired immunodeficiency syndrome (AIDS): a case-control study. BMC Public Health. 2003;3:2.
5. Ponka A, Kotilainen H, Rimhanen-Finne R, Hokkanen P, Hanninen ML, Kaarna A, et al. A foodborne outbreak due to Cryptosporidium parvum in Helsinki, November 2008. Euro Surveill. 2009;14(28):pii=19269. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19269
6. Insulander M, de Jong B, Svenungsson B. A food-borne outbreak of cryptosporidiosis among guests and staff at a hotel restaurant in Stockholm county, Sweden, September 2008. Euro Surveill. 2008;13(51):pii=19071. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19071
7. Blackburn BG, Mazurek JM, Hlavsa M, Park J, Tillapaw M, Parrish M, et al. Cryptosporidiosis associated with ozonated apple cider. Emerg Infect Dis. 2006;12(4):684-6.
8. Quiroz ES, Bern C, MacArthur JR, Xiao L, Fletcher M, Arrowood MJ, et al. An outbreak of cryptosporidiosis linked to a foodhandler. J Infect Dis. 2000;181(2):695-700.
9. Smittskyddinstitutet (SMI). Statistik för cryptosporidiuminfektion. [Statistics for Cryptosporidium infection]. Solna:SMI. Swedish. [Accessed 15 Nov 2012]. Available from: http://www.smi.se/statistik/cryptosporidiuminfektion/
10. Ng JS, Pingault N, Gibbs R, Koehler A, Ryan U. Molecular characterisation of Cryptosporidium outbreaks in Western and South Australia. Exp Parasitol. 2010;125(4):325-8.
11. Waldron LS, Power ML. Fluorescence analysis detects gp60 subtype diversity in Cryptosporidium infections. Infect Genet Evol. 2011;11(6):1388-95.
12. Alves M, Xiao L, Sulaiman I, Lal AA, Matos O, Antunes F. Subgenotype analysis of Cryptosporidium isolates from humans, cattle, and zoo ruminants in Portugal. J Clin Microbiol. 2003;41(6):2744-7.
13. Chalmers RM, Smith RP, Hadfield SJ, Elwin K, Giles M. Zoonotic linkage and variation in Cryptosporidium parvum from patients in the United Kingdom. Parasitol Res. 2011;108(5):1321-5.
14. Grinberg A, Pomroy WE, Squires RA, Scuffham A, Pita A, Kwan E. Retrospective cohort study of an outbreak of cryptosporidiosis caused by a rare Cryptosporidium parvum subgenotype. Epidemiol Infect. 2011;139(10):1542-50.
15. Insulander M, Lebbad M, Stenstrom TA, Svenungsson B. An outbreak of cryptosporidiosis associated with exposure to swimming pool water. Scand J Infect Dis. 2005;37(5):354-60.
16. Xiao L, Singh A, Limor J, Graczyk TK, Gradus S, Lal A. Molecular characterization of cryptosporidium oocysts in samples of raw surface water and wastewater. Appl Environ Microbiol. 2001;67(3):1097-101.
17. Sulaiman IM, Hira PR, Zhou L, Al-Ali FM, Al-Shelahi FA, Shweiki HM, et al. Unique endemicity of cryptosporidiosis in children in Kuwait. J Clin Microbiol. 2005;43(6):2805-9.
18. Glaberman S, Moore JE, Lowery CJ, Chalmers RM, Sulaiman I, Elwin K, et al. Three drinking-water-associated cryptosporidiosis outbreaks, Northern Ireland. Emerg Infect Dis. 2002;8(6):631-3.
19. Macarisin D, Bauchan G, Fayer R. Spinacia oleracea L. leaf stomata harboring Cryptosporidium parvum oocysts: a potential threat to food safety. Appl Environ Microbiol. 2010;76(2):555-9.
20. Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C, et al. Isolation of Cryptosporidium parvum and Cyclospora cayetanensis from vegetables collected in markets of an endemic region in Peru. Am J Trop Med Hyg. 1997;57(6):683-6.
21. Insulander M, Svenungsson B, Lebbad M, Karlsson L, de Jong B. A foodborne outbreak of Cyclospora infection in Stockholm, Sweden. Foodborne Pathog Dis. 2010;7(12):1585-7.
22. Decraene V, Lebbad M, Botero-Kleiven S, Gustavsson AM, Lofdahl M. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. Epidemiol Infect. 2012;140(3):519-27.
23. Buchholz U, Bernard H, Werber D, Bohmer MM, Remschmidt C, Wilking H, et al. German outbreak of Escherichia coli O104:H4 associated with sprouts. N Engl J Med. 2011;365(19):1763-70.
24. Insulander M, Silverlas C, Lebbad M, Karlsson L, Mattsson JG, Svenungsson B. Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden. Epidemiol Infect. 2012:1-12.
25. Waldron LS, Ferrari BC, Power ML. Glycoprotein 60 diversity in C. hominis and C. parvum causing human cryptosporidiosis in NSW, Australia. Exp Parasitol. 2009;122(2):124-7.
26. Hijjawi N, Ng J, Yang R, Atoum MF, Ryan U. Identification of rare and novel Cryptosporidium GP60 subtypes in human isolates from Jordan. Exp Parasitol. 2010;125(2):161-4.
27. Quilez J, Torres E, Chalmers RM, Hadfield SJ, Del Cacho E, Sanchez-Acedo C. Cryptosporidium genotypes and subtypes in lambs and goat kids in Spain. Appl Environ Microbiol. 2008;74(19):6026-31.
28. Amer S, Honma H, Ikarashi M, Tada C, Fukuda Y, Suyama Y, et al. Cryptosporidium genotypes and subtypes in dairy calves in Egypt. Vet Parasitol. 2010;169(3-4):382-6.
29. Silverlas C, Naslund K, Bjorkman C, Mattsson JG. Molecular characterisation of Cryptosporidium isolates from Swedish dairy cattle in relation to age, diarrhoea and region. Vet Parasitol. 2010;169(3-4):289-95.
30. Alves M, Xiao L, Antunes F, Matos O. Distribution of Cryptosporidium subtypes in humans and domestic and wild ruminants in Portugal. Parasitol Res. 2006;99(3):287-92.
31. Xiao L, Zhou L, Santin M, Yang W, Fayer R. Distribution of Cryptosporidium parvum subtypes in calves in eastern United States. Parasitol Res. 2007;100(4):701-6.
32. Smittskyddinstitutet (SMI). Cryptosporidium i Östersund [Waterborne outbreak of cryptosporidiosis in Östersund, Sweden 2010]. Solna:SMI; Nov 2011. Swedish. Available from: http://www.smittskyddsinstitutet.se/upload/Publikationer/Cryptosporidium-i-Ostersund-2011-15-4.pdf
33. Ng J, MacKenzie B, Ryan U. Longitudinal multi-locus molecular characterisation of sporadic Australian human clinical cases of cryptosporidiosis from 2005 to 2008. Exp Parasitol. 2010;125(4):348-56.
34. Pelayo L, Nunez FA, Rojas L, Wilke H, Furuseth Hansen E, Mulder B, et al. Molecular and epidemiological investigations of cryptosporidiosis in Cuban children. Ann Trop Med Parasitol. 2008;102(8):659-69.
35. Elwin K, Hadfield SJ, Robinson G, Chalmers RM. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. Epidemiol Infect. 2012;140(4):673-83.

EMCDDA publishes 2012 report on the state of the drugs problem in Europe

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Today, 15 November 2012, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in Lisbon, published its 17th annual report on the state of the drugs problem in Europe.

In this year's report, the Centre raises concerns over a complex stimulant market with a diversity of powders and pills used today. While cocaine, ecstasy and amphetamines continue to be the main stimulants used, they are now competing with a growing number of emerging synthetic drugs, such as cathinones, one of the largest groups of new drugs being reported in Europe today.

The report also shows a decline in heroin use. While heroin-related problems continue, at lower levels, the Centre states that 'we may now be moving into a new era in which heroin will play a less central role in Europe's drugs problem'.

A chapter dedicated to drug-related infectious diseases and drug-related deaths stresses that regardless of the substance used, drug injecting continues to be an important vehicle for the transmission of infectious diseases, including HIV and hepatitis C, with new HIV outbreaks recently experienced by some European countries underlining the importance of maintaining effective public health response in this area.

The EMCDDA annual report 2012 is available for downloading in 22 languages on the Centre's website: http://www.emcdda.europa.eu/publications/annual-report/2012.