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Legionnaires' disease in a neonatal unit of a private hospital, Cyprus, December 2008: preliminary outbreak report

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We report an outbreak of Legionnaires' disease in neonates, affecting 11 newborn babies. The case fatality rate is currently 27%. The outbreak has been confirmed by detection of *Legionella pneumophila* antigen in eight of the 11 cases. Tests are in progress to determine the source of infection.

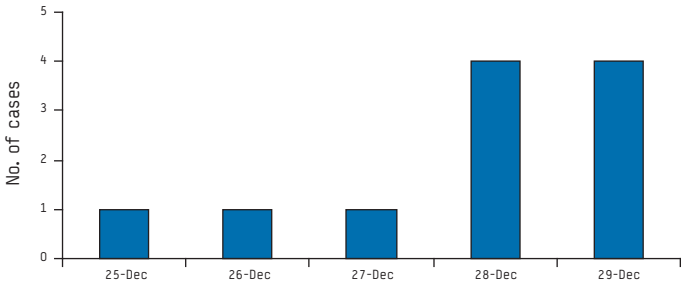
Legionnaires' disease is an established and frequent cause of pneumonia in adults but is thought to be a rare cause in children [1]. It is the acute pneumonic form of disease caused by legionella bacteria, usually *Legionella pneumophila*. About 80-90% of cases are caused by serogroup 1. The incubation period is usually 5-6 days (ranging from 2-10 days). The case fatality ratio has been as high as 39 percent in hospitalised cases, and even higher in immunocompromised persons. Most reported cases in children have involved neonates and children who are immunocompromised [2]. Nosocomial Legionnaires' disease associated with "water birth" (deliveries under water in a pool) has been reported in a few neonates [3].

Outbreak description

A cluster of 11 severely ill newborn babies were admitted to the Intensive Care Unit of the Governmental Hospital Archibishop Makarios III in Nicosia, Cyprus, between 25 and 29 December 2008. All were 7-11 days-old and born in a private hospital in Nicosia between 18 and 22 December 2008. None of the newborns were premature, and all had left the private hospital in a good general condition. They were admitted to the intensive care unit between three and five days after their discharge from the private hospital. The dates of admission of the 11 cases are shown in the Figure.

Figure

Legionellosis in newborns, by date of admission to the intensive care unit, Cyprus, December 2008 (n=11)



Clinical characteristics

The newborns were admitted with the following clinical signs and symptoms: Pyrexia, dyspnoea or tachypnoea and grunting, as well as failure of feeding. Two of them had cyanotic attacks, collapse and shock. Chest X-rays of all babies was positive for pneumonia.

To date, three babies have died (one baby admitted on 25 December died on 31 December, one admitted on 28 December died on 3 January, and one admitted on 28 December died on 7 January). Due to severe pneumonia, one baby is still on a respirator. The general condition of the remaining seven babies has improved, and six of them were discharged from the hospital on 9 January 2009. Immediately upon admission to hospital, all babies were given azithromycin and rifampicin, and the ones in critical condition were additionally given quinolones to cover bacterial infections with sepsis.

Laboratory investigations

Serum samples were tested for antibodies against adenovirus, respiratory syncytial virus, *Mycoplasma pneumoniae* and *L. pneumophila*. Two of the samples were positive for legionella IgM, all other samples were negative.

Urine samples were also tested for detection of *L. pneumophila* antigen. Initially, seven of the samples were positive for *L. pneumophila* serogroup 1 infection. Two negative samples were retested and one of them was then found to be positive, bringing it to a total of eight positive cases.

Bronchoalveolar lavage (BAL) culture was done for three babies who were on respirators. *L. pneumophila* was isolated from all three BAL samples and the serogroup was found to be 2-14.

The first results indicating legionella infection were available on the afternoon of 29 December 2008, and the recommended specific treatment for legionellosis (as described above) was started immediately.

Epidemiological measures

Following detection of the cases, the following measures were taken:

- Epidemiological investigations started immediately after the Unit for Surveillance and Control of Communicable Diseases

(Medical and Public Health Services, Ministry of Health) had been informed.

- The admitted newborns were treated in isolation from other newborns also treated in the intensive care unit.
- Disinfection of the newborn unit of the private hospital and of the water supply system was carried out, while the private hospital called experts in the field of water systems legionellosis management and control from the United Kingdom to come to Cyprus for assistance.
- In parallel with the laboratory investigation of the newborns, blood specimens from personnel working in the maternity ward (delivery room, maternity unit and the nursery) were collected and tested for respiratory syncytial virus infection (all were negative).
- Water samples were collected from various points in the private hospital and from the suspected humidifier that had been placed in the newborns´ unit on 12 December 2008. Water samples were also taken from the main water supply (municipal) and the distributing system in the hospital. *L. pneumophila* was isolated from the humidifier and from parts of the water distributing system in the hospital.
- Investigation of all the patients who had been admitted to the private hospital since 18 December 2008 was carried out in collaboration with the private hospital to rule out further pneumonia cases. No cases were identified.
- At the same time all paediatricians of babies born in the same hospital during the same period, were contacted, informed about the outbreak and requested to liaise with their patients about the situation.
- The private hospital was advised to implement all appropriate control measures to prevent any further nosocomial infections due to legionella.
- The maternity ward and the nursery were closed on 29 December 2008, immediately after the diagnosis of Legionnaires' disease was laboratory-confirmed.

Conclusion

Legionnaires' disease in neonates is extremely rare. This is the first confirmed outbreak affecting this age group and the case fatality rate is currently 27%. The investigation is still ongoing and tests are in progress to determine the source of infection.

It is possible that the discrepancy in the serotypes detected in the urine versus the BAL samples was due to cross-reactivity in the antigen test. Until the investigations are complete, however, we cannot exclude the possibility that both serotypes (1 and 2-14) were involved in the outbreak.

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Rebound of overdose mortality in the European Union 2003-2005: findings from the 2008 EMCDDA Annual Report

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Drug overdose is an important cause of death among young adults in Europe. According to data reported by Member States to the EMCDDA, many of the European Union countries reported a rebound in the numbers of overdose deaths in 2003-2005, following decreases in almost all reporting countries in previous years (2000 to 2003). Further investigations are needed in order to clarify the factor driving these increases and inform policies and interventions aimed at reducing these deaths.

Introduction

An important proportion of mortality of young adults in Europe is attributable directly or indirectly to illegal drug use, and it has been estimated that in some urban areas opiate use can account for over 20% of the overall mortality of the 15-49 year-olds [1,2].

Overdose deaths (ODs) are an important component of drug-related mortality. They are the main cause of death among problem drug users[1] in those European countries in which the prevalence of human immunodeficiency virus (HIV) infection in this group is low [3]. In the European Union, reported ODs outnumber, at present, other reported important causes of death attributable to drug use such as mortality due to HIV infection and acquired immunodeficiency syndrome (AIDS) transmitted through sharing injection equipment [4]. The risk of ODs is particularly high when drugs are injected, and polydrug use (the use of more than one substance in combination, often including alcohol or prescription medicines) is an important additional risk factor [5,6].

We aim to provide an overview of trends in ODs in the European Union from 2000 until 2005. We base ourselves on data recently made available in the 2008 Annual Report from the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [4].

Methods

The EMCDDA annually collects information from each of the 27 European Union (EU) Member States, and from Norway, Croatia and Turkey, on the number and characteristics (age-group, gender and basic information on substances found in the toxicological analysis) of people dying from an overdose of illegal drugs. Information is extracted by national focal points from the general mortality registries (GMR) following a standard protocol and case definition [7] that prescribes a set of ICD-10 codes as underlying cause of death. This set includes mental and behavioural disorders, and accidental, intentional or undetermined intent poisonings due to illegal drugs. Alternatively, in countries where a special mortality registry (SR, based on forensic and/or police sources) is considered of higher reliability, cases are selected when they are classified as poisonings due to illegal drugs.

For the present analysis, we used the data on ODs reported in the period 2000 - 2005 (last year with data available from most countries reporting to the EMCDDA). No data were available for Belgium, Cyprus, Denmark and Slovakia, whilst Croatia and Turkey were not yet participating in EMCDDA activities during the study period. The detailed data on ODs for each country are available in the 2008 EMCDDA Statistical Bulletin [8].

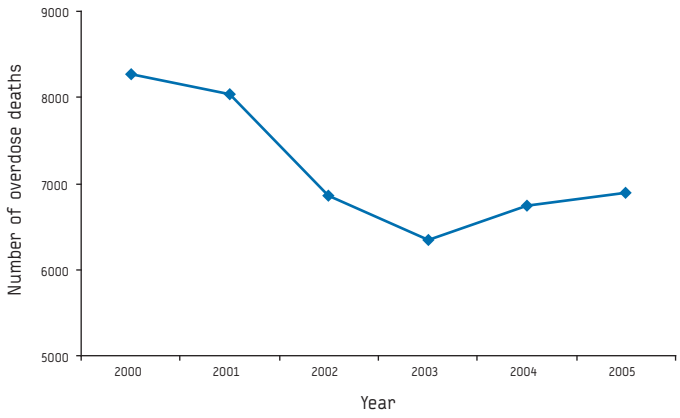
Population mortality rates and proportion of the total mortality attributable to drug overdoses were computed for 2005 using Eurostat population and mortality figures for 2005 [9].

Results

Between 2000 and 2003, the number of reported ODs decreased in 21 of the 24 countries included in the analysis, the total reported

Figure

Number of overdose deaths reported to the EMCDDA by 23 EU Member States and Norway, 2000-2005



Belgium, Cyprus, Denmark and Slovakia were not included in the analysis
In Sweden and Ireland, data for 2005 were missing and 2004 figures have been imputed.

number of deaths dropping from 8,275 to 6,350 (a 23% decline). However, a subsequent increase of ODs was observed between 2003 and 2005 in 16 of the 24 countries, with the total number of deaths increasing from 6,350 to 6,887 (an 8.5% increase) (see Figure).

Regarding toxicological findings, where information was available, opioids were detected in most OD cases reported to the EMCDDA, ranging from 33% to 100% of cases in 2005 (average of all cases in participating countries 80%).

We estimate that in 2005, ODs accounted for an average of 3.9% of all deaths among people aged 15 to 39 years in the countries participating in the analysis. In eight countries this proportion was over 7%, reaching 13.0%* in Malta, 10.6% % in Norway, 9.9% in Greece and 9.6% in the United Kingdom. Population rates of ODs ranged from under five to over 100 deaths per million inhabitants aged 15 to 39 years, with an estimated average of 28 per million in participating countries.

Discussion

A clear increase in reported ODs has been observed between 2003 and 2005 in many EU countries, reverting the previously declining trend seen between 2000 and 2003. A limitation of this analysis is that it was not possible to include more recent years because of incomplete data, indicating a need for more timely monitoring of OD. The factors influencing these trends are not well understood. Improved reporting is unlikely to explain this phenomenon, as the reporting methodology in most of the countries remained the same during the entire study period and therefore does not explain that the majority of countries recorded a reversal in the trend halfway through the observation period.

The number of ODs in a community is related to the size of the population at risk (problem drug users, in particular opiate users), but also to important risk factors such as injecting drug use and polydrug use as well as intensity and regularity of use [5,10]. In addition, the state of health of drug users such as deteriorated liver and respiratory functions could influence the risk of suffering a fatal overdose [11]. Comprehensive information on the prevalence of these factors and how they evolved during the study period is not available for many of the participating countries.

On the other hand, in the case of opiate users treatment (substitution and other) for their drug dependence provides a strong protection (by a factor of 10: hazard ratio 0.09; 95% confidence interval: 0.04-0.19) against OD as long as they continue the treatment, whereas the risk of OD is particularly high immediately after leaving treatment [12]. Overdose risk is also high immediately after prison release [13], possibly because of relapse to drug use of people that have lost tolerance to opiates. Provision of drug treatment has increased considerably in the past years in most EU countries and it will likely be a key element of any strategy to reduce the number of ODs. However, the quality of the treatment provided has to be further investigated, as well as the question if it is appropriately accessible for specific vulnerable groups (e.g. prisoners, immigrant or homeless drug users) [14].

Finally, specific interventions to reduce ODs are being implemented in some countries (e.g. peer education, family support groups, naloxone distribution to users, supervised consumption facilities), and further research is needed to assess their effectiveness [15].

Conclusions

A worrying change in the trend of reported ODs was observed between 2000 and 2005 in many of the reporting countries. This trend should be monitored more in-depth and in a more timely fashion. It is important to improve data availability on the factors influencing OD, in order to develop effective prevention interventions and policies.

1 Note: Problem drug use (PDU) is defined by the EMCDDA as "injecting drug use or long duration/regular use of opioids, cocaine and/or amphetamines"

* Authors' correction: Due to divergences in extraction procedures in Eurostat database resulting in extraction of incorrect figures for some countries, a wrong number was originally published for the proportion of deaths due to overdoses in Malta. The sentence "In eight countries this proportion was over 7%, reaching 20.0% in Malta,..." was corrected on 22 January 2009 to read "In eight countries this proportion was over 7%, reaching 13.0% in Malta,...". The authors apologise for the mistake.

Aknowledgements

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Long-term *Cryptosporidium* typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003

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To improve understanding of the aetiology and epidemiology of human cryptosporidiosis, over 8,000 *Cryptosporidium* isolates were submitted for typing to the species level over a four year period. The majority were either *Cryptosporidium parvum* (45.9%) or *Cryptosporidium hominis* (49.2%). Dual infection occurred in 40 (0.5%) cases and six other known *Cryptosporidium* species or genotypes were found in 67 (0.9%) cases. These were *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, and the *Cryptosporidium* cervine, horse and skunk genotypes. The remaining 3.5% were not typable. Epidemiology differed between infecting species. *C. parvum* cases were younger, although *C. hominis* was more prevalent in infants under one year and in females aged 15 to 44 years. Spring peaks in cases reported to national surveillance were due to *C. parvum*, while *C. hominis* was more prevalent during the late summer and early autumn as well as in patients reporting recent foreign travel. Temporal and geographical differences were observed and a decline in *C. parvum* cases persisted from 2001. Typing of isolates allowed outbreaks to be more clearly delineated, and demonstrated anthroponotic spread of *C. parvum* as well as *C. hominis*. Our findings suggest that national surveillance for *Cryptosporidium* should be conducted at the species level.

Introduction

The aetiological agent of the diarrhoeal disease cryptosporidiosis in humans has been traditionally ascribed to the protozoan parasite *Cryptosporidium parvum*, on the basis of microscopical identification of oocysts or detection of oocyst wall antigens in faeces, and the assumption that all oocysts detected were monospecific [1]. However, *C. parvum* variants, recognised initially phenotypically (designated human or H and cattle or C types) and latterly through genomic studies, segregate into two genotypes (1 and 2), of which genotype 1 is host-adapted for humans [2]. This is now assigned species status and called *Cryptosporidium hominis* and genotype 2 remains *C. parvum* [3]. Although these are the most commonly found species in human cryptosporidiosis worldwide, the distribution varies temporally and geographically [1]. Six other *Cryptosporidium* species have also been found in this host (*Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium suis*, *Cryptosporidium muris* and *Cryptosporidium andersoni*), as have *C. hominis* monkey genotype, *C. parvum* mouse genotype and the *Cryptosporidium* cervine, chipmunk genotype I, skunk, horse and rabbit genotypes [4, 5, 6]. The site-specific occurrence and pathogenicity of these unusual *Cryptosporidium* species/genotypes in humans appears to depend on a combination of endemnicity, exposure and parasite-related factors rather than host immune status [7].

Discrimination between *Cryptosporidium* species/genotypes is not possible by methods traditionally applied in routine diagnostic laboratories and national cryptosporidiosis surveillance is usually undertaken and reported without account of the aetiology. One exception is Scotland, where reference laboratory typing results have been incorporated in national surveillance since 2004 [8]. In England and Wales, the parasite is routinely identified at the genus level only [9] and surveillance data show that in the ten years between 1998 and 2007, the number of laboratory confirmed cases reported annually ranged from 3,010 to 5,863 [10]. More cases are reported in one to two year old children and cases are unevenly distributed over time, with peaks in the spring and autumn [11].

Although data have been published on the species identification and occurrence of *Cryptosporidium* spp. in human isolates, numbers studied are often small and / or from selected patient groups, and are rarely representative of community cases routinely seeking medical assistance [1]. The distribution of *C. parvum* and *C. hominis* cases mainly in England between 1998 and 1999, has been shown to vary geographically and temporally [12]. *C. parvum* was detected in 56.1%, *C. hominis* in 41.7%, and the remaining 2.2% comprised *C. meleagridis*, *C. felis*, *C. andersoni*, *C. canis*, *C. suis* and the *Cryptosporidium* cervine type, and samples containing both *C. parvum* and *C. hominis* [13]. While these studies contributed to knowledge of the epidemiology and transmission of *Cryptosporidium* species, national surveillance remained at the genus-level.

In order to improve our understanding of the aetiology and epidemiology of human cryptosporidiosis, and investigate changes over time, an on-going, representative, national collection of *Cryptosporidium* oocysts was established for the whole of England and Wales in January 2000. Here we describe the establishment, baseline aetiology and epidemiological analysis of the national

collection for the first four years (2000 to 2003), and assess the value of *Cryptosporidium* typing for epidemiological and surveillance purposes.

Methods

Between January 2000 and December 2003, faecal samples in which *Cryptosporidium* was detected during routine diagnosis of diarrhoeal disease in publicly funded laboratories through out England and Wales were referred to the Cryptosporidium Reference Unit (CRU) in Swansea for typing to the species level. Briefly, oocysts were separated from faecal debris by salt flotation, and disrupted by boiling, and DNA was extracted by a spin column technique (QIAamp DNA Mini Kit, Qiagen Ltd.) as described previously [14]. The *Cryptosporidium* oocyst wall protein (COWP) gene was investigated for all isolates using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) [15] and isolates where no amplicons were obtained were further tested by PCR-RFLP analysis of the small sub-unit (SSU) rRNA gene [16]. If amplicons were still not obtained, the stool was examined by microscopy following modified Ziehl-Neelsen staining of fixed smears [17] or immunofluorescence staining (Crypto-Cel, TCS Water Sciences) according to the manufacturer's instructions. PCR products with equivocal or unusual RFLP profiles were purified (Qiaquick, Qiagen Ltd), sequenced in both directions (GeneService Ltd) and edited, consensus sequences compared with published sequences in the GenBank database using the National Institutes of Health National Centre for Biotechnology Information basic local alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were verified and >99.5% similarity, in the region targeted by the PCR, to a published sequence was considered homologous.

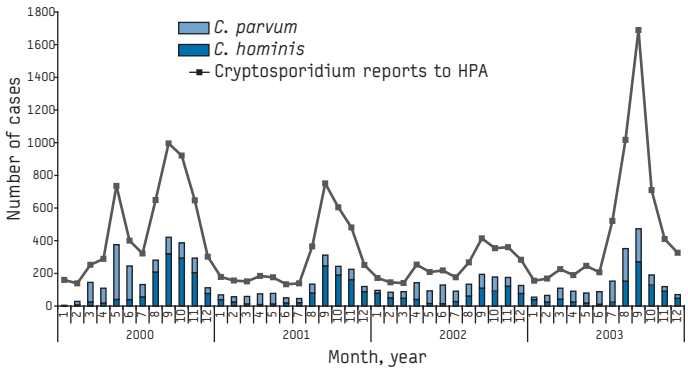
Patient demographics (locality, date of birth or age, and sex), clinical details, specimen collection date, history of recent foreign travel and whether the case was considered to be part of a family or household cluster or a general outbreak, was collected from the diagnostic laboratory on a form submitted with each sample and outbreaks verified with the investigating authority. For specimens where the collection date was missing, a proxy date was calculated by deducting from the date of receipt at the CRU the modal time delay for this interval (five days). Cases were geographically located using the Government Office Region of the submitting laboratory. Countries visited by patients reporting recent foreign travel were grouped according to the health advice provided in Health Information for Overseas Travel [18].

To confirm that the submitted samples were representative, the dataset was compared by specimen date, patient age and sex distribution with primary diagnostic laboratory surveillance reports to the Health Protection Agency, using the Mantel-Haenszel version of the chi-squared test and Mann-Whitney two sample test for sex and age distribution respectively.

Differences in demographic data and patient history of first time, confirmed cases of *C. parvum* and *C. hominis* in the whole dataset were compared by univariate logistic regression analysis and age distribution investigated using the Mann-Whitney two-sample test. Patients infected with *C. parvum* were designated as "cases" while patients infected with *C. hominis* were designated as "controls". Further analyses were undertaken separately for each infecting species. Patients co-infected with both species were excluded from these analyses. All statistical analyses were undertaken using EpiInfo (Version 6, Centers for Disease Control and Prevention, Atlanta, GA) and STATA 7 (Stata Corporation, College Station, TX).

FIGURE 1

Monthly total numbers of cases of *Cryptosporidium* in humans in England and Wales, 2000 to 2003, comparing laboratory surveillance reports and *C. parvum* and *C. hominis* cases identified in the sub-set submitted for typing



Source: Health Protection Agency and United Kingdom Cryptosporidium Reference Unit data

Results

Specimen submission

During the four year period from 1 January 2000 to 31 December 2003, a total of 8,075 faecal specimens were received from 133 primary diagnostic laboratories throughout England and Wales, representing 44.3% of the 18,235 *Cryptosporidium* cases reported to national surveillance over the same time period. The monthly distribution of submitted isolates reflected the number of cases reported to national surveillance (Figure 1).

The specimen collection date was available for 7,732 of the 8,075 (95.8%) specimens, and the time delay to date of receipt by the CRU ranged from 1 to 311 days (mean = 6 days, mode = 5 days, median = 5 days). The age of the patient was known for 8,003 (99.1%) specimens. The youngest patient was two months old and the oldest 98 years (mean = 16 years, mode = 1 year, median = 9 years). This was not significantly different from the cases reported to national surveillance (Mann-Whitney two-sample test =-0.031, df=1, p=0.9752).

Of the 8,075 specimens received by the CRU, 3,965 (49.1%) specimens were from males, 4,072 (50.4%) were from females and for just 38 (0.5%) the sex of the patient was not known. This was not significantly different from the ratio of male to female cases (1:1.02) reported to national surveillance ($\lambda 2$=0.06, P>0.05, df=1). Foreign travel was indicated on the submission form for 1,049 (13.0%) specimens in the CRU collection compared with 3% of cases reported to national surveillance.

Microbiological and genotyping results

Cryptosporidium was confirmed by microscopy or PCR in 7,829 (97.0%) specimens. Of the remaining 246 (3.0%) specimens, seven were identified by microscopy as Cyclospora cayetanensis, 44 were insufficient in volume for confirmation and the remaining 195 contained yeast cells, mushroom spores, pollen grains or unidentified staining artefacts.

Of the 7,829 confirmed isolates, 7,560 (96.6%) were typable by PCR-RFLP. The positivity rate for COWP PCR-RFLP was 88% on initial test, rising to 92% when a repeat test was included. The overall positivity rate rose to 96.6% following testing of COWP negative samples by SSU rDNA PCR-RFLP. The remaining 3.4% of specimens were confirmed by microscopy, but were not amplified or showed equivocal results (e.g. bands too faint to assign to species/genotypes or multiple bands present) by the PCR methods described here. A total of 141 repeat specimens were received from 70 patients. None of these sequential samples demonstrated a change in the *Cryptosporidium* species from that detected in the initial specimen.

Of the 7,758 first specimens from each patient, 3,817 (49.2%) were *C. hominis*, 3,564 (45.9%) were *C. parvum*, 40 (0.5%) were dual infections with *C. parvum* and *C. hominis*, other *Cryptosporidium* species/genotypes were identified in 67 (0.9%) and 270 (3.5%) were not typable. The unusual species/genotypes were *C. meleagridis* (n=56), *C. felis* (n=4), *Cryptosporidium* cervine genotype (n=4), *C. canis* (n=1), *Cryptosporidium* horse genotype (n=1) and *Cryptosporidium* skunk genotype (n=1). The finding of the horse and skunk genotypes has been described by Robinson et al., [6] and the epidemiology of cases other than *C. parvum* and *C. hominis* is being prepared for publication elsewhere.

Demographics

The patient demographics for *C. parvum* and *C. hominis* are compared in Table 1. The mean age of *C. parvum* cases (15 years, range 0 to 92 years, median 8 years, mode 1 year) was lower than that of *C. hominis* cases (17 years, range 0 to 97 years, median 9 years, mode 1 year) (Mann-Whitney two-sample test=9.69, df=1, p=0.002). Both species were linked to young age (0 to 9 years). There was an excess of *C. parvum* in 10 to 19 year olds, whereas *C. hominis* was common in adults, particularly those between 30 and 39 years of age. More detailed examination of the age-related data (Figure 2) showed that *C. hominis* was also more prevalent than

Table 1

Comparison of demographics and history of 7,381 cases with *Cryptosporidium parvum* and *Cryptosporidium hominis* in England and Wales over four years from 2000 to 2003: analysis using case-control methodology

Variable		*C. parvum* "cases" n=3,564	*C. hominis* "controls" n=3,817	Odds Ratio (95% CI)	p
10 year age group*	0 to 9 years	1,917 (53.8%)	1,946 (51.0%)	1.00	-
	10 to 19 years	579 (16.2%)	494 (12.9%)	**1.19 (1.04 to 1.36)**	**0.012**
	20 to 29 years	362 (10.2%)	352 (9.2%)	1.04 (0.89 to 1.22)	0.597
	30 to 39 years	354 (9.9%)	577 (15.1%)	**0.62 (0.54 to 0.72)**	**0.000**
	40 to 49 years	172 (4.8%)	182 (4.8%)	0.96 (0.77 to 1.19)	0.709
	50 to 59 years	88 (2.5%)	123 (3.2%)	**0.73 (0.55 to 0.96)**	**0.026**
	60 + years	68 (1.9%)	107 (2.8%)	**0.65 (0.47 to 0.88)**	**0.006**
Sex**	Female	1,819 (51.0%)	1,931 (50.6%)	1.00	-
	Male	1,733 (48.6%)	1,866 (48.9%)	0.98 (0.90 to 1.08)	0.761
Immuno-compromise reported	Yes	21 (0.6%)	29 (0.8%)	1.00	-
	No	3,543 (99.4%)	3,788 (99.2%)	0.77 (0.44 to 1.36)	0.373
Foreign travel reported	Yes	304 (8.5%)	621 (16.3%)	1.00	-
	No	3,260 (91.5%)	3,196 (83.7%)	**0.48 (0.41-0.56)**	**0.000**
Outbreak	Yes	276 (7.7%)	226 (5.9%)	1.00	
	No	3,288 (92.3%)	3,591 (94%)	**1.33 (1.11 to 1.61)**	**0.002**
Household cluster	Yes	223 (6.3%)	331 (8.7%)	1.00	-
	No	3,341 (93.7%)	3,486 (91.3%)	**0.70 (0.59-0.84)**	**0.000**
Government Office Region***	West Midlands	286 (8.0%)	253 (6.6%)	1.00	-
	London	37 (1.0%)	155 (4.1%)	**0.21 (0.14 to 0.32)**	**0.000**
	North West	1,044 (29.3%)	911 (23.9%)	1.01 (0.83 to 1.23)	0.889
	South East	191 (5.4%)	435 (11.4%)	**0.39 (0.30 to 0.50)**	**0.000**
	Yorkshire and The Humber	234 (6.6%)	333 (8.7%)	**0.62 (0.49 to 0.79)**	**0.000**
	North East	68 (1.9%)	96 (2.5%)	**0.63 (0.43 to 0.91)**	**0.009**
	East	293(8.2%)	454 (11.9%)	**0.57 (0.45 to 0.72)**	**0.000**
	East Midlands	236 (6.6%)	339 (8.9%)	**0.62 (0.48 to 0.79)**	**0.000**
	South West	621 (17.4%)	456 (11.9%)	1.20 (0.97 to 1.49)	0.079
	Wales	548 (15.3%)	358 (9.4%)	**1.35 (1.09 to 1.69)**	**0.006**

Source: United Kingdom Cryptosporidium Reference Unit data
p: significant values are indicated in bold
* Age not known for 60 cases
** Sex not known for 32 cases
*** Apart from baseline, ranked by decreasing population density per hectare

C. parvum in infants under one year of age. Although *C. parvum* and *C. hominis* cases overall did not differ with regard to sex, this was affected by age with more *C. parvum* in young boys and more *C. hominis*, especially in females, in the 30 and 39 years age group (Figure 2). There was no difference in the distribution of these *Cryptosporidium* species in immunocompetent and immunocompromised patients.

More patients with *C. parvum* belonged to recognised outbreaks but fewer belonged to family or household clusters where *C. hominis* was more common.

Travel history

C. parvum cases were less likely to have reported travel outside the United Kingdom (UK) prior to illness than *C. hominis* cases. The locations visited were Europe (207 *C. parvum*; 378 *C. hominis*), Indian subcontinent (18 *C. parvum*; 42 *C. hominis*), North Africa and the Middle East (18 *C. parvum*; 42 *C. hominis*), sub-Saharan and southern Africa (13 *C. parvum* and 27 *C. hominis*), the Caribbean (6 *C. parvum* and 18 *C. hominis*), South East Asia and Far East (4 *C. parvum* and 2 *C. hominis*), North America, Australia and New Zealand (5 *C. parvum* and 7 *C. hominis*), Central America (3 *C. parvum* and 8 *C. hominis*), South America (2 *C. parvum* and 6 *C. hominis*), mixed locations or country not stated (29 *C. parvum* and 36 *C. hominis*).

Geographical distribution

Regional differences were observed when compared with the West Midlands which had similar numbers of *C. parvum* and *C. hominis* cases. Government Office Regions on the eastern side of the country (i.e. London, South East, Yorkshire and the Humber, North East, East of England and the East Midlands) were more likely to have increased numbers of *C. hominis* while Wales, on the western side, had more *C. parvum* cases. The proportion of *C. parvum* and *C. hominis* cases in the North West and the South West were similar.

Seasonality

The annual proportion of cases of *C. hominis* (49.2% in 2000, 57.5% in 2001, 46.0% in 2002 and 45.1% in 2003) and *C. parvum* (47.1% in 2000, 35.7% in 2001, 49.3% in 2002 and 49.9% in 2003) was approximately equal each year, with the exception of 2001 when there was a much lower proportion of *C. parvum* cases, particularly in the spring. Because of this change over time, and the epidemiological differences highlighted here between *C. parvum* and *C. hominis*, the following data are presented annually and separately for each infecting *Cryptosporidium* species.

All ages were affected by the spring decline in *C. parvum* cases in 2001 (Figure 3), and the spring peak was only partially restored in 2002 and 2003 (Figure 1). During each of the four years most isolates were received during September, this peak being mainly composed of *C. hominis* and to a lesser extent *C. parvum* (Figure 1).

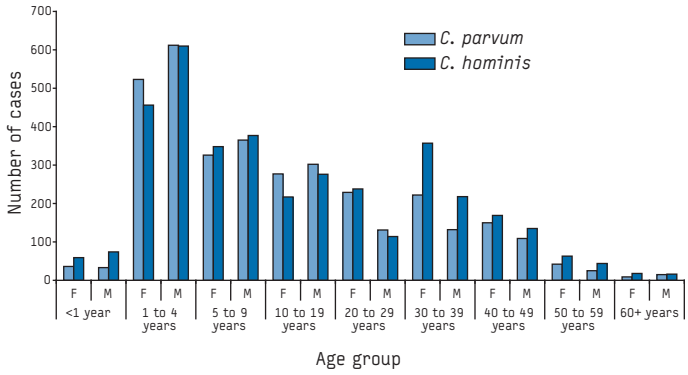
The spring peak in *C. parvum* was almost exclusively composed of indigenous cases, whereas the late summer / autumn *C. hominis* peak included patients who had reported foreign travel (Figure 4). In 2003 there was a substantial peak in *C. parvum*, probably linked to an outbreak originating among holiday makers in Majorca (Table 2). The younger ages particularly were affected by the unusual autumnal peak in *C. parvum* in 2003 (Figure 4).

Cryptosporidiosis outbreaks

Specimens were received from 508 cases linked to 29 locally or nationally recognised outbreaks of cryptosporidiosis during the four year period (Table 2). Outbreaks were caused by *C. hominis*

FIGURE 2

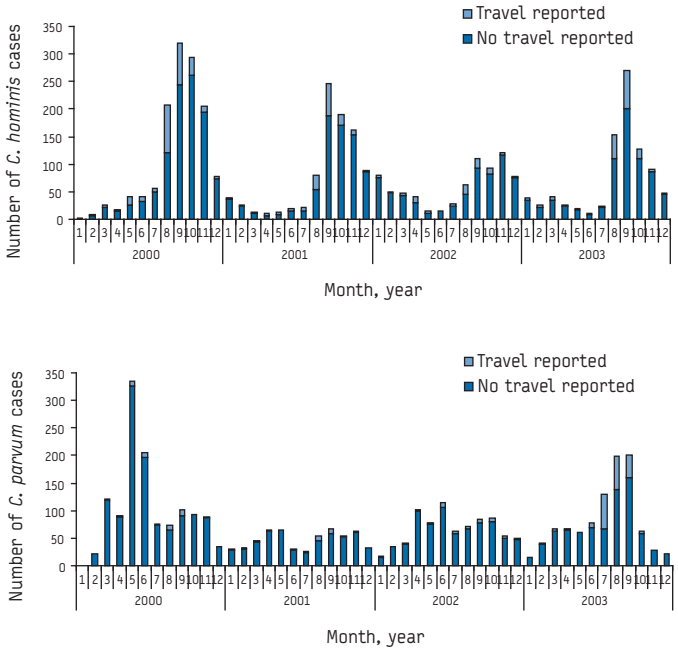
Age and sex distribution of *Cryptosporidium parvum* and *Cryptosporidium hominis* cases in England and Wales over four years from 2000 to 2003 (n= 7,381)



Source: United Kingdom Cryptosporidium Reference Unit data
F: Female; M: Male

FIGURE 4

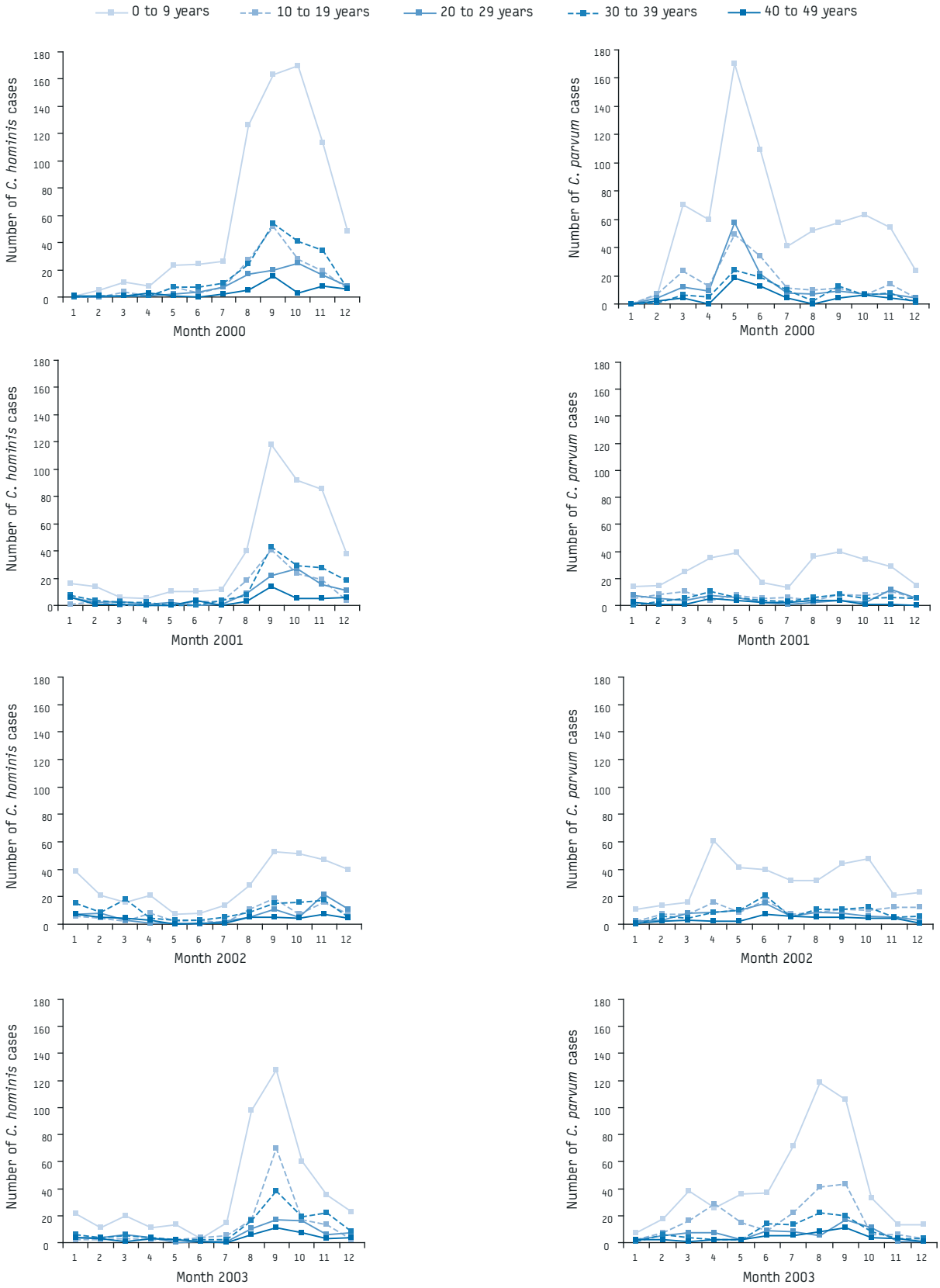
Distribution of *Cryptosporidium hominis* and *Cryptosporidium parvum* in cases reporting travel and not reporting travel outside the United Kingdom over four years from 2000 to 2003



Source: United Kingdom Cryptosporidium Reference Unit data

FIGURE 3

Age distribution of *Cryptosporidium parvum* and *Cryptosporidium hominis* cases in England and Wales, by month, in 2000, 2001, 2002 and 2003 (n=7,381)



Source: United Kingdom Cryptosporidium Reference Unit data

Table 2

***Cryptosporidium* species identified in outbreaks of cryptosporidiosis in England and Wales, from January 2000 to December 2003**

Year	Month	HPA outbreak database number	Government Office Region	Type of supply; source or contact	Cases ill (laboratory confirmed)	Isolates submitted for typing	*C. parvum*	*C. hominis*	Other	References
Drinking water										
2000	March	00/219	North West	Public supply; Spring	58 (58)	48	47	0	1 NT	[19, 20]
2000	May	00/413	North West	Public supply; surface water	207 (207)	134	119	14	1 NT	[21]
2000	May to June	00/440	South West	Private supply at a farm holiday centre	8 (3)	3	3	0	0	[19]
2002	May	~	Wales	Private supply at a child minder's premises on a farm	4 (4)	4	4	0	0	[22]
2002	November	02/1547	South East	Public supply	21 (21)	18	0	18	0	[23]
2002	November to December	02/1701	South East	Public supply; surface and borehole	31 (31)	28	0	28	0	[23]
2002	March	02/018	North West	Private supply; well at a college	50* (1)	1	1	0	0	[23, 24]
Swimming pools										
2000	May to June	00/406	Yorkshire and The Humber	Public pool	41 (41)	34	34	0	0	[19, 22]
2000	July to August	~	London	Club pool	9 (8)	7	1	6	0	Unpublished data
2000	July to August	00/723	London	Public pool	5 (5)	1	0	1	0	[19]
2000	September	00/656	London	Public pool	10 (10)	8	0	8	0	[19]
2000	September	00/870	South West	Public pool	12 (7)	1	1	0	0	[19]
2000	October to November	00/972	South West	Club pool	5 (5)	5	1	4	0	[19]
2001	June	01/347	South East	Outdoor school pool	152* (10)	5	0	5	0	[19, 24]
2001	October to November	01/528	South West	Club pool	3 (3)	3	0	3	0	[24]
2002	September to February	02/1877	South East	Public pool	20 (20)	5	1	4	0	Unpublished data
2003	January to April	03/220	Yorkshire and The Humber	Public pool	66 (48)	21	0	21	0	[23]
2003	August to September	03/409	South East	Public pool	17 (17)	2	0	2	0	[25]
Other water										
2001	August	01/440	South West	Contact with a stream at a beach	14 (6)	5	3	2	0	[24]
2003	August	03/411	West Midlands	Fountain in public park	122 (35)	32	0	31	1 *C. meleagridis*	[25]
2003	September	03/401	South West	Interactive water feature at an animal attraction centre	63 (27)	31	29	2	0	[25, 26]
Farm										
2003	March	03/167	East of England	Open farm, general public	7 (7)	2	2	0	0	[22]
2003	March	03/197	Wales	Open farm, school visit	17 (6)	6	6	0	0	[22]
2003	April	~	Wales	Residential farm centre, school visit	36 (12)	10	10	0	0	Unpublished data
Institutions										
2000	October	00/806	London	Day care nursery	13 (13)	13	0	13	0	Unpublished data
2001	September	01/442	South East	Day care nursery	30 (10)	8	0	8	0	Unpublished data
2002	November	02/1794	Yorkshire and The Humber	Day care nursery	47 (12)	9	0	8	1 NT	Unpublished data
International										
2000	Summer	~	Majorca	Hotel pool	>250	48	0	48	0	[27]
2003	July	~	Majorca	Hotel pool	179 (75)	16	14	0	2 NT	[28]

Source: United Kingdom Cryptosporidium Reference Unit, Health Protection Agency (HPA) and National Public Health Service for Wales data
~ Not recorded in the HPA outbreak database
* Concurrent community outbreaks of Norovirus may account for a proportion of cases.
NT = not typable

(13 outbreaks), *C. parvum* (10 outbreaks) and both species were detected in six outbreaks. Public drinking water supplies were associated with two outbreaks caused by *C. hominis*, one caused by *C. parvum* and one outbreak where both species were detected. Three outbreaks were linked to private water supplies and all three were caused by *C. parvum*.

Although more swimming pool-associated outbreaks in England and Wales were caused by *C. hominis* (n=6) than *C. parvum* (n=2), the largest indigenous outbreak linked to a swimming pool was caused by *C. parvum*. Both species were detected in three outbreaks linked to swimming pools. All swimming pool-associated cryptosporidiosis outbreaks except one were at indoor pools, the most common type in the UK. Furthermore, two international outbreaks were investigated, both linked to hotel pools in Majorca, one was caused by *C. hominis* in 2000 and the other by *C. parvum* in 2003, which may have influenced the subsequent increase in *C. parvum* in the autumn that year (Figure 1).

Water features were associated with two outbreaks, one linked to a fountain in a public park caused by *C. hominis* and the other associated with an interactive water feature at an adventure park featuring a petting zoo caused by both *C. parvum* and *C. hominis*. Three outbreaks linked to open or residential farms were caused by *C. parvum*. One outbreak linked to environmental contact was caused by *C. parvum* and *C. hominis*. Three outbreaks at day care nurseries were caused by *C. hominis*.

Discussion

In this paper, long term, *Cryptosporidium* species-specific epidemiological analysis is described for the first time at a national level, demonstrating that aetiological identification of a large proportion of cryptosporidiosis cases is possible, and furthermore, enhances the surveillance data provided by routine genus-level reporting. The epidemiology of *C. parvum* and *C. hominis* differs, and there is evidence for distinct sources and transmission routes. *C. parvum* infections occur all year round but mainly in the spring, although the spring peaks have declined since 2001. Outbreaks caused by *C. parvum* were linked to farm visits, environmental contact, drinking and recreational water. *C. hominis* cases occur mainly in the summer and autumn, in infants under one year of age and in adult females between 30 and 39 years, and in people who travelled abroad. *C. hominis* outbreaks were linked to day care nurseries, drinking and recreational waters. Some of these risks were identified in a case-control study of sporadic cases undertaken in 2001 which found contact with farmed animals as the significant risk factor for *C. parvum* and travel abroad, contact with another person with diarrhoea and changing young children's nappies to be significant risk factors for *C. hominis* [29]. Thus the epidemiology supports human transmission of *C. hominis*, and both zoonotic and anthroponotic transmission of *C. parvum*. Although household clusters of cases were more commonly caused by *C. hominis*, *C. parvum* was also involved. Human transmission of *C. parvum* was also demonstrated in outbreaks linked to indoor swimming pools, indicating a human source of contamination and infection with this species.

Multi-locus genotyping of a subset of *C. parvum* isolates from this collection, analysed with enhanced patient data, has demonstrated a predominance of some alleles linked to anthroponotic transmission, and others linked to zoonotic transmission [30].

The temporal distribution, with *C. parvum* predominating in the spring and *C. hominis* in the autumn, which has been reported in some other temperate climates [31] are shown to have changed over time in England and Wales. Although the national reduction in the spring peak in 2001, driven by *C. parvum*, showed strong association with control measures for the foot and mouth disease epidemic that year [32] it clearly continued after the control measures were lifted and data from this archive for the North West of England demonstrated links to improvements in drinking water quality [33]. This demonstrates the value of typing isolates in identifying interventions for disease reduction. The regional differences observed, reflecting population densities, have been further explored in analysis of the socio-economic risk factors [34]. This showed significant association between *C. hominis* and higher social economic status, young children and urban areas, and for *C. parvum* faecal application to land [35].

Although the cases in our dataset were representative of those reported to national surveillance, a higher proportion of our cases reported foreign travel. This is not considered to be submission bias but due to improved reporting since our submission form actively sought this information whereas it is reported passively to national surveillance. Travel data is under-reported in national surveillance and to a lesser extent to CRU, compared with enhanced data collection in a case-control study [29]. Travel-related cryptosporidiosis was mainly caused by *C. hominis* but this is influenced by the most frequently visited areas and differences may reflect variations in the endemic *Cryptosporidium* species of the host countries (about which little is known), or differences in behaviour and exposure during travel to different destinations. It is also possible that outbreaks among holiday makers may occur independently of the indigenous population, particularly if hotel swimming pools are involved [30]. It appears that foreign travel has a role in initiating the autumn peak, although this has not been investigated and should be studied further to investigate community spread and identify risk factors and interventions for disease reduction.

The typing methods used in this study enabled investigation of a vast number of specimens with very little loss in resolution [22]. Potential mis-identifications in the COWP assay that are currently known include the *Cryptosporidium* rabbit genotype confounding for *C. hominis* [6] and the mouse genotype mistaken for *C. parvum* [36]. Enhanced testing of a subset of our isolates indicates that the rabbit genotype is a rare human infection (unpublished data) and there is only one report from elsewhere of human infection with the mouse genotype [5]. *Cryptosporidium* species/genotypes not amplified by the COWP primers were further investigated at the SSU rRNA locus. PCR amplification of isolates not typable in this algorithm may have been inhibited by substances in the faecal samples or represent genotypes not amenable to amplification with the primer sets used in this study.

We identified 40 (0.5%) cases with dual *C. parvum* and *C. hominis* infections. This proportion is comparable with that found previously in England [13]. The disadvantage of any PCR-based system using common primers is the probable under-ascertainment of dual or multiple alleles within a sample. However, a subset of our isolates have been tested using separate species-specific primers and by multi-locus typing and showed little evidence of mixed infection [37]. The likelihood of dual infections is also driven by the endemicity of the parasite and exposure, as higher proportions have been detected in high-prevalence regions of the UK [38]. Unlike studies investigating only immunocompromised patients,

we investigated both immunocompetent and immunocompromised populations and found no difference in the distribution of *C. parvum* and *C. hominis*, and other species/genotypes were not more prevalent in immunocompromised patients (unpublished data).

Conclusion

Cryptosporidium species-specific risk factors have been identified as a result of this work. Although zoonotic risks regarding handling animals have been well described, indirect exposures are less well documented and in January 2004, the focus of national collection was changed to a sentinel laboratory scheme for the study of zoonotic cryptosporidiosis. The work presented in this paper facilitates the development of more rapid methods for *Cryptosporidium* species identification is facilitated by this work, not only providing an archive of material for assay development and evaluation but also by identifying that the key targets in the UK, and probably elsewhere in northern Europe, are *C. parvum* and *C. hominis*. In conclusion, species-level analyses are critical to the investigation and explanation of changes in incidence over time.

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An outbreak of measles in orthodox Jewish communities in Antwerp, Belgium, 2007-2008: different reasons for accumulation of susceptibles

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From August 2007 to May 2008, an outbreak of at least 137 cases of measles occurred in some orthodox Jewish communities in Antwerp, Belgium. The outbreak was linked to outbreaks in the same communities in the United Kingdom and in Israel. The reasons for this outbreak were diverse: cultural factors, misinformation on vaccination by some medical doctors and the lack of a catch-up vaccination programme in private Jewish schools. The identification of smaller susceptible groups for measles transmission and vaccination of these groups represent a major challenge for the measles elimination programme.

Introduction

Outbreaks of measles have been described in several European countries in 2007 and 2008. Travelling played an important role in several of these outbreaks. Roma populations and Irish travellers are some of the susceptible groups for measles transmission. Other susceptible groups identified in 2007 were orthodox Jewish communities [1,2].

These latter communities were also affected in an outbreak of measles in Belgium that occurred in Antwerp between August 2007 and May 2008 [3].

Measles vaccination in Belgium (trivalent measles-mumps-rubella (MMR) vaccine) has been offered free of charge since 1985 through the routine childhood immunisation programme (first dose at the age of 12 months) and through school health centres (second dose at the age of 10-13 years since 1995 and catch-up vaccination for both doses). Following the introduction of routine immunisation, the incidence of measles in Belgium has decreased from 998 cases per 100,000 inhabitants in 1982 to six cases per 100,000 in 1999 [4]. The national incidence of measles in Belgium in 2006 was estimated at between five and 10 cases per million inhabitants, based on data reported by a voluntary surveillance network of paediatricians and general practitioners (GPs), a laboratory network and mandatory notification of measles cases in schools [5]. The current surveillance of measles does not allow estimating incidences per province, but since 2002, only sporadic cases of measles have been notified in Antwerp.

Overall vaccine coverage for the first dose of measles vaccine (MMR1) in Antwerp is 94% according to a vaccine coverage study in children aged 18-24 months in 2005 [6]. Separate information on measles vaccine coverage in particular groups such as Jewish communities in Belgium is not available.

Antwerp is home to several Jewish communities, all residing in the same part of town. Orthodox Jewish communities are very isolated, with children going to Jewish schools.

In October 2007, a school health service in Antwerp reported several suspected cases of measles in two Jewish schools in the city. The objectives of this study were to describe the outbreak and to identify reasons for non-vaccination and accumulation of susceptible communities in Antwerp, in order to implement control measures and prevent outbreaks in the future.

Methods

Investigation of the cluster was carried out by the Public Health Surveillance of Flanders, in collaboration with the Scientific Institute of Public Health (IPH). Cases were reported by school health services in Antwerp (mandatory notification), by paediatricians and GPs (voluntary notification), through the Jewish communities (after an awareness campaign) and by the national laboratory for measles and rubella (IPH).

All cases that met with the clinical case definition of measles (rash and fever and at least one of the following symptoms: coryza, cough or conjunctivitis) and were either member of a Jewish community or had an epidemiological link with a case associated with the outbreak, were included. The diagnosis of measles was confirmed on saliva and nasopharyngeal samples (IgM and/or PCR) on as many cases as possible. Genotyping was performed by the national laboratory for measles and rubella (IPH) and the World Health Organization (WHO) regional reference laboratory in Luxembourg.

Epidemiological data were collected through a structured questionnaire, administered by the outbreak investigation team to cases or their parents, during a house visit or a phone interview.

Patients or their parents were questioned on demographical data, clinical data, contact with other patients, stay abroad and vaccination status (validated when possible by vaccination card). The electronic vaccination database of Flanders (Vaccinnet) was used to complete missing information on the vaccination status (for all cases).

Results

At least 137 cases of measles were identified in this outbreak between August 2007 and May 2008. The questionnaire was filled in for 128 cases (93%).

Epidemiological investigation indicated that the two first cases of measles, two children belonging to an orthodox Jewish community, had attended a summer camp in the United Kingdom (UK) (Figure 1). Both fell ill on their return. Further spread among ultra-orthodox Jewish communities may have been reinforced at different moments, as the outbreak points out, with possible re-importation of the virus from the UK and from Israel.

Almost all cases of the outbreak (96%) lived in the same neighbourhood in Antwerp, and 129 cases (94%) belonged to orthodox Jewish communities.

The age distribution for the measles cases is presented in Figure 2. The majority of cases (81%) were younger than 10 years of age. Of the 16 children that were under 12 months of age, three (19%) were between three and six months-old, seven (44%) were between six and nine months-old and six (37%) were between nine and 11 months-old. Two children (one four and one 11 months-old) were infected by their mother. Half of the non-Jewish cases were adults. 71 of 135 cases (for whom sex was known) were male. Complications (otitis, bronchitis, pneumonia) occurred in 14% of cases and 7% were hospitalised (n=130 for whom the information was available).

The diagnosis of measles was confirmed for 27% of cases. Genotyping was performed on 25 samples (18%) (Figure 1). The virus isolated was of genotype D4.

Data on vaccination status was collected for 129 measles cases (94%), of whom 28 children (22%) were vaccinated with one dose of measles containing vaccine, according to their parents. However, this information could only be validated for 15 children (12%). Of the 101 unvaccinated cases (according to the parents), 78 (77%) were eligible for vaccination according to their age.

For 69 (88%) of these cases, information on the reason for non-vaccination could be collected. Reported reasons were: 'on advice of the GP or paediatrician' for 26 cases (38%), 'by omission' for 18 cases (26%), and 'out of fear of side-effects, allergy or frequent disease in childhood' for 16 children (23%). Opposition to vaccination as reason for non vaccination was reported for only nine cases (13%), representing three families (5% of all Jewish families involved in the outbreak). 56% of the non vaccinated eligible cases were patients of the same GP, known to be opposed to vaccination. None of the families mentioned religious beliefs as reason for non-vaccination.

The majority of cases (40%) in this outbreak were identified by active case investigation and contact tracing. Mandatory notification in schools identified 21% of cases, although 67% of cases were school-aged children. The other cases were reported by the national laboratory for measles (19%), the Jewish communities (12%) and paediatricians and GPs (8%). Percentages pertain to the initial source of information.

Control measures

Awareness among the Jewish communities was raised through publications in a local paper (in Yiddish and in Flemish), with the help of Jewish doctors, rabbis and a Jewish health organisation. GPs and paediatricians in Antwerp were informed about the outbreak and invited to perform laboratory testing (on saliva) for confirmation of the diagnosis in suspected measles cases, to report the cases to the division of infectious disease control of at the Public Health Surveillance of Flanders and to check the measles vaccination status of all patients.

In response to the notification of the first measles cases in October 2007, vaccination was offered by the school health service to non-vaccinated children in the two affected subsidised Jewish schools in Antwerp. As the epidemic continued in spring 2008, a second vaccination campaign was carried out in May 2008 in all subsidised Jewish schools. Setting up a catch-up vaccination campaign in private Jewish schools was more difficult and time consuming, and took place in June 2008. Although no recent cases had been identified, about 500 school aged children were

Figure 1

Epidemic curve of measles cases from the outbreak in Antwerp, August 2007-May 2008 (n=133 cases with known date of onset)

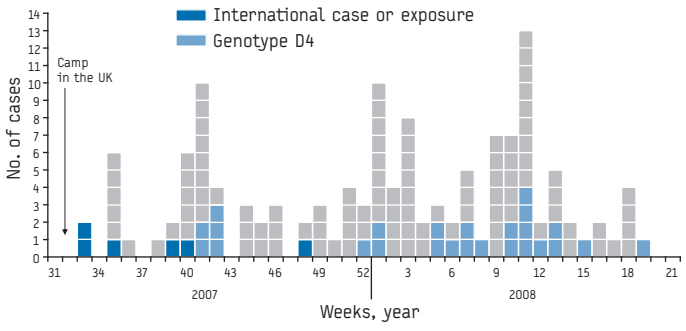
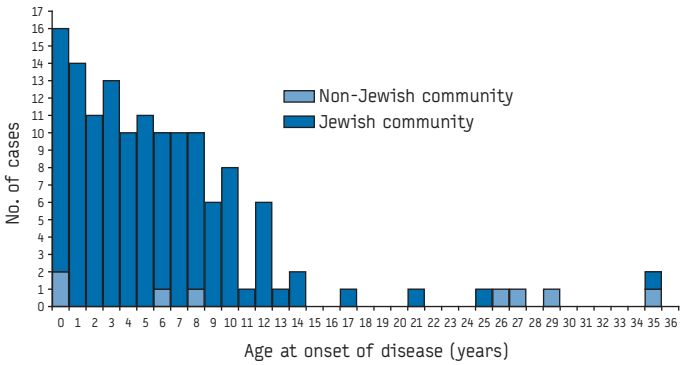


Figure 2

Age distribution of measles cases from the outbreak in Antwerp, August 2007-May 2008 (n=137)



vaccinated, to avoid new import of measles during the summer holidays by the remaining susceptible children.

Discussion

Similar to other culturally closed communities such as Roma and Irish travellers, orthodox Jewish communities belong to the group of hard-to-reach populations identified in Europe, as contact with "outsiders" is regarded with suspicion. Building up contact with representatives of these communities took time, but once established, investigation and control activities were carried out with their support.

As measles is not a mandatorily notifiable disease, some doctors refused to report cases. It is therefore likely that some cases were not identified. Nevertheless, active case finding through house visits allowed the description of the outbreak, and parents of cases collaborated well, which resulted in a high response rate to the questionnaires.

The virus strain circulating in Antwerp (D4) was of the identical genotype as the strain responsible for the outbreak in Jewish communities in the UK [1] and in Israel [2]. Although D4 strains have recently been implicated in major outbreaks in Europe [7,8] and information on circulating genotypes in Belgium or Antwerp before the outbreak is not available, it is most probable that the virus was imported from the UK.

Transmission of the virus within the Jewish communities occurred mainly at school, with further spread to the non-protected younger siblings at home. The high MMR1 coverage in the general population and the socially isolated way of life of ultra-orthodox Jewish communities avoided spread of the outbreak to the whole town or country. In total, only eight non-Jewish individuals were infected with measles during this outbreak. Except for two vaccinated children, the affected non-Jewish cases were either too young or too old to have taken part in the routine vaccination programme. Transmission to non-Jewish individuals occurred in the neighbourhood, through work or in the waiting room for paediatric consultation at a hospital in the area. Non-Jewish adult cases were initially diagnosed as having an allergic rash in response to antibiotics prescribed for a supposed respiratory tract infection.

The outbreak investigation highlighted that there were no religious reasons for opposition to vaccination. Similar to findings of a qualitative study among the orthodox Jewish community in London, many families had partially immunised their children [9]. Cultural factors (routine vaccination schedule started later and with a longer interval between vaccines, large families with omission of vaccination for one or two children) and lack of information or misperception of possible side effects or interaction with other diseases were important reasons why children did not get a first dose of MMR vaccine during their childhood. In subsidised schools where follow-up of health and vaccination status is provided by public health services of school medicine, catch-up vaccination is offered to the children at each of their regular consultation appointments (every 2-3 years). Pupils of private schools that do not have a school health service are not offered (catch-up) vaccination and have to rely on their paediatricians and GPs for information on vaccines and for vaccination itself. The investigation revealed that two physicians in Antwerp, known to serve a high proportion of the orthodox Jewish communities, are advising mothers not to vaccinate their children. Within families, the index case of measles was generally infected at (primary) school. Early catch-up vaccination for the first dose of MMR in schools, and systematically offering the second dose of MMR, not only in subsidised schools but also in private schools might have avoided this outbreak.

None of the measles cases in the outbreak was vaccinated with two doses of measles-containing vaccine, highlighting the importance of giving a second dose of vaccine.

Because of the outbreak and the vaccination campaigns, we can expect that all the individuals that had been susceptible to measles in these communities are now protected – through natural disease or vaccination. To avoid new accumulation of susceptibles, an agreement must be found to offer the routine childhood vaccines to pupils of private schools that do not yet have a school health service.

Conclusion

Very diverse reasons have led to an accumulation of people susceptible to measles within part of the population in the centre of Antwerp. The nature of social behaviour in Jewish communities, with frequent travelling and lots of international contacts, led to the importation of measles among these susceptibles, leading to an outbreak of at least 137 cases.

The orthodox Jewish communities were previously not identified as a risk group for measles transmission in Belgium. Other unidentified groups may exist.

The identification of smaller susceptible groups for measles transmission and systematic vaccination of these groups represent a major challenge for the measles elimination programme in Europe.

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High rates of community-acquired, Panton-Valentine leukocidin (PVL)- positive methicillin-resistant *S. aureus* (MRSA) infections in adult outpatients in Greece

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Staphylococcus aureus was isolated in 88 (30. 8%) of 286 adult patients suffering from various skin and soft-tissue infections examined in the outpatient department of a 650 bed tertiary-care hospital of Athens, Greece between January 2006 and December 2007. Twenty-seven (30.7%) of the *S. aureus* infections were caused by methicillin-resistant *S. aureus* (MRSA). All MRSA isolates were also resistant to tetracycline, fucidic acid and kanamycin, but were sensitive to gentamicin and tobramycin, as well as to to cotrimoxazole, chloramphenicol, quinolones, clindamycin and erythromycin. All isolates belonged to staphylococcal cassette chromosome *mec* elements (SCC*mec*) type IV, and were found to carry the *luk*F-PV and *luk*S genes coding for Panton-Valentine leukocidin (PVL). Pulsed-field gel electrophoresis (PFGE) and *spa*-typing revealed high genetic similarity among all MRSA isolates and with the PFGE pattern of the well-described ST80 clone that seems to be spreading through Europe. The high prevalence of MRSA among *S. aureus* infections in the community signify that empiric therapy in Greece, when clinically indicated, should exclude β-lactam antibiotics. Moreover, the establishment of an active screening for PVL-positive community-acquired (CA)-MRSA carriage and the adoption of a search and destroy strategy for CA-MRSA in all patients admitted with purulent skin and soft-tissue is of high priority in Greece as well as in all European countries which face high rates of CA-MRSA infection.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-recognised major cause of healthcare-associated infections. Over the past 10 years the epidemiology of this pathogen has changed throughout the world and infections caused by it have emerged in the community [1,2]. First reports of MRSA infections in the community were described predominantly in children without established risk factors for MRSA acquisition and were defined as community-acquired MRSA (CA-MRSA) [3]. Further infections have been reported among selected populations, including sports teams and correctional facility inmates. Moreover, infections in outpatients, mainly healthy, non-immunocompromised adults without risk factors have also been documented [1,2]. CA-MRSA isolates primarily cause skin and soft-tissue infections but serious, life-threatening, invasive infections such as bacteraemia and necrotizing pneumonia have also been described [4].

In Greece, 20 to 40 % of all *S. aureus* skin and soft-tissue infections in paediatric outpatients are found to be due to CA-MRSA [5,6]. In the presented study we investigated the prevalence of Panton-Valentine leucidine (PVL)-positive CA-MRSA among *S. aureus* infections in adult outpatients in a tertiary-care hospital of Athens.

Materials and methods

Between January 2006 and December 2007, 286 patients suffering from various skin and soft-tissue infections and with no history of hospitalization or any contact with a hospital during the past twelve months were examined in the outpatient department of a 650 bed tertiary-care hospital of Athens.

Laboratory testing

Microbiological examination of the respective clinical specimens and the identification of the species were performed by standard procedures. Resistance to oxacillin in *S. aureus* was assessed by the disc diffusion method, through cefoxitin resistance, according to the Clinical and Laboratory Standards Institute (CLSI) criteria [7]. The same criteria were used to determine resistance levels to other antibiotics (tetracycline, kanamycin, tobramycin, gentamicin, fucidic acid, chloramphenicol, erythromycin, ciprofloxacin and cotrimoxazol).

Molecular testing

Staphylococcal cassette chromosome elements (SCC*mec*) typing as well as detection of the *mec*A gene was performed by PCR, as described by Oliveira and de Lencastre [8]. The *luk*F-PV and *luk*S-PV genes coding for the PVL toxin, were detected by PCR, as described by Lina et al. [9]. To determine the genetic relatedness of the isolates, SmaI restriction fragments of genomic DNA were separated by PFGE as described previously [10] and analysed by BioNumerics software, version 4.6 (Applied Maths, Sint-Martens-Latem, Belgium), using Dice coefficients and the unweighted-pair group method by means of average linkages. *Spa*-typing was performed as described by Harmsen et al. [11] and *spa* types were determined using Ridom StaphType software version 1.4 (Ridom GmbH, Würzburg, Germany).

Results

S. aureus was isolated from 88 (30.8%) of 286 patients presenting with skin infections without history of hospitalisation or any contact with a hospital during the last year. Upon sensitivity testing the infection was found to be caused by MRSA in 27 (30.7%). Fourteen of the affected were men and 13 women. The mean age of these patients was 43 years, ranging from 29 to 56 years. Abscesses (skin abscesses 7, soft-tissue abscesses 9) dominated the clinical presentations, followed by furuncles (6), wound infections (4) and folliculitis (1) (Table). No statistically significant difference was found between the rates of methicillin-sensitive *S. aureus* (MSSA) and MRSA isolated from the various types of skin and soft-tissue infections. Moreover, there was no difference in age or sex between patients suffering from MSSA or MRSA infections (data not shown).

All MRSA isolates were resistant to tetracycline, fucidic acid and kanamycin, but they were sensitive to tobramycin and gentamicin, as well as to cotrimoxazole, chloramphenicol, quinolones, clindamycin and erythromycin.

All isolates belonged to SCC *mec* type IV and carried the *luk*F-PV and *luk*S-PV genes. PFGE revealed high genetic similarity among all MRSA strains (Table). The PFGE patterns of 18 isolates were identical and shared 100% similarity with the PFGE pattern of the well-described ST80 clone that seems to be spreading through Europe [12,13]. The remaining nine isolates revealed differences in one to three bands and were allocated into four subpatterns, comprising 5, 2, 1 and 1 isolates respectively. *Spa*-typing of the 27 strains, allocated 26 into *spa* type t044, a type closely associated with the ST80 clone and one to *spa* type t131 which is also associated with the ST80 clone (Table).

Discussion

MRSA has become a significant cause of community-acquired skin and soft-tissue infections in many parts of the world [12,13]. The worldwide spread of PVL-positive CA-MRSA clones that were initially described at the beginning of this decade to be continent specific [12] has been documented [12]. Furthermore, new lineages of PVL-positive CA-MRSA strains have also been detected [13].

It is well recognised that the high prevalence of MRSA among *S. aureus* skin and soft-tissue infections observed in the USA, is due to the spread of a single clone that can be identified on the basis of PFGE and other genotyping characteristics. This clone, a result of recent clonal expansion and diversification of a subset of isolates [14] is designated as the USA300 clone by the United States Centres for Disease Control and Prevention (CDC) in Atlanta. It belongs to MLST (ST8) and *spa* type (t008) which are different from the ones described in this study [15,16]

In Europe although CA-MRSA skin and soft-tissue infections have been reported from most countries, the prevalence of infections due to CA-MRSA appear to vary across the continent [17-27]. However, reports of prevalence rates of MRSA among *S. aureus* infections are, to the best of our knowledge, lacking. The currently prevailing genetic type among CA-MRSA in Europe is the PVL-positive, t044/ST80-SCC*mec* type IV [12]. In a recent Danish study, travel to or residing in countries abroad, especially in the Mediterranean region, the Balkans (Serbia) and the Middle East, where there is a high prevalence of CA-MRSA infections caused by t044/ST80-SCC*mec* type IV, have been associated with infections with this type [17]. Moreover, in some European countries strains with USA300 genotype are starting to be isolated with increasing frequency: The emergence of clones that are related to the USA300 has been associated with increasing rates of CA-MRSA in Spain. These clones were primarily isolated from immigrants from South America [25]. Further increasing isolation rates of the USA300 clone have been reported in Germany [26].

Contrary to the high degree of molecular diversity among CA-MRSA that has been shown in various parts of Europe [17, 24, 27], our study documented high genetic relatedness among the PVL-positive CA-MRSA isolates, which might indicate a successful and rapid spread of this clone in Greece. The study has some limitations since it focuses on patients presenting at the outpatient department of a large hospital, a fact that might be a selective factor for more serious infections. Nevertheless, the high prevalence of PVL-positive CA-MRSA has implications for both antimicrobial

Table

Main characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains* isolated in a tertiary-care hospital in Athens, Greece, January 2006 - December 2007 (n=27)

No	Sex**	Age	Disease	PFGE type	*spa* type	Resistance Phenotype***
1	M	45	Furuncle	A1	t044	Oxa Tet Km FA
2	M	43	Abscess (skin)	A	t044	Oxa Tet Km FA
3	F	45	Abscess (skin)	A	t044	Oxa Tet Km FA
4	M	34	Furuncle	A1	t044	Oxa Tet Km FA
5	F	43	Abscess (soft tissue)	A	t044	Oxa Tet Km FA
6	F	46	Folliculitis	A	t131	Oxa Tet Km FA
7	M	51	Furuncle	A2	t044	Oxa Tet Km FA
8	F	32	Abscess (soft tissue)	A	t044	Oxa Tet Km FA
9	M	45	Abscess (soft tissue)	A	t044	Oxa Tet Km FA
10	M	32	Abscess (soft tissue)	A	t044	Oxa Tet Km FA
11	F	38	Abscess (skin)	A	t044	Oxa Tet Km FA
12	F	31	Wound infection	A1	t044	Oxa Tet Km FA
13	F	29	Abscess (skin)	A	t044	Oxa Tet Km FA
14	M	35	Wound infection	A	t044	Oxa Tet Km FA
15	F	48	Furuncle	A	t044	Oxa Tet Km FA
16	M	39	Abscess (skin)	A3	t044	Oxa Tet Km FA
17	F	46	Abscess (skin)	A	t044	Oxa Tet Km FA
18	M	51	Abscess (soft tissue)	A2	t044	Oxa Tet Km FA
19	M	56	Abscess (soft tissue)	A1	t044	Oxa Tet Km FA
20	F	51	Furuncle	A	t044	Oxa Tet Km FA
21	M	45	Abscess (soft tissue)	A	t044	Oxa Tet Km FA
22	M	47	Abscess (soft tissue)	A	t044	Oxa Tet Km FA
23	M	43	Wound infection	A	t044	Oxa Tet Km FA
24	F	46	Abscess (soft tissue)	A	t044	Oxa Tet Km FA
25	F	51	Wound infection	A4	t044	Oxa Tet Km FA
26	M	34	Furuncle	A	t044	Oxa Tet Km FA
27	F	51	Abscess (skin)	A1	t044	Oxa Tet Km FA

* Note: All stains were sensitive to tobramycin and gentamicin, cotrimoxazole, chloramphenicol, quinolones, clindamycin, erythromycin.
** M=male; F=female
*** Oxa=Oxacyclin; Tet=Tetracyclin; Km=Kanamycin; FA=Fucidic acid

treatment and MRSA surveillance in Greece. Our results indicate that in this country empiric therapy when clinically indicated, should exclude β-lactam antibiotics. Moreover, empiric use of macrolides for purulent skin and soft-tissue infections should be monitored closely. Clindamycin, trimethoprim–sulfamethoxazole, or linezolid, because of their good activities against all *S. aureus* in general, are potential alternatives to β-lactams for oral application. However, routine microbiologic workup should be performed for all community-acquired skin and soft-tissue infections in this country.

In contrast to the well documented nosocomial spread of CA-MRSA in the USA, outbreaks of nosocomial infections due to CA-MRSA have so far been reported only sparsely in Europe, with eight cases in Germany in 2005 [28]. This might be due to an overall low prevalence of CA-MRSA in the European population, and thus a rare introduction of such strains to the hospitals by admission of colonised carriers on the one hand. On the other, the high clinical manifestation index of CA-MRSA might lead to an earlier detection of patients infected with CA-MRSA. The phenomenon may also indicate that ST80-MRSA type IV isolates are less well adapted to be sustained in hospital environments [17]. However, in Greece, PVL-positive ST80-MRSA type IV CA-MRSA have been introduced in at least one hospital since 2000 [29, 30], a fact of great public health significance. These strains are associated with increased disease severity mainly due to the presence of PVL genes, and a possible adaptation in the hospital environment would result in outbreaks of serious nosocomial infections. This perspective is of immense importance in a country already suffering from high rates of infections due to multidrug-resistant organisms (see Greek System for the Surveillance of Antimicrobial Resistance www.medne.gr/whonet and ERASS http://www.rivm.nl/earss/).

In conclusion we believe that the establishment of an active screening programme for PVL-positive CA-MRSA carriage and adopting a search and destroy strategy for CA-MRSA in all patients admitted with purulent skin and soft-tissue is of high priority Greece as well as in all European counties who face high rates of CA-MRSA infections.

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