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Peer-reviewed European information on communicable disease surveillance and control

Eurosurveillance

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Accepted for the impact factor – what is the impact of Eurosurveillance?

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Publications in impact factor journals have become an essential determinant of scientific careers and their listing plays a central role in the evaluation of grants, promotions etc. The scientific community judges the quality and importance of a journal and of individual publications by the impact factor which is awarded by a company called Thompson Reuters.

After several recent improvements to the journal, such as the merging of the former monthly and weekly editions into one weekly edition in January 2008 and the launch of a new website in April 2008, we embarked on an evaluation, in collaboration with a medical librarian, of whether our journal met the inclusion criteria for an impact factor [1]. We applied for the impact factor in October 2008 and are pleased to announce that Eurosurveillance has recently been selected for coverage by Thomson Reuters and is now indexed and abstracted in the Science Citation Index Expanded (also known as SciSearch®) and in the Journal Citation Reports/Science Edition, beginning with Volume 14(1) 2009.

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The basis for the calculation of the impact factor is the frequency with which the average article in a given journal has been cited in a defined period [2]. For Eurosurveillance, we expect the allocation of our first official impact factor for 2011, after the two-year evaluation period. It will be calculated as a ratio with the total number of citable articles published in Eurosurveillance in 2009 and 2010 as the denominator and the number of citations these articles receive in indexed journals in 2011 as the numerator. Generally, citable items are articles, reviews, proceedings or notes, while editorials or letters to the editor are excluded.

Obvious challenges lie ahead of us, and we will maintain our efforts to select the most interesting articles of high quality for our readers while at the same time supporting capacity building across Europe by lending assistance to less experienced authors. However, in the field of public health and communicable diseases, the real impact of a journal is determined by more than the calculated value of the impact factor. When dealing with outbreaks or emerging diseases, it is important that authoritative information is disseminated rapidly and reaches a wide range of stakeholders. Public health experts and policy makers require scientifically sound information that will allow them to choose necessary and appropriate public health actions.

In the past months, Eurosurveillance has proven to have an impact on public health by documenting the emerging H1N1 influenza pandemic in numerous reports not only from Europe but also from North and South America, Asia, Australia and New Zealand. In 61 articles to date, we have covered relevant aspects of the pandemic from modelling and phylogenetic analysis to antiviral treatment and vaccination. The majority of articles were rapid communications that we were able to process within one week from submission thanks to authors and peer reviewers who agreed to work to tight deadlines despite their already high work load. We are grateful for this support, and the efforts have paid off. Papers published in Eurosurveillance were further disseminated through channels such as ProMEDMail (http://promedmail.oracle.com/pls/otn/f?p=2400:1000:) and the Lancet H1N1 flu resource centre (http://www.thelancet.com/H1N1-flu). They featured widely in the general media and caught the attention of many experts and high level policy makers. Articles in Eurosurveillance were cited by a co-chairman of President Obama's Council of Advisors on Science and Technology [3].

We are confident that authors, reviewers and readers will continue to support us in our efforts to publish relevant and influential information of high quality, and that in two years time, these efforts will be manifest not only in the assigned impact factor but also in our actual impact for public health in Europe.

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A foodborne outbreak due to Cryptosporidium parvum in Helsinki, November 2008

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We report the first foodborne outbreak caused by *Cryptosporidium parvum* in Finland. The outbreak occurred among personnel of the Public Works Department in Helsinki, who had eaten in the same canteen. 72 persons fell ill with diarrhoea, none was hospitalised. Four faecal samples obtained from 12 ill persons were positive for *Cryptosporidium* by an antigen identification assay and microscopy. The vehicle of infection could not be identified with certainty but a salad mixture was suspected.

Introduction

Cryptosporidium infection is transmitted by the faecal-oral route and results from the ingestion of *Cryptosporidium* oocysts through the faecally contaminated water or food or through direct person-to-person or animal-to-person contact [1]. The infectious dose is low, 10-30 oocysts [2,3]. The reported foodborne outbreaks are not as common as those caused by swimming in water. In the United States, *Cryptosporidium* is the leading cause of reported recreational water-associated outbreaks [1,4,5].

On 12 November 2008, the Food Control Unit and the Epidemiology Unit of the Helsinki City Health Department were alerted of a gastroenteritis outbreak among the clients of the canteen of Public Works Department. Tens of people had fallen ill within the two weeks since 31 October. The main symptoms were watery diarrhoea, which lasted approximately one week, abdominal pain, fatigue and nausea. All persons affected had eaten at the canteen of the Public Works Department.

Materials and methods

The canteen of Public Works Department belongs to a large chain of catering services. The daily lunch includes three dishes of warm food, salad buffet and bread. Approximately 100-150 persons of the total personnel of 400 use daily the services of the canteen.

According to the standard procedures, the Food Control Unit listed the foods served between 22 and 31 October. The number of different foods and drinks served in the canteen was about 30 per day. A retrospective cohort study was carried out among the personnel by the Food Control Unit. A detailed questionnaire on symptoms and consumption of canteen food during the period of 22 to 31 October was e-mailed to all 400 staff members on 18 November. Completed questionnaires were obtained from 127 persons (response rate 32%). A case was defined as a person with diarrhoea (at least four loose stools a day) or laboratory-confirmed Cryptosporidium infection during the period from 31 October to 14 November. Associations between food items and illness were assessed by univariate analysis using the chi-squared test.

In late November, the health inspector examined the consignment records of the canteen and found that some salads had not been included in the questionnaires. These included a mixture of lettuce packed of red and green colour by a Swedish company. The salad mixture had been served during two or three days on the week before the beginning of the outbreak. A separate case-control study was carried out on 19 December. In order to find out about the consumption of the salad mixture, 30 cases and 30 controls randomly identified from the cohort study were interviewed by phone. Of the cases 29, and of the controls 30 replied.

The canteen was inspected on 14 November and 19 samples of foods and spices used between 27 and 31 October were taken. The food samples of the previous week had already been disposed. The food samples were analysed for *Escherichia coli, enterococci, Staphylococcus aureus, Campylobacter*, yeasts and molds, and later for *Cryptosporidium*. Some specimens were analysed also for total aerobic bacteria count, *Bacillus cereus* and *Enterobacteriaceae*. Two drinking water samples were taken on 11 November and analysed for total number of aerobic microbes, faecal coliforms, *Escherichia coli* and free and total chlorine, and estimated for colour, taste, odour and appearance as part of the internal quality control. No irregularities in the kitchen conditions, functions of the staff or in complying with internal quality control were found.

Stool samples were taken on 12 to 14 November from 10 ill quests of the canteen and from two ill members of the kitchen staff. The samples were initially tested for *Campylobacter, Salmonella, Shigella* and *Yersinia* spp. as well as norovirus. On 17 November, the investigating team requested stool samples to be analysed for *Cryptosporidium*. The samples were analysed by using Remel's (Lenexa, US) ProSpecTRGiardia/Cryptosporidium and ProSpecTR. Presence of *Cryptosporidium* was further verified from all positive

samples by modified Ziehl-Nielsen staining. Faecal DNA samples of three patients were available for PCR analysis [6].

Results

Seventy-two persons (41 women, 31 men) met the case definition. The mean age was 48 years. The outbreak peaked on 3 to 4 November when 38 cases fell ill (Figure). Two members of the kitchen staff reported diarrhoea with the onset on 3 November. Watery diarrhoea (100%), fatigue (85%), abdominal pain (76%), nausea (69%) and headache (61%) were the most common symptoms. Fever (31%) and vomiting (21%) were reported less often and some patients reported arthralgia or myalgia. The epigastric pain was often described as very severe. Two persons had to visit hospital emergency services, but none was hospitalised.

Four stool samples of 12 persons were found positive for *Cryptosporidium*. None of them belonged to kitchen staff. No other pathogens were found. Control samples taken from the infected persons approximately two weeks later were found negative for *Cryptosporidium*. In one sample, the amplification of *Cryptosporidium*-specific PCR product was successful and the sequence had 100% similarity with the sequence of *C. parvum*.

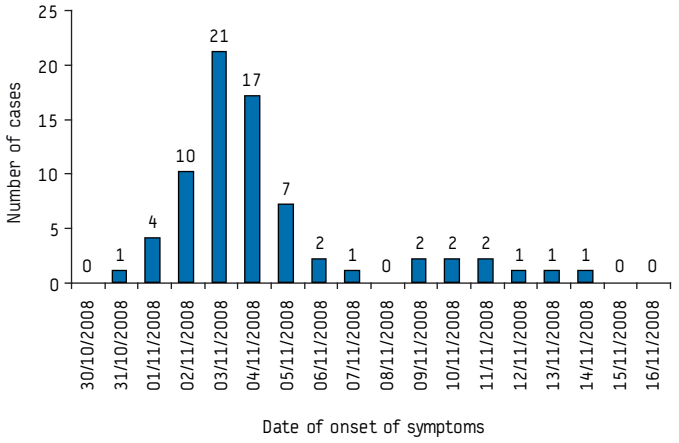
Food samples were negative for *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Salmonella, Campylobacter* and enterococci. Methods to analyse *Cryptosporidium* from food samples have not been built up and validated in Finland, but the salads were examined by using the same method as for stool samples. These results were negative, too. Analyses of drinking water suggested no faecal contamination. The total number of aerobic bacteria was 1 and 0 cfu/ml.

The analysis of the cohort study did not show significant association between any of the foods served and the illness. In the case-control study, the odds ratio for consumption of the salad mixture was 22.5 (95% CI 3.5–177.9).

The imported lot of the salad mixture weighted 486 kg and consisted of two batches. The batches contained salads from Denmark, France, Spain, Italy and Sweden. According to the records, the lot was divided and sold in small quantities to 130 premises in various municipalities all around Finland. The Building Department canteen received 1.5 kg of the salad mixture possibly originating from both of the batches. Thus, the exact tracing was not possible.

FIGURE

Date of onset of symptoms in persons affected by an outbreak of cryptosporidiosis, November 2008, Helsinki, Finland (n=72)



Discussion

More than 70 guests of a canteen of the Public Works Department of Helsinki fell ill with gastroenteritis in October 2008. The symptoms were compatible with cryptosporidiosis and *Cryptosporidium* spp. was detected in stool specimens of four patients. Genotyping of one isolate showed that the causative agent was *C. parvum*. A case-control study suggested that mixed salad was the source of the outbreak. This was the first time that *Cryptosporidium* was found to cause an outbreak in Finland.

All workers of the Public Works Department, personnel of occupational health authorities and the National Public Health Institute were immediately informed about the outbreak. Persons having diarrhoea were instructed about their personal hygiene and were forbidden to use public swimming pools until the end of November. On 21 November, a press release about the outbreak was issued by the local authorities.

Vegetables, and especially salads, have been shown to be an important source of foodborne outbreaks recently [7,8,9]. Specifically, *Cryptosporidium* was linked to consumption of vegetables in Nordic countries [10,11]. In addition, *Cryptosporidium* was found in samples from fresh produce [12,13]. In our outbreak, the vehicle transmitting *Cryptosporidium* was unfortunately not found. Information about the outbreak came so late to the municipal authorities that relevant food samples were no longer available. Food Control Department of Helsinki recommends that in institutional kitchens, frozen samples of 200 g from all served foods should be stored for two weeks to enable microbiological investigations after possible outbreaks. Operators of either the producer or the importer of the suspected salad did not comply with the legislation of the European Union. The Article 18 of the Regulation 178/2002 of the European Parliament and of the Council states that the traceability of food or any substance intended to be, or expected to be, incorporated into a food product shall be established at all stages of production, processing and distribution. Food business operators should be able to identify the operators from whom they have been acquiring food and also the ones where food has been delivered to. In addition, the salad finally suspected to be the vehicle, was not included in the initial questionnaire due to an error of the kitchen personnel.

The outbreak described here shows that the public health authorities should be aware of the possibility of foodborne infections caused by protozoa, not only by bacteria and viruses. Testing for *Cryptosporidium* should be included in the panel of tests performed in gastrointestinal illness and appropriate methods to detect *Cryptosporidium* in food samples should be developed. It is also imperative that food handlers are aware that proper handling of vegetables is an important method to prevent transmission.

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Outbreak of Salmonella enterica serotype Muenster infections associated with goat's cheese, France, March 2008

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Salmonella enterica serotype Muenster (hereafter referred to as S. Muenster) is rare in France and in Europe. In France, a nationwide outbreak of gastrointestinal illness due to S. Muenster occurred during March and April 2008. Twenty-five laboratory-confirmed cases of S. Muenster were documented by telephone using a trawling questionnaire. Four patients were admitted to hospital and no death was recorded. Among the 21 interviewed cases, 16 reported consumption of goat's cheese in the days prior to symptoms. The investigation incriminated goat's cheese from producer X as being the most likely source of the outbreak. S. Muenster was isolated from both cases and the incriminated goat's cheese. The pulsed-field gel electrophoresis profiles of the food isolates of producer X and the isolates from cases were indistinguishable. Following the withdrawal of the contaminated batch of cheese, the number of cases decreased to its usual level. To our knowledge, this is the first published outbreak of S. Muenster associated with food consumption in Europe.

Introduction

In France, the surveillance of *Salmonella* isolates of human and non-human origin is laboratory-based. The National Reference Centre (NRC) for *Salmonella* at the Institut Pasteur in Paris collects human isolates through a voluntary network of approximately 1,500 medical laboratories (corresponding to 30% of all French clinical laboratories). Animal, food and environmental isolates are collected by the French Food Safety Agency (Afssa) through a national voluntary network of 160 veterinary and food laboratories. Moreover, clusters of suspected food poisoning are subject to mandatory notification and must be reported to the relevant district health office (Direction départementale des affaires sanitaires et sociales, DDASS). An outbreak investigation is then conducted by the DDASS and veterinarians from the district veterinary service (Direction Départementale des Services Vétérinaires, DDSV), if necessary with the assistance of the French Institute of Public Health Surveillance (Institut de veille sanitaire, InVS).

Although salmonellosis is the largest documented cause of foodborne infections in France [1], *Salmonella enterica* serotype Muenster (hereafter referred to as S. Muenster) is rarely identified from humans, foods or animals. The NRC for *Salmonella* identified an annual average of 12 cases in the past three years. A total of 21 S. Muenster isolates had been received by the Afssa between January 2006 and February 2008. Among them, four were food isolates (poultry); the other 17 strains were from different origins (meat and bone meal, environmental isolates). A documented food poisoning outbreak caused by S. Muenster occurred in Canada in 1982 and implicated cheddar cheese made from unpasteurised milk as the source of infection [2]. On 18 March 2008, the NRC for *Salmonella* reported three laboratory-confirmed cases of S. Muenster to the InVS. An investigation was conducted in order to confirm the outbreak, determine its extent, identify the source of infection and put in place control measures.

Methods

Epidemiological information

A case was defined as a person living in France with S. Muenster isolated from a stool or a blood specimen since 25 February of 2008 (week 9). Cases were reported by the NRC for *Salmonella* and clusters of cases were identified through the mandatory notification of suspected food poisoning. Basic epidemiological data (age, gender, district of residence, address of the medical laboratory) was available. Cases were interviewed by the relevant district health office or the InVS by telephone using a trawling questionnaire, in order to inquire about the onset of illness, type of symptoms, hospitalisation, and exposures during the week before the onset of illness such as contact(s) with other symptomatic individual(s), or with animal(s) or water, recent travel abroad, food consumption and the places where they had purchased food.

European investigation

The European Food- and Waterborne Diseases Network of the European Centre for Disease Prevention and Control (ECDC) was informed on 28 March of the ongoing outbreak in France, and the network members were requested to report any recent increase in number of cases of S. Muenster or any cases possibly linked to the French outbreak.

Microbiological investigation

Antimicrobial drug susceptibility was determined by disk diffusion as previously described [3]. Human and food isolates of S. Muenster linked to the outbreak as well as isolates not related to the outbreak (isolates received by the NRC in 2006 and 2007) were characterised by standard pulsed-field gel electrophoresis (PFGE) analysis of XbaI-digested chromosomal DNA [3]. Each profile that differed by at least one clear band >100 kb was considered as a distinct profile. BioNumerics software (Applied Maths) was used to compare the PFGE profiles [4].

Figure 1

Geographical distribution of *S. enterica* serotype Muenster infections according to Département of residence, France, February-April 2008 (n=25 identified cases)

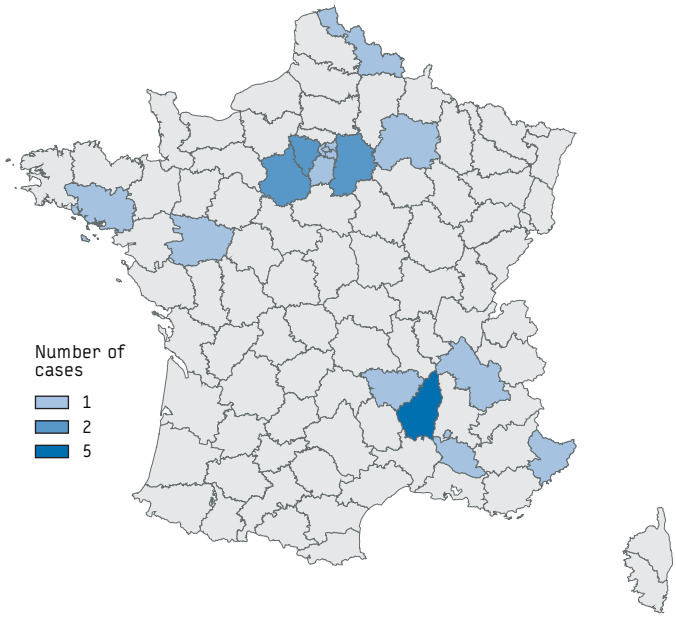
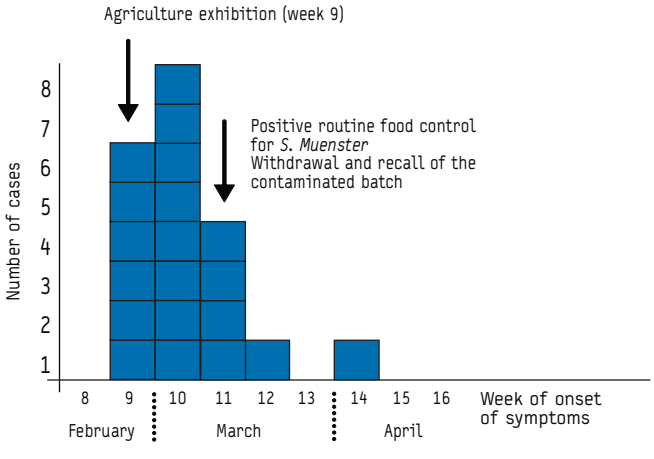


Figure 2

Epidemic curve by week of onset of symptoms, *S. enterica* serotype Muenster, France, February-April 2008 (n=20)



Results

Epidemiological information

Between 28 February and 24 April 2008, a total of 25 laboratory-confirmed cases of S. Muenster were reported by the NRC to the InVS, and among them six cases were reported as clusters of food poisoning through the mandatory notification. Four of them were isolated from children (8-12 years-old) and 21 from adults (median age 58 years). Only nine cases were male. The cases lived in 17 different administrative "Départments" spread across the country (Figure 1).

Of the 25 reported cases, 21 could be interviewed. The dates of onset of symptoms were from 27 February (week 9) to 3 April 2008 (week 14) (Figure 2). The most frequently reported symptoms were fever (20/21), diarrhoea (20/21), abdominal pain (17/21) and nausea (12/21). None of the interviewed cases had underlying medical conditions such as chronic illness or immunosuppressive therapy. Four patients were admitted to hospital and no death was recorded.

Among the 21 interviewed cases, 16 reported consumption of goat's cheese in the days before the onset of symptoms. The place of purchase of the goat's cheese was known for 10 cases: Seven cases had purchased unpasteurised goat's cheese at an agriculture exhibition that was held in Paris from 23 February until 2 March, and three cases had purchased this type of cheese at a local market in south-eastern France. Other food products frequently consumed were beef, ham, Emmentaler cheese and chicken (Table).

During the same period, a household cluster of salmonellosis involving three cases was reported through the mandatory notification system. The investigation of this cluster incriminated unpasteurised goat's cheese (consumed on 8 February 2008) as the source of infection. The isolates of these cases were later shown to be positive for S. Muenster.

In parallel, a routine food control was carried out on 14 March 2008 at a producer X, based in south-eastern France, and was positive for *Salmonella* for one of the batches of unpasteurised goat's cheese. This producer had delivered 360 unpasteurised goat's cheeses to the agriculture exhibition in Paris and supplied several local markets in south-eastern France. Control measures were taken by the producer immediately after this positive routine control: the contaminated batch and, as a precautionary measure, of all the other batches on the market were withdrawn and recalled. Following the withdrawal, the number of cases decreased to its usual level, around two isolates per month.

European investigation

A notification was made to the Rapid Alert System for Food and Feed (RASFF) on 20 March 2008 because the product had also been distributed to Belgium, Germany the Netherlands, and Sweden. No cases related to the French outbreak nor an unusual increase of S. Muenster isolates was reported from the ECDC's Food- and Waterborne Diseases Network.

Microbiological investigation

The human outbreak isolates of S. Muenster were susceptible to all antimicrobials tested. The PFGE profile (XMUENS-11) of the 20 isolates related to the outbreak was identical to the one of the food isolates of producer X. Ten different profiles other than

XMUENS-11 were observed for the 12 isolates found in 2006 and 2007 from cases not related to the outbreak (Figure 3).

Discussion

We describe a nationwide salmonellosis outbreak involving 25 cases of infection with the uncommon serotype Muenster that occurred in France between February and April 2008. The investigation incriminated unpasteurised goat's cheese from producer X as being the most likely source of the outbreak.

The incrimination of this goat's cheese was supported by the following findings: Firstly, a high proportion of cases (16 of 21) reported having eaten goat's cheese from the same small producer X. Secondly, a cluster of cases followed the consumption of goat's cheese from producer X. Thirdly, there was a concordance between the temporal (March 2008) and the geographical occurrence (agriculture exhibition in Paris and the south-eastern France) for the majority of the cases, and the distribution of goat's cheese of the producer X. Moreover, S. Muenster is a rare *Salmonella* serotype that was isolated from both cases and the incriminated goat cheese. The PFGE profiles of the food isolates of producer X and the isolates from the cases were identical. All isolates from related cases had an indistinguishable PFGE profile not previously identified. It was decided not to carry out an analytical study because of the findings discussed above.

This event was picked up by three surveillance systems. The NRC for *Salmonella* and the mandatory notification of clusters of food poisoning performed well by reporting cases of S. Muenster simultaneously to the InVS. The positive routine food control of producer X allowed early withdrawal of the contaminated batch, resulting in a limited number of cases.

In the literature, unpasteurised dairy products have been shown to cause outbreaks of salmonellosis, campylobacteriosis, listeriosis and Shigatoxin-producing *Escherichia coli* (STEC) infections, including cases of haemolytic-uraemic syndrome. In spite of the large amounts of many different types of raw milk cheeses consumed in France, foodborne outbreaks related to these cheeses remain relatively rare [5-8]. To our knowledge, this is the first published outbreak of S. Muenster associated with food consumption in Europe. This outbreak highlighted also the importance of routine food controls in order to prevent community-wide outbreaks of salmonellosis and other foodborne infections.

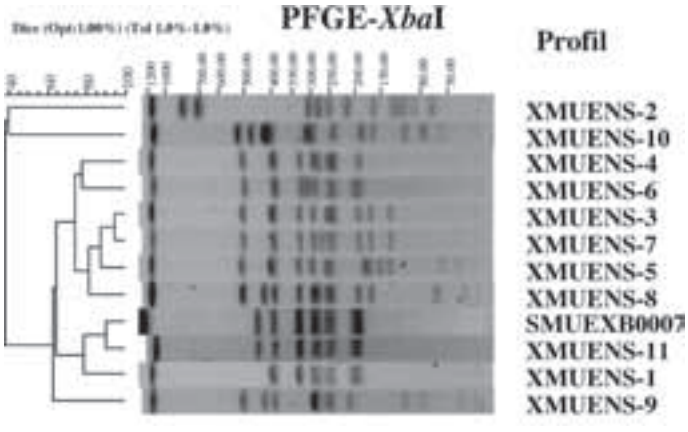
Table

Food exposures of interviewed cases of *S. enterica* serotype Muenster, France, February-April 2008 (n=21)

Food exposure	Number/total
Goat's cheese	16/21
Beef	11/21
Ham	10/21
Emmentaler cheese	9/21
Chicken	9/21

Figure 3

Pulsed-field gel electrophoresis profiles of XbaI-digested DNA from *S. enterica* serotype Muenster isolates, France



XMUENS-11: isolate from a case in this outbreak;
SMUEXB0007: food isolate of producer X;
XMUENS-2-10: isolates from patients identified in France in 2006 and 2007 that were not associated with this outbreak.
Source: French National Reference Centre for Salmonella and French Food Safety Agency.

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Increase in reported gonorrhoea cases in Sweden, 2001 - 2008

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Gonorrhoea is on the rise in Sweden and in many other European countries. The present report describes and evaluates the gonorrhoea trends in Sweden from 2001 to 2008 when an increase of 32% was reported. Up to 86% of the cases were reported in men, with the highest proportion among heterosexually infected men (41-59% during these years). Heterosexually infected men more often acquired gonorrhoea abroad, especially in Thailand, whereas women and men who have sex with men were more likely to acquire the infection within Sweden. The recent increase in gonorrhoea cases in Sweden is most likely due to adoption of more risky sexual behaviour (e.g. an increase in the number of sexual partners and the number of new/casual sexual partners and/or low use of condoms) in the Swedish population. Further research regarding more effective identification and description of sexual transmission chains and sexual networks is needed in order to follow the spread of infection and to recognise more effective interventions to prevent the spread of gonorrhoea and also other sexually transmitted infections.

Introduction

Gonorrhoea is a bacterial sexually transmitted infection (STI) that showed a steady decline in incidence during the 1970s, 1980s and early 1990s in Sweden. This epidemiological trend was also seen in many other, especially high- and middle-income, countries worldwide [1]. However, after an all-time low incidence in 1996 (2.4 per 100,000 population) with most of the cases acquired abroad, the gonorrhoea incidence in Sweden started to increase again (Figure 1) [2].

A similar increase has also been described from many other high- or middle-income, industrialised countries since the mid- or late 1990s. In north-western Europe, this re-emergence of gonorrhoea was primarily due to outbreaks among men who have sex with men (MSM), but also due to increased transmission among young heterosexuals of both sexes [2-4]. In 2005, it was estimated that 95 million gonorrhoea cases among adults occurred worldwide, with the majority of cases in Sub-Saharan Africa, South and South-East Asia, Latin America and the Caribbean [5].

Resistance of the aetiological agent of gonorrhoea, the bacterium Neisseria gonorrhoeae, to antimicrobials used in the traditional treatment (penicillin, tetracycline, and fluoroquinolones) of the infection is now prevalent worldwide. Most worrying, the level of resistance and/or reduced susceptibility of *N. gonorrhoeae* also to newer treatment alternatives, such as azithromycin and extended-spectrum cephalosporins (cefixime and ceftriaxone), has increased worldwide [6-8].

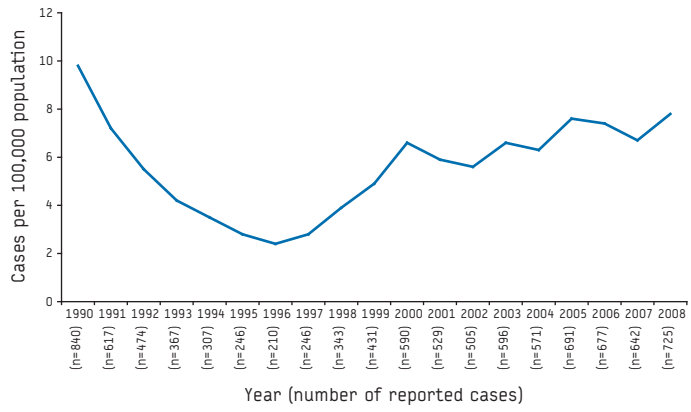
This report summarises the gonorrhoea surveillance data in Sweden for the last eight years (2001-2008).

Methods

Gonorrhoea is a notifiable infection in Sweden, in accordance with the Swedish Communicable Diseases Act, and the present surveillance system has been described elsewhere [2,9]. The gonorrhoea case definition used in Sweden since 1997 includes any person meeting the laboratory criteria. The laboratory criteria are as follows: a) *N. gonorrhoeae* has been isolated from a clinical specimen using culture, b) *N. gonorrhoeae*-specific antigen or nucleic acid has been demonstrated in a clinical specimen, and/or c) *N. gonorrhoeae* Gram-negative intracellular diplococci have been identified in a urethral smear from a symptomatic male. The Swedish laboratory confirmation also requires use of appropriate diagnostics. Quality-assured culture remains the recommended diagnostic method and accounts for most of the reported cases during each year. Positive nucleic acid amplification tests (NAATs) are recommended to be confirmed (using other method or a NAAT targeting another suitable gene). The Swedish gonorrhoea case

Figure 1

Incidence of reported gonorrhoea cases in Sweden, 1990–2008



definition is identical to the case definition of the European Union (EU) [10,11].

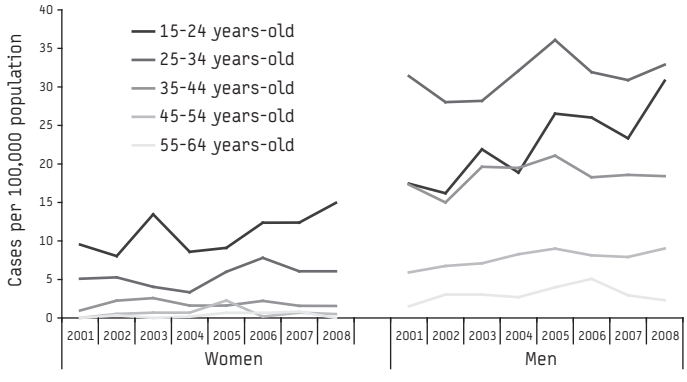
Data from the national computer-based surveillance system SmiNet was used to describe epidemiological trends for the period from 2001 to 2008. In this system, gonorrhoea cases are described by age, sex, reporting county, self-reported route of transmission (divided into heterosexual transmission, homosexual transmission and vertical transmission (mother to child), and country of acquisition (consistent with incubation period and anamnesis). Unfortunately, the electronic database can contain only one laboratory notification, and notifications from other sites for the same case are disregarded, which makes it impossible to draw any conclusions from the site of infection (therefore these data are not presented).

In this paper, we present data on the self-reported sexual route of transmission (not on sexual identity) when we are referring to homosexually infected men (MSM) and heterosexually infected men. Furthermore, the number of people tested and the number of people positive for *N. gonorrhoeae* are reported on a voluntary basis to the Swedish Institute for Infectious Disease Control, by the 29 laboratories in Sweden performing diagnostics for *N. gonorrhoeae*. These data are presented as number of people tested (by sex) and as positivity rate (proportion of people positive for *N. gonorrhoeae*). The annual incidence was calculated using all reported gonorrhoea cases per 100,000 population/men/women (population data from Statistics Sweden, www.scb.se).

The presented antimicrobial resistance data are from the Swedish Reference Laboratory for Pathogenic Neisseria, Örebro University Hospital, Örebro, which annually reports trends and characteristics including antimicrobial resistance data of all examined Swedish *N. gonorrhoeae* isolates [12,13]. It is recommended by the Swedish Reference Laboratory that all gonococcal isolates should be examined for antimicrobial resistance. Although most isolates are actually tested, the results from a few laboratories are not available for this report.

FIGURE 2

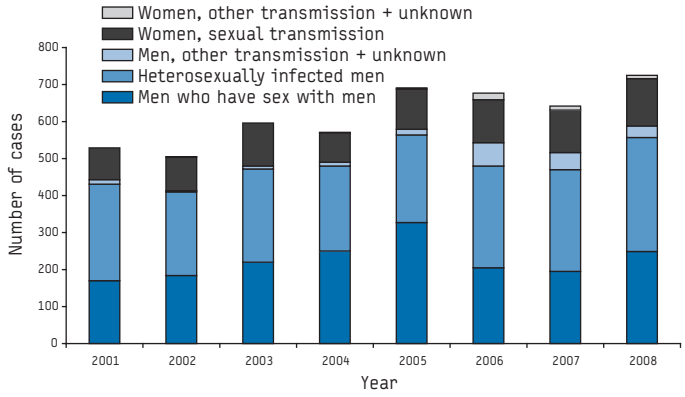
Gonorrhoea incidence in women (n=877) and men (n=4,020) by age group in Sweden, 2001–2008*



* Cases under 15 years and over 64 years (n=39) are not included in the figure.

FIGURE 3

Reported gonorrhoea cases by sex and self-reported route of transmission in Sweden, 2001–2008 (n=4,936)



Results

In the period from 2001 to 2008, a total of 4,936 gonorrhoea cases were reported to the national electronic surveillance system SmiNet. The gonorrhoea incidence during this period increased by 32% from 5.9 to 7.8 cases per 100,000 population (notably, this corresponds to a 225% increase since 1997) with several smaller incidence peaks in 2000 (6.6/100,000), in 2003 (6.6/100,000), in 2005 (7.6/100,000) and in 2008 (7.8/100,000). Overall during the study period, a steady upward trend in the incidence was observed (Figure 1).

Age

During 2001-2008, the median age for infected women was 27 years (range: 14-61 years), for heterosexually infected men 34 years (range: 15-80 years), and for MSM 32 years (range: 15-77 years). The highest incidences as well as the largest increase in incidence in both sexes were observed in the age groups of 15-24 year-olds and 25-34 year-olds, and were consistently higher among men (Figure 2).

Sex and self-reported route of transmission

Between 2001 and 2008, the male-to-female ratio varied from 4.1 to 6.2. The mean proportion of men in general and MSM was 83% (range: 80-86%) and 44% (range: 38%-56%), respectively (Figure 3). The proportion of female cases increased from 16% in 2001 to 20% in 2008.

Geographic spread

The majority of the gonorrhoea cases between 2001 and 2008 were reported from the counties with the highest number of population. Accordingly, Stockholm county (21% of Sweden's population) reported a mean of 68% of all gonorrhoea cases per year (range during 2001-2008: 61-73%), Skåne county (13% of Sweden's population) reported 10% (range: 6-16%), and Västra Götaland county (17% of Sweden's population) reported 10% (range: 5-16%).

Country of acquisition of the infection

In the period from 2001 to 2008, a mean of 60% of the cases had acquired the infection in Sweden, 34% abroad, and for 6% this information was not available. Women and MSM more often acquired gonorrhoea in Sweden (71% of the female cases and 79% of MSM). In contrast, heterosexually infected men more often acquired infection abroad (56%). No major longitudinal trends were identified regarding the country of acquisition of

gonorrhoea and way of transmission (Figure 4). For men who had acquired gonorrhoea abroad by heterosexual transmission, the most common countries of infection were Thailand (22-32% of these cases over the years) and the Philippines (3-5% of these cases). The other heterosexually infected male cases acquired gonorrhoea in countries worldwide that were implicated less frequently, e.g. 1-2% in northern European countries (Denmark, Finland, Iceland, Norway and Sweden), 1-4% in western European countries (United Kingdom, Spain, France, Germany, Portugal, Italy), and 0-1% in eastern European countries (Baltic States, Poland, Bulgaria) (range for 2001-2008). MSM who acquired gonorrhoea abroad most frequently acquired it in Denmark (1-8% of the cases), Spain (1-4% of the cases) and Germany (0.5-3% of the cases). Among men with unknown route of transmission, the majority had acquired gonorrhoea in Sweden (range for 2001-2008: 5-67%) and in Thailand (range: 0-33%).

Laboratory-based reporting of test volumes (voluntary)

According to the voluntary reporting from the laboratories, the number of persons tested for *N. gonorrhoeae* in Sweden increased by 15% from 48,925 in 2001 to 56,084 in 2008. The peak in the number of people tested in 2007 was likely due to the reports in late 2006 of the new variant of *Chlamydia trachomatis* (nvCT), which resulted in high numbers of false-negative results. In 2007, when new genetic assays detecting the nvCT had been introduced, many people were re-tested (testing volumes for *C. trachomatis* significantly increased in 2007), and were most probably also tested for gonorrhoea at the same time. All 29 laboratories performing testing for *N. gonorrhoeae* reported most of the requested data and, accordingly, the coverage was as high as 97-100% in the period from 2001 to 2008, although reporting was voluntary. Of those tested, 60-64% were women. Despite the fact that more women were tested for *N. gonorrhoeae*, only 0.3-0.4% were found to be positive. In contrast, 2.2-2.9% of the tested men were positive (Figure 5), which may also reflect that gonorrhoea is more commonly symptomatic in men than in women. In general, no major trends were seen in the positivity rates for women or men from 2001 to 2008. Furthermore, during the study period, there has not been any major change in the laboratory methods used for diagnosis.

FIGURE 4

Gonorrhoea reported to be acquired in Sweden and abroad by sex and route of transmission, 2001–2008 (n=4,936)

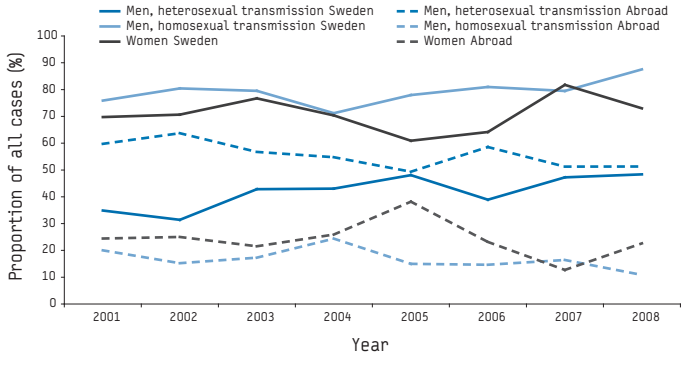
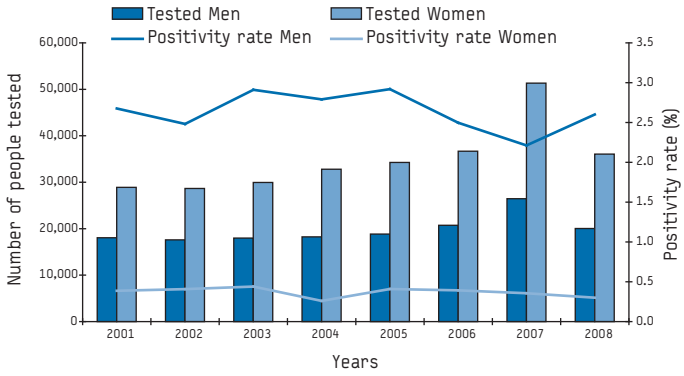


FIGURE 5

Number of persons tested and positivity rate for *Neisseria gonorrhoeae* among men and women in Sweden, 2001–2008



Antimicrobial resistance of Swedish *Neisseria gonorrhoeae* isolates

Between 2001 and 2008, all Swedish isolates reported by the Swedish Reference Laboratory (n=2,242) were susceptible to spectinomycin (100%), 99.96% to ceftriaxone (i.e. only one isolate in 2008 displayed an intermediate susceptibility/resistance in vitro), 98.7% to cefixime, and 94.8% to azithromycin. However, the level of intermediate susceptibility to cefixime increased from 0% to 4% and the resistance to azithromycin increased from 0% to 3% (0-10% intermediate susceptibility), over the years. The level of beta-lactamase production, intermediate susceptibility and resistance to ampicillin, and intermediate susceptibility and resistance to ciprofloxacin varied from 22% to 39%, 66% to 82%, and 50% to 71%, respectively, over the study period [12,13].

Discussion

The incidence of reported gonorrhoea cases in Sweden has increased by 32% over the last eight years (2001-2008), from 5.9 to 7.8 cases per 100,000 population, an increase of 225% compared to the all-time low incidence in 1996 (2.4 per 100,000 population) [2]. Similar increasing patterns have also been observed in other Nordic countries such as Denmark and Norway [4,14] as well as in other EU countries [3,15,16]. The main contributors to the recent increasing trend in Sweden, in particular in the period form 2005 to 2008, were heterosexually infected men but also women: the proportion of heterosexually infected men increased from 41% to 59% and the proportion of female cases increased from 16% to 20% during these years. MSM also contributed to the increase in gonorrhoea cases. However, the proportion of these cases decreased from 56% to 42% in the past four years (2005-2008).

The majority of the heterosexually infected men acquired gonorrhoea abroad, with the majority of cases acquired in Thailand, sometimes through sexual contacts with female commercial sex workers (FCSWs; it is occasionally but not consistently possible to collect these data). This is most worrying because Thailand has a high prevalence of human immunodeficiency virus (HIV) infection and many other STIs among commercial sex workers. For instance, recent estimates among FCSW in Thailand revealed an HIV prevalence of 4.7% among venue-based FCSW and of 43% among street-based FCSW [17]. Accordingly, the heterosexual Swedish men may, in addition to gonorrhoea, also acquire HIV and other STIs that they could transmit to others after their return to Sweden. A similar pattern has also been observed in Norway [4]. This provides support for targeted prevention interventions

among Swedish men going abroad, especially to Thailand and the Philippines.

The high proportion of MSM (38-56% during the study period) among the men gonorrhoea cases in Sweden is also a reason for concern. Some MSM have not consistently adopted safe sex practices and therefore maintain continuous possibilities for transmission of STIs and HIV [3]. An increasing number of casual sexual partners, anonymous sexual partners, and non-use of condoms are likely to have contributed to the recent increases in STIs among MSM [3,18]. Preventive programmes with adapted educational messages tailored specifically to MSM would be beneficial.

From 2001 to 2008, gonorrhoea cases were reported from all over Sweden with a higher number of cases reported from the counties with the largest cities, such as Stockholm county, Skåne, and Västra Götaland. This correlates well with the reported syphilis cases in Sweden [9], suggesting that cities with a large population provide an environment where free sexual behaviour is more readily accepted. In addition, sexual networks tend to be larger in the cities and the chances of contact with risk groups for STI transmission are higher.

The increase in gonorrhoea and other STIs in Sweden could be due to several reasons. One of the most important reasons might be the adoption of more risky sexual behaviour which has been observed in studies among MSM in Sweden [19]. For example, practise of unprotected anal sex during the last 12 month was reported by 59% of responding MSM with an average number of three to four partners during the last 12 month, as well as practise of unprotected anal sex with a partner with unknown HIV-status (during the last sexual contact) [19]. The present study observed more risky sexual behaviour not only among MSM but also among heterosexually infected men and women. The increase in the number of sexual partners overall and in the number of new/casual sexual partners combined with an insufficient use of protection is certainly one of the factors contributing to the spread of gonorrhoea. Regular assessments and studies of sexual knowledge, behaviour, attitudes, and the risks of HIV/AIDS and STIs that have been performed in Sweden since 1989 provide comprehensive and valuable insights into these factors [20,21]. These studies showed that in the years from 1989 to 2003, the prevalence of casual sexual contacts (unspecified type of sexual contacts) without condom use rose significantly, especially in the age groups under 35 years (both men and women) [20]. Furthermore, the proportion of 18-19 year-old men and women who had more than three sexual partners during the last 12 months increased between 1989 and 2007 from 17% to 23% in men and from 13% to 26% in women [21]. These studies, as well as surveillance data for gonorrhoea (and other STIs), support the need for targeted prevention interventions in vulnerable groups of the Swedish population.

The upward trend of gonorrhoea in Sweden during the period analysed in this study cannot be explained by changes in the national gonorrhoea case definition or the diagnostic methods. Nevertheless, another possible contributing factor is a rise in the number of people tested for *N. gonorrhoeae* (by 59% in 2001-2008), which is partly a result of an improved access to health care.

Gonorrhoea is on the rise in many European countries [3,4,14,22,23]. There are also major concerns worldwide regarding the high level of antimicrobial resistance in *N. gonorrhoeae*, and it is crucial for effective treatment to perform antimicrobial resistance surveillance locally, nationally and internationally. Accordingly, gonorrhoea needs special attention from health care professionals, health promoters, surveillance facilities and diagnostic laboratories. Further research regarding more effective identification and description of sexual transmission chains and sexual networks is needed in order to follow the spread of infection and to recognise more effective interventions to prevent the spread of gonorrhoea as well as other STIs.

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Tuberculosis in a child – search for the infected adult nearby; case report, Portugal, 2007

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Tuberculosis (TB) transmission in a non-household setting is difficult to detect, because contact with the source case is often not obvious. Here, we report on a case of a four-year-old child who got infected through sporadic non-household exposure at a coffee shop. The source case was a woman who had suffered from weight loss, productive cough and fatigue for two months before being diagnosed with TB. Screening the child's contacts revealed two active TB cases within its family. Overall 148 contacts were screened for both cases and 18 cases of latent TB infection detected. The connection between the child and the source case, who were not aware of their contact, was confirmed by molecular fingerprinting. Our case report illustrates the difficulty in detecting non-household transmission between individuals that do not have significant contact, and draws attention to the need to look for the infected adult whenever a child falls ill with TB. This report is a reminder of the importance to consider possibly neglected ways of TB transmission and highlights once again the need of early diagnosis of TB.

Introduction

Childhood tuberculosis (TB) is an indicator of public health programme performance and all cases in children can be considered signals of missed opportunities. The most important missed opportunities are:

- failure to detect the infectious case;
- delayed reporting of an adult source case;
- failure to identify contacts of infectious cases with children;
- delayed or incomplete evaluation of children exposed to TB; and
- inadequate treatment of latent TB infection (LTBI) [1].

TB in young children (under five years) is often transmitted from a family or household member [2]. Therefore, screening procedures in cases of childhood TB should always start with the search for the source case within the family/household [3]. The transmission of TB to children through non-household exposure is difficult to ascertain. In many instances, public health authorities experience difficulties in establishing the contact with the source case as most of the affected are not aware of the contact which leads to the infection.

This paper describes a case of a four-year-old child who became infected through exposure at a coffee shop, which she visited sporadically in the company of a family member. It illustrates the difficulty in detecting non-household transmission between individuals that did not have significant contact. Moreover, it demonstrates the need to look for the infected adult whenever a child is diagnosed with TB.

Case reports

Case 1 (child)

In September 2007, a four-year-old child came to our practice after having had a temperature around 37,S5-38ºC for 15 days (Figure 1).

Chest radiography showed a right hiliar enlargement. TB was confirmed by bronchoalveolar lavage analysis, which was smear and culture positive. The *Mycobacterium tuberculosis* (MTB) isolate was susceptible to all first-line drugs. The clinical strain was genotyped using mycobacterial interspersed repetitive unit-variable-number tandem repeat (MIRU-VNTR) typing [4] and IS6110 restriction fragment-length polymorphism (IS6110-RFLP) typing [5]. The patient was treated with isoniazid, rifampicin and pyrazinamide by means of directly observed treatment (DOT).

Contact tracing after diagnosis

Upon diagnosis, the parents were interviewed and asked to describe the daily activities and routines of the child at home, at school and in its social environment. The parents were not aware of any contact with TB patients. Initially we considered three relatives (the parents and grandmother) and later on an additional 11 close members of the family as well as 30 pupils and nine staff from the school as contacts of the child. All identified contacts were offered a screening programme which included a symptom questionnaire, a tuberculin skin test (PPD RT23 SSI) and chest radiography. The tuberculin skin text was considered positive if after 72 hours an induration >10 mm was visible, and an interferon gamma assay was performed in all positive cases. One case of LTBI was identified among the contacts, a school staff who was subsequently treated with isoniazid for 6 months.

Case 2 (adult source case)

In October 2007, a woman in her early forties presented at our clinic with progressive weakness, weight loss and a persistent productive cough for the past two months (Figure 1). Chest

radiography showed bilateral pulmonary infiltrates and cavities. The sputum was smear and culture positive for *M. tuberculosis* and the isolate was susceptible to all first-line drugs. The strain was genotyped using MIRU-VNTR [4] and IS6110-RFLP. The patient began treatment with isoniazid (INH), rifampicin (RIF) and pyrazinamide (Z) and ethambutol (E) in form of DOT.

Contact tracing

Eight close non-household contacts were identified and offered screening as described above. Two of them had LTBI and were treated with isoniazid for six months. Afterwards, contact screening was broadened to include casual contacts defined as exposure/contact for less than eight cumulative hours during the symptomatic period from August 2007. An additional 20 persons were identified and screened. LTBI was diagnosed in five contacts and active pulmonary TB was diagnosed in two.

Case 1 and case 2 were apparently unrelated. However, it was noticed that both patients lived in the same geographic area. Further questioning identified a coffee shop as a place where both individuals would spend time occasionally. At first, it seemed there was little chance of a connection between them. The visits to the coffee shop were rare and short. In order to clarify the situation and to enable other public health measures, clinical isolates of both patients were genotyped. Strain typing was done through the use of MIRU-VNTR and IS6110-RFLP standard methods [4, 5]. Both fingerprinting results showed that the two clinical isolates had identical fingerprinting (Table 1 and Figure 2), and therefore were epidemiologically related.

Lane 1: Molecular marker (1kb marker): 10.000 bp, 8000 bp, 6000 bp, 5000 bp, 4000 bp, 3500 bp, 3000 bp, 2000bp
Lane 2: M. tuberculosis isolate from case 1 (child)
Lane 3: M. tuberculosis isolate from case 2 (adult source case)

Contact tracing after results from genotyping

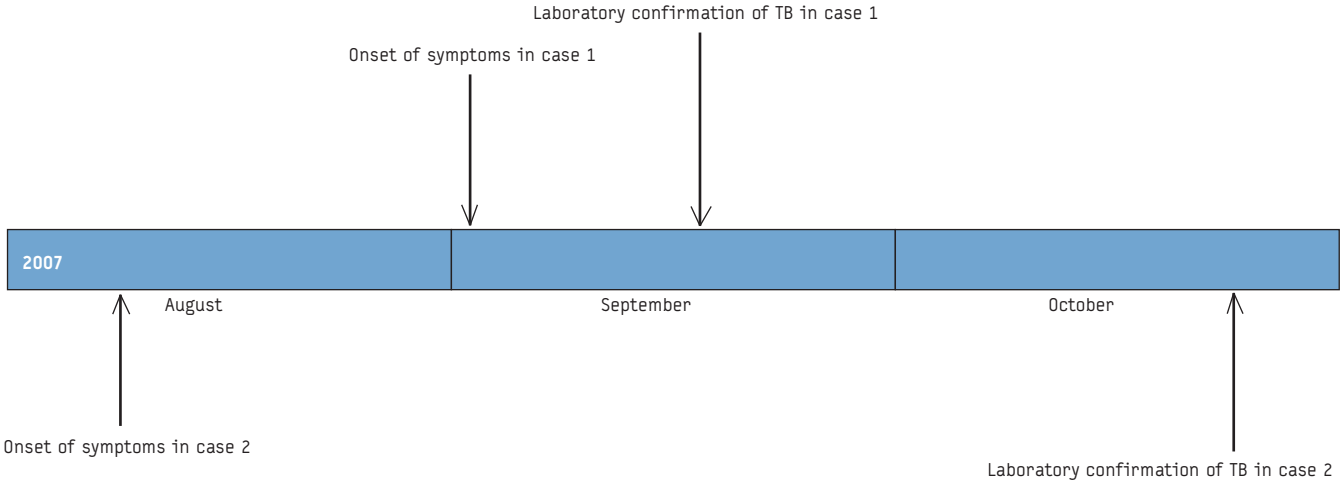
After the results from genotyping had revealed the connection between the two cases, and the coffee shop was identified as the place where the two cases had met, a new investigation was conducted directed at the coffee shop customers. 67 people were screened and LTBI was diagnosed in ten of them. Consequently, eight individuals were treated with isoniazid for 6 months. Two cases with LTBI had underlying conditions such as alcoholism and chronic hepatic disease which meant that they were at risk for developing hepatic toxicity from being treated with isoniazid. They were kept under clinical surveillance by the clinic as outpatients for 2 years.

Discussion and conclusion

The two main factors determining the risk of progression from latent to active TB are patient age and immune status. Immaturity of both the innate and adaptive immune systems of young children plays a critical role in increased susceptibility to active TB. Children below five years of age who are infected, have the highest risk of

Figure 1

Timeline of onset of symptoms and of diagnosis of tuberculosis in case 1 (child) and case 2 (adult source case), Portugal 2007



Table

Results of mycobacterial interspersed repetitive unit-variable-number tandem repeat (MIRU-VNTR) typing (15 loci), tuberculosis cases Portugal, 2007

	Mtub04	ETRC	MIRU04	MIRU40	MIRU10	MIRU16	Mtub21	QUB11b	ETRA	Mtub30	MIRU26	MIRU31	Mtub39	QUB26	QUB4156
Case 1 (child)	1	4	2	1	N/A	1	3	2	2	1	4	3	2	N/A	2
Case 2 (adult source case)	1	4	2	1	N/A	1	3	2	2	1	4	3	2	N/A	2

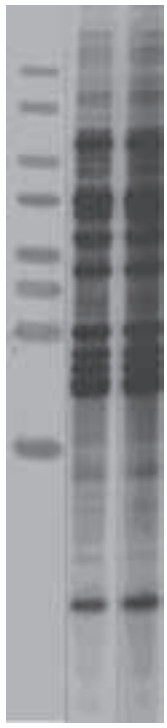
Legend: N/A= not available

progression to disease [6, 7]. The two strategies for managing TB in children are searching for secondary cases in close contacts and searching for the infectious adult source case. Whenever a TB case in a small child is diagnosed, family members are interviewed to identify contacts in the sphere of the patient's daily activities. The public health authority investigation team visits the child's home, school or other identified places where the child has social contacts in order to identify those at risk and to find the infected adult. Progression from primo-infection to illness in children under five years old is very fast, so one can be sure that there is an infectious adult nearby who transmitted the disease to the child [6, 7].

Due to the low probability of transmission between very young children, screening of other children is usually largely unproductive. There is however evidence that TB in young children is occasionally transmissible particularly in the presence of cavitation, consolidation or bronchial lesions [8, 9]. Although case 1 did not present cavities in the chest radiography, she was smear and culture positive so the risk of transmission could not be excluded. Therefore, all family and school contacts were screened. This activity did not prove to be fruitful as all children tested negative, only one adult was found to have LTBI (probably not connected with this situation) and it did not lead to the identification of the source case. At this point in time, we were reconsidering our strategy when another case appeared in the same geographic area. Further inquiries revealed a seemingly improbable contact between the adult and the child in an unusual place for a child to be – a coffee shop. Genotyping of the *M. tuberculosis* isolates from both cases however, confirmed the connection.

Figure 2

IS6110 restriction fragment-length polymorphism (IS6110-RFLP) typing fingerprints, tuberculosis cases Portugal, 2007



The delay of the diagnosis of TB in case 2, by two months and a prolonged infectious period were the causes of the high rate of TB transmission, resulting in three cases of pulmonary TB (two family members and case 1) and 18 LTBI among contacts which can most probably be attributed to the event.

Transmission of TB in bars has been described before and may pose a risk to public health [10,11]. This case report is intended as a reminder to health professionals that all TB transmission scenarios are possible and need to be considered in an investigation around a case, even the less likely. Moreover, a delay in the diagnosis of the infectious case results in transmission of the disease. Finally, the link between the child and the adult would not have been proved without the use of the *M. tuberculosis* fingerprinting techniques.

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StatFlu - a static modelling tool for pandemic influenza hospital load for decision makers

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The emergence of a new influenza virus strain setting off a global epidemic can put considerable strain on the current hospital capacity. The task of estimating hospital load during an influenza pandemic event remains difficult, despite a number of tools that are publicly available for decision makers today. The estimate depends on a multitude of parameters, each with associated uncertainties. We provide a new tool, StatFlu, combining advances in static modelling using historic influenza data with a pedagogical interface designed to highlight propagation of parameter settings and uncertainties in the output. StatFlu provides graphs of the load on hospital wards as well as primary care units as a function of time, aiding the user in decision making. Here we present the model and software. We also demonstrate it with an example and compare the results with a similar tool.

Introduction

Many successful models using dynamic simulations to estimate the effect of an influenza pandemic have been presented and have been essential in providing the knowledge needed for pandemic preparedness [1-9]. All models have their inherent ailments and there is always a great deal of uncertainty in the estimates they are able to produce. This depends on the data used to calibrate the model as well as the assumptions made by the model itself. The virulence of a new viral strain can only be guessed at. Contact patterns and other social dynamics contribute a similar uncertainty. Conveying the difficulties of modelling and the effect of the many uncertainties in the estimates provided should be a primary objective for modellers.

From the attack rate estimates that dynamic models produce it is possible to estimate the hospital load, using assumptions of how many of the clinically ill are expected to seek medical care. This could be done by extrapolation from historic data on past epidemics or pandemics. It is this last step that is the focus of many static models.

Many publicly available software applications use static modelling. Whether using simulation output or presumed attack rate scenarios, these models translate outbreak data into variables of interest, such as hospital load, cost of treatment or loss of lives. Static models have the advantage that assumptions about social networks and similar factors are already implicit in the data, which makes them very reliable. Generally, fewer assumptions are made which need to be accounted for and the results are more transparent. The two main sources of uncertainty in static models are in the parameters of the extrapolation and in how historic data from a particular region and time is relevant and plausible in another region and another time. The first of these uncertainties can be handled using statistical methods. All models described in this paper have this in common.

A notable example of a static modelling approach available on the internet is FluSurge [10], released by the United States (US) Centers for Disease Control and Prevention (CDC), which can be used to project the total hospital load over the duration of the pandemic. This software, its predecessor without time projection – FluAid [11], as well as slightly adapted versions thereof, have been used by authors in published articles predicting hospital load in several regions and countries [12-17].

The StatFlu project was initiated to bridge the gap between researcher and decision maker and to replace FluSurge, amending several of its flaws, to be detailed below. StatFlu is currently in use at the National Board of Health and Welfare in Sweden. The excess hospital and primary care load due to a pandemic is calculated without intermediate steps using a closed formula, at a resolution of one day. The full variance of the possible scenarios generated by the uncertainties in the input is displayed using a colour gradient in the plots. We have built our model with a bottom-up approach, incorporating the time-distribution from the start. We also allow the user to specify the age-dependent risk of contracting infection, relative to the other age groups, rather than a distribution of the attack rate among age groups, making the model independent of differences in age distribution.

Using StatFlu, the user can immediately see the effects that changing assumptions in attack rate, average susceptibility of age groups, duration of the pandemic and length of hospital stay will have on the hospital load and primary care visit frequency. The uncertainties of other parameters in the model, in particular the risk of hospitalisation of infected individuals, are taken into account by use of a Monte Carlo-type sensitivity analysis [18-20]. The output estimates of 10,000 such simulations are collected and presented so that probable and less probable outcomes are apparent. The objective is for the user to acquire an intuitive understanding for the assumptions behind the estimates.

StatFlu can be downloaded and used freely from www.s-gem.se/statflu.

Previous research

Meltzer *et al.* used Monte Carlo methods to express the uncertainty in a study to evaluate the economic impact of a pandemic influenza outbreak in the US [21]. Using predefined probability distributions they could model a range of estimates. Essential parameters for age group-specific attack rates were collected from various studies of outbreaks of seasonal and pandemic influenza. Parameters were assumed to be either triangular or uniform in their uncertainty distributions. The distributions were randomly sampled and used to calculate mean economic impact. Mean hospital admittance and mortality was calculated with 90% confidence bounds. They also compared results with and without the use of vaccination.

A similar setup was used in France by Doyle *et al.* with slightly different background variables and with a focus on hospital admittance and mortality [22]. Many parameters were taken from the study by Meltzer *et al.* Also in this study the authors compared scenarios with and without the use of intervention programmes, in this case both vaccination and antiviral pharmaceuticals.

Van Genugten *et al.* used detailed national data collected from seasonal influenzas [23]. Their approach was a scenario analysis. This approach is more pedagogical and the results are more readily applicable. The lack of sensitivity analysis means that only the expected outcome is shown of each scenario. Information on the variability of the output is not provided. This may or may not be problematic depending on the parameter values used.

A missing piece in the first two studies mentioned above is how the hospital load varies over time. It is important to point out that the predicted increase in patient load during a pandemic, whatever the degree of uncertainty, will not happen in one day. The frequency of visits will follow the epidemic incidence curve, which means that the estimated total increase cannot directly be translated into a required capacity.

Reasonable adjustments have been made to amend this. Bonmarin *et al.* [24] published a follow-up calculation to the French study, assuming the shape of the time function would be similar to that of previous seasonal influenza outbreaks as gathered from sentinel data. Van Genugten *et al.* included a time plot in the original study where the estimated attack rate was distributed along a Gaussian (normal) curve [23]. FluSurge also plots the output on a time axis, although it is not clear what the rationale behind their choice of algorithm is.

In the Results section of this paper, we compare output from StatFlu and FluSurge. In our opinion, the latter is flawed. We are concerned about the appearance of the admissions plot as well as some of the calculations concerning the death rate described in the manual [25]. Most importantly, however, FluSurge will give unexpected results unless the age group proportions in the target country or region matches those of the United States. Both Doyle *et al.* [22] and van Genugten *et al.* [23] have used data from Meltzer *et al.* without the flaw.

It should also be noted that the contribution of static models to understanding the effect of vaccination and antiviral pharmaceuticals is questionable. Usually, the effectiveness of the drug is quoted and used simply as a reduction factor on final outcome [21-23]. However, as with any intervention strategy, antiviral drugs (as prophylaxis or therapy) as well as vaccination, can at best completely halt the spread, but may also have an insignificant impact. The end result is in part due to chance, but more specifically, each prevented case will not spread the disease further and one dose can have a wide-reaching effect in the transmission chain. At the very least a pharmaceutical effect should be used, covering both the effectiveness of the drug and the dynamic effects. Due to the difficulty in this, we decided not to include the feature in the currently available release of StatFlu. However, users should be cautioned against the assumption that antiviral drugs and vaccines are not effective.

Table 1

Variables used in StatFlu, their sources and implementation

Variable	Description	Source	Uncertainty	Implementation/treatment
Gross attack rate	Fraction of population infected	User-specified	Hypothetical	User-specified 5-50%
Duration of epidemic	From first infected to last	User-specified	Hypothetical	User-specified 10-150 days
Population	Population in age groups 0-19, 20-64, >64, by region	Population register [37]	High certainty	Fixed, specified in text file
Duration of hospital visit	Average length of treatment at hospital	User-specified	Attainable, partly hypothetical	User-specified for all ages 1-14 days
Age group-dependent relative risk of infection		User-specified	Hypothetical	Specified for age group relative to the other age groups
Size of risk group	Fraction of age group at elevated risk for complications	Provided by [35]	Definition-dependent, attainable in theory	ca. 2% for the whole population; specified in text file
Risk of hospitalisation	Risk per age and risk group of hospitalisation given infection	Provided by [30,32,33,38] and expert opinion, see Table 3 in [38]	Uncertain, dependent on risk group definition	Sampled from beta-distribution; hard-coded
For primary care load only				
Primary care visits	Yearly primary care visits per region under normal circumstances	Provided by [39]	High certainty	Fixed, editable in text file
Hospitalisations associated with influenza-like illness	Hospital patients coded with influenza	Provided by [35]	Highly uncertain, coding-dependent	Fixed, editable in text file
Risk of primary care visit	Risk per age and risk group of hospitalisation given infection	Provided by [21,32,40] and expert opinion, see Table 3 in [38]	Uncertain, dependent on risk group definition	Sampled from beta-distribution, hard-coded
Fear factor	Deterrence from primary care due to pandemic	User-specified	Hypothetical	User-specified 0-40%

Both Meltzer *et al.* and Doyle *et al.* provide estimates of incidence reduction following either vaccination or antiviral drugs. Wallinga *et al.* [26] have developed the model by van Genugten *et al.* with a dynamic model approach, further developed by Mylius *et al.* [27].

Aims of the StatFlu project

Our priorities in developing StatFlu were:

1. Pedagogical input and output,
2. Full time resolution,
3. Transparency,
4. Visualised variance/uncertainty combined with scenarios analasys,
5. Independence from age distribution.

In our opinion, this work represents an improvement over previous attempts in terms of presentation and, in some aspects, of validity. It should be pointed out that the outcome is still highly uncertain and the intention with StatFlu is to highlight the uncertainty, not conceal it. There is still a danger that the user over-interprets the results. StatFlu is a tool for aiding intelligent decision-making, and the results must always be interpreted by the user based on input and experience.

In a recent version of the model an addition was made to provide figures for primary care load whereby we also explored the possibility of a decrease in load due to public awareness of transmission risk within the health care system. We call this the *fear factor*. Studies conducted during the epidemic of severe acute respiratory syndrome (SARS) support such assumptions [28,29]. A reduction in visits as large as 35% was seen in Taiwan during following the peak of the epidemic.

Methods

A detailed description of the model is given in a separate section at the end of the article.

Data and input

As much of the required epidemiological data is not available in Sweden, as far as we know, we have incorporated many of the figures found in Meltzer *et al.* [21], and references therein, into StatFlu, as we regarded this paper to be the standard in the field [30-34]. Results can therefore be compared with that of other studies based on the same parameters, differences resulting primarily from differences in demographics and less from differences in epidemiological assumptions. A summary of the variables used in StatFlu is given in Table 1.

Regarding the size of the risk groups in Sweden, we used the Swedish Hospital Discharge Diagnosis Register [35] for a rough estimate of the prevalence of certain chronic diseases including heart, kidney and lung disease that increase the risk of developing complications and being hospitalised subsequent to an influenza infection. These data are stratified by age, county and the number of distinct diagnoses. Our results may be considered low compared to estimates in other countries.

In the estimation of the number of primary care visits we used data from the Swedish Hospital Discharge Diagnosis Register on influenza diagnosis in each county during a normal influenza season. We also included estimates on the total primary care visits, taken from Otterblad Olausson [36].

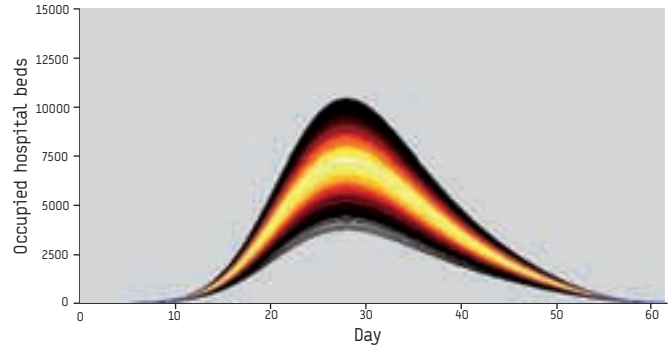
Estimates from Meltzer *et al.* used in our model included the risk of being hospitalised and visiting primary care depending on age group and risk group. We use the estimated lower and upper bounds including the conversion factor used to convert from population risk to risk among afflicted [21].

The users themselves enter the population size and demographics by selecting one of the predefined counties or the whole country. It is also possible to customize the demographics with data from other countries or regions by editing a text file. The user also sets the duration of the pandemic, the average duration of a hospital visit, the fear factor, and, as discussed in the introduction, the age group-dependent relative risk of infection. The user has at their disposal a flexible graphical user interface functional under the Windows operating system.

Table 1 shows the input variables used in the model, the details of which are described below in the Model section.

FIGURE 1

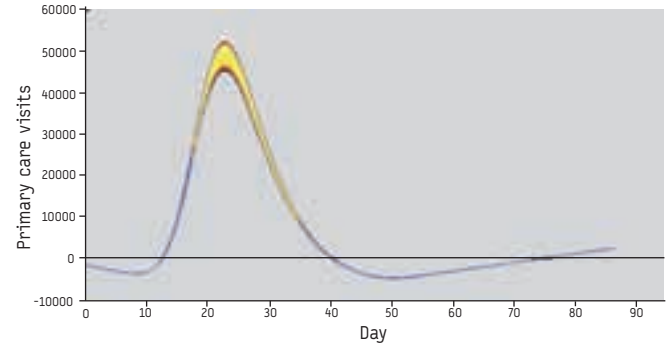
Example of hospital load output from StatFlu, i.e. simultaneously occupied hospital beds



We used Swedish risk group estimates. Blue lines indicate 95% confidence intervals.

FIGURE 2

Example of primary care load output from StatFlu, i.e. visit frequency per day



We used Swedish risk group estimates. Depending of the fear factor, in this case 20%, the curve may initially decrease due to deterrence of visiting the facilities for other reasons than severe influenza illness.

Results

Output of StatFlu and comparison with FluSurge

We provide here (Figure 1) sample outputs from StatFlu based on the whole Swedish population, using Swedish values for risk group distribution as described above, an attack rate of 25%, a duration of 90 days of the epidemic, and 10 days average time in hospital (full colour figures are available from the StatFlu website, www.s-gem.se/statflu). We also used the values from Meltzer *et al.* for age group-dependent relative risk of infection [21]. The most probable scenario estimates the number of simultaneously occupied beds to about 7,500 at the peak of the outbreak, at 28 days.

Figure 2 shows the primary care visit load, i.e. the number of visitors per day. We set the fear factor to 20%, resulting in an initial decrease in the patient rate. 23 days into the outbreak, the increased rate of patients is just short of 50,000 in the most probable scenario.

For comparison with FluSurge we chose the same settings between the two applications as far as was possible. This included population size, attack rate, hospital visit duration and duration of pandemic. In StatFlu, we used values from Meltzer *et al.* for age group-dependent relative risk of infection [21]. We also used the risk group partition from Meltzer *et al.* In FluSurge we set the probability for intensive care unit and ventilator requirements to =0, because the types of care are not differentiated in the current version of StatFlu. The results are slightly higher than in the previous scenarios modelled for Sweden, probably due to the size of the risk groups (see Discussion).

Table 2 shows the weekly admission rate as modelled in FluSurge. The last row shows the number of patients in hospital, and these values can be compared to the plotted output from StatFlu in Figure 2.

The two applications give similar estimates, as is to be expected in this comparison scenario. The daily distribution of admissions given by FluSurge is the interpolated curve in Figure 3.

Figure 4 shows the corresponding plot from StatFlu accomplished by setting the duration of stay =1.

The results provided by StatFlu represent an improvement over FluSurge in terms of graphic display of the load and the uncertainty, the daily resolution of the results and the robustness of the calculations.

Discussion

Regarding the size of the risk groups in Sweden, we used the Swedish Hospital Discharge Diagnosis Register [35] for a rough estimate of people inflicted with certain chronic diseases including heart, kidney and lung disease. Persons so diagnosed were assumed to have an increased risk of developing complications and being hospitalised subsequent to an influenza infection. We calculated that roughly 2% of the population belong to the high-risk group. This in contrast to 15% in Meltzer *et al.* and 10% in van Genugten *et al.* [21,23]. The difference has to do with limiting the number of diseases included in the query, including persons over the age of 64 years based only on the discharge data in the high-risk group and not including pregnant women, infants and institutionalised persons. Meltzer *et al.*, for example, included by default 40% of the population above the age of 65 years.

All individuals registered at a Swedish hospital during 2006 with one or more of a predetermined set of symptoms or diseases were counted. Ultimately, our goal was to sample the entire population that had at one point in time carried such disease, i.e. the prevalence. The prevalence is generally very hard to estimate accurately without conducting a large scale study. We restricted our method to taking hospital discharge frequency to be an estimate of the prevalence. The sample period of one year was chosen arbitrarily. An extension of the sampling period would probably yield a higher number of cases but would also make it likely that a significant number is lost due to death during the sampling period.

It might be considered a flaw that we used many epidemiological parameters from an American study, as the relevant figures should reflect conditions for the nation in which they are applied. But the figures in Meltzer *et al.* [21] are boundary values reflecting a range of possible values. Based on these values we performed an uncertainty analysis as described above. As a benefit we have the opportunity to compare results with Meltzer *et al.* and all others using the same values.

Model

Occupancy

We postulated that the pandemic incidence is normally (Gaussian) distributed over time, adopted from [23]. Notation is according to.

$$Ae^{-(t-\mu)^2/2\sigma^2}$$

This gives a symmetrical distribution with thin tails at either end. Adjusting the appearance to a more recognisable form can be accomplished by time substitution with a cubic spline, as

Table 2

Tabular weekly output data from FluSurge

Weeks	1	2	3	4	5	6	7	8	Total
Weekly admissions	2,027	3,379	5,068	6,420	6,420	5,068	3,379	2,027	33,789
Minimum scenario	893	1,489	2,233	2,829	2,829	2,233	1,489	893	14,889
Maximum scenario	2,667	4,446	6,669	8,447	8,447	6,669	4,446	2,667	44,458
Peak admissions/day				1,000	1,000				
No. of influenza patients in hospital	2,027	4,299	6,602	8,721	9,438	9,182	7,296	5,038	

The row "Number of patients in hospital", corresponds to the curve from StatFlu plotted in Figure 2.

explained below. As the incidence is normally distributed, so is the number of daily admissions. The procedure gives the curves a more recognisable form but may give false confidence in the output.

As the incidence is normally distributed, so is the number of daily admissions. We have complete control of the mean and standard deviation of this distribution. The mean m is the point in time when the pandemic is expected to reach its peak. μ is the standard deviation and controls the horizontal distribution over the duration of the outbreak. A is a normalising constant. The duration of the pandemic, the average length of each hospitalisation and the risk of admission, given symptoms, are assumed independent of the attack rate. All these parameters are assumed given at the start and can be altered by the user.

The number of admissions during the period t_1 till t_2 is normally distributed according to

(1)

$$S(t_1,t_2) = A\int_{t_1}^{t_2} e^{\frac{(t-\mu)^2}{2\sigma^2}}\,dt$$

The total admittance during the pandemic, S, is the integral taken over the whole time axis which is shown to be .

$$S = \sqrt{2\pi}\sigma A$$

The Gaussian is defined on an infinite axis in both directions, but we took the end of the epidemic t_e to be the point in time when the number of remaining admissions drops <1, i.e.

(2)

$$1 = A\int_{t_e}^{\infty} e^{-\frac{(t-\mu)^2}{2\sigma^2}}\,dt.$$

Symmetry similarly defines the start of the pandemic t_0. Setting the peak of the epidemic to $\mu = t_e/2$ and $t_0=0$, σ can be extracted by solving the integral.

The hospital load was considered by introducing the average duration of each hospital visit into the model and calculating the number of simultaneously occupied hospital beds, the occupancy. Let τ be the average duration of each hospital visit. The number of simultaneously occupied hospital beds, the occupancy B(t), is then given by

(3)

$$B(t) = A\int_{t-\tau}^{t} e^{-\frac{(t-\mu)^2}{2\sigma^2}}\,dt.$$

Time substitution

The Gaussian distribution, though a good starting point and easy to manipulate mathematically, is decidedly not realistic enough with its symmetric shape. Epidemics are not symmetric. The most familiar shape is one that climbs quickly, almost exponentially, and reaches a peak before declining with a long tail. This is the shape that is generated by the standard SIR (Susceptible – Infectious – Recovered) model [41]. To accommodate the user's expectations, we choose to manipulate the form to resemble something recognised from classical epidemic models, using a one-to-one function $\hat{t} = g(t)$ on the interval $[t_0,t_e]$. This function must obey

$g(t_0)=0$
$g(t_e)=t_e$
$g'(t)=1$, $t\leq 0$ and $t>t_e$

in order not to change the tail values. The definite solution to the integral (1) now must carry with it a correction e. We chose a piecewise continuous spline:

Figure 3

Hospital load in comparison scenario from StatFlu, to be compared to the output from FluSurge in Table 2

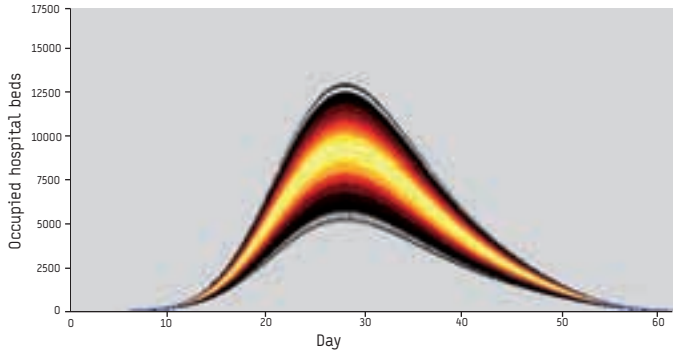


Figure 4

Daily admission rate in StatFlu along a more realistic epidemic curve, to be compared with Figure 3

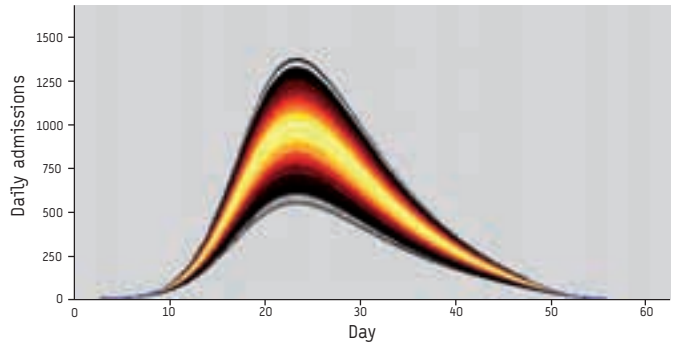
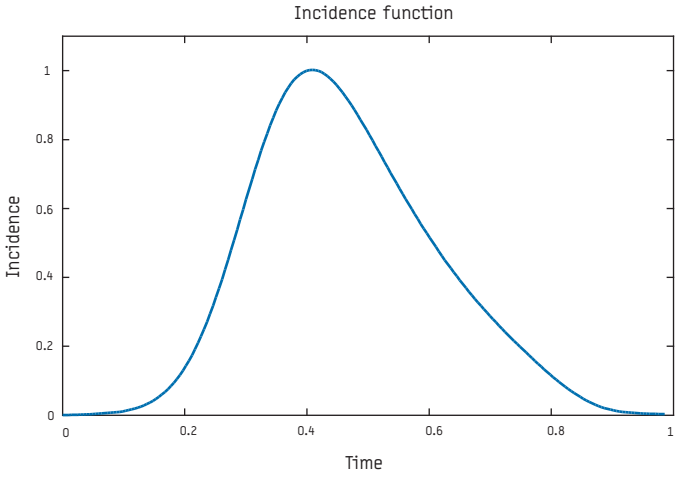


Figure 5

The new incidence curve, a normal distribution with time substitution $\hat{t}=g(t)$.



$g(t)=t$, $t \le t_0$ and $t \ge t_e$
$g(t)=-2.9t^3+1.8t2+t$, $t_0 < t \le .4t_e$
$g(t)=1.9t^3-1.7t2+t+.50$, $.4t_e < t \le .74t_e$
$g(t)=8.2t^3+.21t2+.53t+.73$, $.74t_e < t \le .94t_e$
$g(t)=-112t^3+.5.1t2+1.6t+.91$, $.94t_e < t \le t_e$

A correction was calculated numerically for feasible integer values of t_e ensuring that the total number of cases remains constant. The resulting distribution is depicted in Figure 5.

The other definitions in the previous sections were redefined by replacing t with $\hat{t}$.

The time substitution is implemented in the current release of StatFlu without the possibility to turn it off. This possibility will be an important amendment to upcoming releases, to make sure the user recognises the artificiality of this approach. Otherwise, there is a danger of too much confidence in the graph.

Monte Carlo simulation

The uncertainty in the risk of succumbing to illness upon infection and being admitted to hospital is modelled using a beta-distribution over a given uncertainty interval (see introduction). The beta-distribution was chosen for its applications in Bayesian sensitivity analyses [42], opening the possibility of creating a distribution based on point value estimates of risks from an expert panel. The intervals are specific for each combination of age group and risk group, six in total. 10,000 random values are picked from each distribution producing a value for the total admittance, S, according to equation (1). This value in turn is used to calculate according to equation (2).

It should be noted that the values from the size distributions are coupled, i.e. not considered independent. More specifically this entails that a single value is picked randomly from a uniform distribution and then transformed to each of the six beta-distributions, giving rise to six different value of risk for admission. The admittance is calculated separately and then added. The purpose of the coupling is done to minimise the variance and is justified by the fact that the uncertainty in the risk of admission originates in our ignorance. It is less probable that we overestimate the risk for one group and at the same time underestimate it for another [18-20,42].

Numerical model

A number of measures have been taken to maximise the speed of calculation, making StatFlu quite efficient. Despite the complexity of the numerical calculations, it is the graphic output that proves to be the major bottle neck.

σ is calculated for 10,000 values of admittance originating from a beta-distribution. First the attendance values are sorted. A large repository of random beta distributed values comes pre-calculated for each age/risk group and σ according to equation (2) is solved numerically by StatFlu using binary search. A standardised normal distribution is read from file as a lookup table with a resolution of 10^{-4} for parameter t<10. The table is searched using binary search down to the two closest t values and then linearly interpolated between them. The s values are calculated from both ends of the sorted list. The results of the previous calculation can thereby be exploited to narrow even further the binary search interval.

The σ values are then binned to desired resolution. The central values in the bins are what produces the plotted curves, in other words the integration in equation (3) is made for these central values only. The integrations are carried out with Simpson's formula with a resolution of hospital visit length τ =1 day. By saving intermediate partial results, all the integrations can be carried out in a single sweep.

Primary care and fear factor

StatFlu also outputs the expected increase in primary care visits during an influenza pandemic. These calculations work along the same lines as hospital load. The main difference is that a visit to a primary care unit does not have duration as such. We have also included the concept of deterrence from approaching the health care system as a consequence of a pandemic scare, the so called *fear factor*, $\tilde{\alpha}$. Studies conducted during the SARS-epidemic support such assumptions [28,29]. A reduction in visits as large as 35% was seen in Taiwan following the peak of the epidemic. The effect of the fear factor is to attenuate the increase of primary care visits. The fear factor is set by the user, between 0% and 40%. The total resulting reduction is distributed over the whole duration, linearly increasing up to the peak of the pandemic and then decreasing to zero again.

If μ_0 is the probability of visiting a primary care unit given disease, excluding those with influenza, μ_{i0} is the same risk including influenza, and N and N_i is the population at risk of disease including and excluding influenza, the total number of primary care visits can be expressed as:

(4) $$n_0 = (N - N_i)\,\mu_0 + Ni\mu_{i0}.$$

We might as well assume that the whole population is at risk for disease, thereby setting N to the population size. We also know the frequency of primary care visits [36]. The frequency of primary care visits due to symptoms of influenza-like illness (ILI) is estimated using the number of admitted with ILI symptoms and the associated risk given in Meltzer *et al.* [21]. The unknown risk mi0 can now be extracted from the above expression (4).

During a pandemic we assume that m0 and indeed also mi0 are valid, modified by the fear factor a:

(5) $$n_p = (N - N_{ip})\,\mu_0\alpha + N_{ip}\mu_{i0}\alpha.$$

As detailed in the section on Monte Carlo simulation, we form a beta-distribution for the uncertainties in m0 and mi0. The difference in primary care visits in the pandemic versus non-pandemic case is np - n0. This value will be different for all combinations of age group and risk group. We calculate, as before, each of these separately and then sum them up. The result is treated in the same way as the total admittance S in the subsection on occupancy, including time substitution and distribution over time. The difference is that the visit does not have duration in time in the sense that the value on the graph should be interpreted as *visits per day*. Hospital visit duration τ is always set =1.

What remains to be explained is how the fear factor a is treated over time. To assume a constant fear factor would merely offset the output curve downwards – a clearly unrealistic immediate cut in primary care visit frequency from the first day of the pandemic. We have opted for a model where the reduction increases linearly to peak at the same time as the incidence, and then decreases to zero again. To this end we calculate the total reduction due to the fear factor and distribute it accordingly over time. Finally we subtract this function from the output number of primary care visits. The fear factor model makes it possible for the curve to increase initially, but to decrease, even below zero, towards the epidemic peak.

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Foresight Infectious Diseases China Project - A novel approach to anticipating future trends in risk of infectious diseases in China: methodology and results from an initial application

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The project devised a simple but novel methodology for identifying possible future trends in infectious diseases in animals and humans in China, of priority concern to the Chinese authorities. It used a model of disease drivers (social, economic, biological or environmental factors that affect disease outcomes, by changing the behaviour of diseases, sources or pathways) devised for the Foresight Programme in the United Kingdom. Nine families of drivers were adapted to Chinese circumstances and matrices were constructed to identify the likely relationship of single infectious diseases or families of diseases to the drivers. The likely future trends in those drivers in China were determined by interviews with 36 independent Chinese experts. These trends included not only potentially adverse animal and human movements but also opportunities for innovative surveillance methods, more use of hospitals, antimicrobials and vaccines. Some human behaviours and social trends were expected to increase the risk of infections (in particular sexually transmitted and healthcare-associated infections) while at the same time the experts thought the awareness of risk in the Chinese population would increase. The results suggested a number of areas where the Chinese authorities may experience difficulties in the future, such as rising numbers of healthcare-associated infections, zoonoses and other emerging diseases and sexually transmitted infections (including HIV). Not making firm predictions, this work identifies priority disease groups requiring surveillance and consideration of countermeasures as well as recommending strengthening basic surveillance and response mechanisms for unanticipatable zoonoses and other emerging disease threats.

Introduction

In 2006 the United Kingdom (UK) government published the final results of the *Foresight Project on the Detection and Identification of Infectious Diseases* (September 2004 - April 2006). This produced a vision on risks from infectious diseases in plants, animals and humans over the next 10 to 25 years [1-5]. Particular emphasis was placed on how external factors or *drivers* (defined as social, economic, biological or environmental factors, see Table 1) could lead to changes in patterns of disease [6]. The project was international in scope with an intention to inform practical policies by showing how health threats can be anticipated, detected, prevented and controlled or at least how their effects can be mitigated in any country.

Based on this experience, a Project Group, including the authors, applied this future risks approach to China where there was both a recent history of emerging and changing infectious diseases and an especially rapid social change and therefore there was particular relevance for such an application [7]. These preliminary results were used to predict the more likely changes in infectious diseases and thus inform surveillance priorities, while at the same time refining and improving the Foresight methodologies for a later and larger application. The objective of this paper is to describe the methodology that was developed for the Foresight Infectious Diseases China sub-Project (hereafter referred to as *the China Project*) and the results of its initial application in China.

Methods

A workshop was held at the Health Protection Agency in the UK where objectives for the China Project work were agreed. The overall goals reflecting the policy priorities of the Government of China were to improve human health, to sustain economic development and to promote social stability as stated by the Chinese authors [8]. The specific objective was then to identify groups of human and animal infections that would be most likely to pose problems and challenges to these policy priorities in the next two decades. The rationale was that this would allow authorities to prioritise these groups for purposes of surveillance, prevention and control or mitigation.

The approach developed by the Future Risks component of the main Foresight Infectious Diseases Project was to have a simple model of drivers, sources, pathways and outcomes (Figure) [5]. *Drivers* would be a range of factors, social and otherwise (Table 1) that directly or indirectly can influence the incidence of infectious diseases. Sources were defined as phenomena or biological events that give rise to potential new diseases, enable existing diseases to become more harmful, enable existing diseases to infect new hosts, or enable existing diseases to spread to new areas, *pathways* were

Table 1

Nine groups of societal drivers (total = 96). Foresight Infectious Diseases China Project

A. Governance and social cohesion
• Biosecurity governance of technology (drugs and pesticides)
• Social cohesion as an enabler or constraint on identification and control of infectious diseases
• Illegal practices and consequent spread of diseases of 'pest' species such as myxomatosis
• International/national/regional interactions affecting governance
• Lack of interaction between policy and regulatory agencies leading to delays in detection and identification
• Inter-ministerial agencies
• Openness with the public
• Marginalisation of some groups
• Political leadership on health issues
B. Demography and population change
• Immigration
• Urbanisation
• Migrant labour
• Overall population
• Ageing population
• Dietary and occupation changes (affecting exposure and susceptibility of population to disease risks)
• Population movements (e.g. from rural to urban or from developing to developed world)
• Animal immigration
• Overall animal populations
• Urbanisation of animals
• Animal population movements
• Movement of animals around the country
C. Conflict
• Difficulties in maintaining administrative systems and so loss of effective identification and surveillance systems
• Movement of refugees spreading diseases
• Internal conflict
• Loss of effective identification and surveillance systems for animals
• Unrestricted movements of animals around the country
D. Technology and innovation and their governance
• Impact of innovation on disease identification and treatments
• Ability to control infections; control strategies, e.g. for diseases that are easier (SARS, smallpox) or more difficult ('flu, AIDS) to control
• Impact of GM crops on agriculture and development of plant diseases
• Emergence of drug or pesticide resistant strains of infectious organisms; half lives of existing drugs and pesticides
• Role of technology in disease surveillance systems (detecting new, emerging diseases or monitoring movements of existing pathogens)
• Dissemination of information
• New, faster identification of organisms
• Development of new antivirals and vaccines
• Improved diagnostics, leading to more accurate, less costly and more rapid detection of diseases
• Transplant surgery
• Other high technology medicine
• More use of antimicrobials for humans
• Longer survival of patients with chronic diseases
• Longer survival of patients with chronic diseases
• Ability to control infections and improved control strategies in animals
• Drug or pesticide resistant strains in animals
• New surveillance systems for animal diseases
• Greater information dissemination (web-based information for disease diagnosis, for alerting experts to existence of new diseases, for providing faster and better public dissemination of disease-related information)
• Faster identification of infections in animals
• Use of antimicrobials in animals
• Improved diagnostics for animal infections

E. Changes in agriculture and land use
• Changes in animal husbandry methods, e.g. intensive rearing methods or closer missing of animal and human populations as part of urbanisation
• Greater genetic uniformity in animal and plant populations; less 'biodiversity', less varied crop mosaics
• More intensive farming systems
• Development of new crops
• New developments in production economics involving greater movement of animals and hence more exposure to diseases such as foot and mouth disease
• More frequent proximity of different farming systems
• Changing patterns of land use
F. Economic factors (income, prosperity, employment)
• Overall wealth
• Income disparity
• Education levels in the general population
• Future oil and other energy supplies
• Quality of sanitation and water supplies
• Background pollution levels affecting the natural immunity of animals and humans
• Poverty and malnutrition
• Waste disposal as a source of disease spread (humans)
• The availability of a pool of experts to detect and identify infectious diseases
• Unemployment
• Waste production and disposal in animals
• Pool of experts in animal health
G. Trade and market related factors
• Changing patterns of trade in crops and animals
• Behaviour and structure of markets
• Future diets and demands for exotic products
• Illegal trading in human foods
• Food preservation technology
• The misuse of disease surveillance systems as trade barriers
• Changing patterns of trade in animals
• Illegal trade in animals
•Trade barriers to trade in animals
H. Transport and tourism
• International movement of drug or vaccine resistant strains of organisms
• Changes in the rates of internal movements of people, food, animals etc
• Future levels of tourism to and from China
• Levels of internal tourism
• Changes in patterns of stock-keeping and so movement of diseases; compressed time scales
• Internal migration
I. Human activity and social pressures
• Demands for more healthy food
• Demands for more 'sustainable' production systems
• Changes in sexual practices
• Changing life styles – consumerist, individualist, communitarian
• Public perceptions of risk and willingness to change behaviours
• Public demands for greater levels of safety
• Demands for lower levels of pollution
• Ecological awareness in the public
• Public willingness to accept change
• Media reporting as a driver of how governments react to disease
• Crowding in hospitals
• Farmers and producers perception of risk and biosecurity
• Willingness to change farming practices
• Media reporting on animal health issues

mechanisms or routes by which a disease-causing organism can be transferred from one host to another, within or between species and outcomes were the infectious diseases themselves [5]. For example, changes in the way animals are reared for food production favouring intensive farming and the keeping of animals in close proximity in large numbers would lead to the spread of zoonoses that by definition affect humans.

For the China Project the Future Risks model was developed to make predictions relevant to the Chinese situation. The Project Group identified drivers, considered what was known of their relationship to important groups of animal and human infections (plant infections were outside their expertise). It then determined through consultation with Chinese experts what was thought to be likely to happen to the drivers in the next two decades in China and hence assessed qualitatively what might be expected to occur in regards to the spread and prevalence/incidence of these infections in China over that time.

Figure

Basic Foresight risk model for infectious disease risks

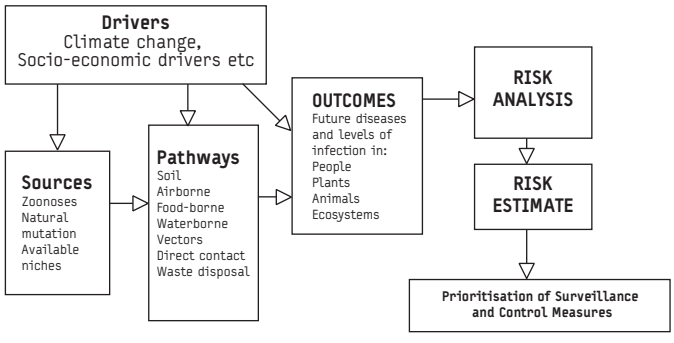

Table 2

Selected animal and human diseases. Foresight Infectious Diseases China Project

Exemplar animal infections
Foot and mouth disease
Avian influenza
Classical swine fever
Bovine spongiform encephalopathy
Selected groups of human infections
Gastrointestinal infections
HIV and other sexually transmitted infections
Malaria and other vector-borne infections
Influenza
Severe acute respiratory syndrome (SARS)
Parasitic infections
Vaccine preventable diseases
Antimicrobial resistant organisms
Zoonoses (taken to include novel infections and novel variants of previous infections)
Healthcare-associated infections
Bloodborne infections

In detail, the Project Group used the drivers established for the main Foresight Project, and adapted these to reflect changes known to be underway in China focusing only on animal and human diseases. As a result a list of 96 drivers grouped into nine families was obtained (Table 1). These included:

- Factors that affect the sources of the infectious disease (e.g. changes in patterns of animal husbandry)
- Factors affecting how infectious diseases are spreading (e.g. changes in the movement of people and changes in institutional structures)
- Factors affecting the assets at risk (businesses, people, animals)
- Factors that are likely to influence vulnerabilities to infectious diseases (e.g. increasing numbers of elderly people and people living with chronic diseases)
- Changing priorities and requirements for surveillance to detect anticipated risks and changes in risks (e.g. detection of healthcare-associated infections)
- Priorities and opportunities for control of risks and diseases (e.g. appreciation of the need for biosecurity around the controlled use of dangerous pathogens in laboratories and industry).

For animal diseases, four exemplar infections were chosen by the veterinary experts in the Project Group to represent both known infections (foot and mouth disease and classical swine fever) and emerging or novel infections (avian influenza and bovine spongiform encephalopathy). While the approach taken for human diseases was to identify 11 important families of infectious diseases or single diseases (Table 2).

The Project Group then used their expert knowledge to populate the two-dimensional matrices establishing the causative relationships or associations between the drivers and the infectious diseases (examples in the Appendix*). For instance, recognised drivers increasing the risk of human immunodeficiency virus (HIV) infection and other sexually transmitted infections included adverse changes in sexual behaviours, increasing migrant labour, decline in educational levels and falls in the earning capacity of women. Conversely, the opposite trends in these drivers might be expected to lead to decreases in sexually transmitted infections, including HIV. These relations are shown in Table 3 for HIV and other sexually transmitted infections as an example [9]. The Group recognised that the relationships between some of the drivers and the animal and human infections were uncertain and therefore these cells were not populated in the matrices.

The main data gathering consisted in obtaining expert opinion from Chinese scientists on the likely future trends in the drivers in their country. A detailed structured questionnaire was developed and piloted within the Project Group itself. Some questions were asked more than once in different forms in order to check on the consistency of answers. Following approval from the Ethics Committee of the Chinese Academy of Medical Sciences, Peking Union Medical College (PUMC), 36 Chinese experts (four per each family of drivers) were identified by the Chinese collaborators in the Project Group from the Chinese academic community (personal details of the experts are not disclosed in this paper but are available from the authors upon request). The selection was based on relevant expertise in the families of drivers in China rather than knowledge about infectious diseases.

The expert opinions were then derived from face-to-face interviews undertaken by a team of postgraduate students from PUMC using the questionnaire. The experts were asked whether

in their opinion the drivers were going to worsen, improve or stay the same in the next two decades. For example, a question from the section on Transport and Tourism (Family H of the drivers) was phrased as follows: *Concerning internal migration in the next 15 to 20 years do you expect this to increase, decrease or stay the same?* Experts could also say that the future situation was genuinely uncertain, or that they had no opinion. Notes were kept of additional remarks and comments made by the experts. The students who performed the interviews were trained so as to achieve consistency in the process and this was checked by repeating 10% of interviews with a different student.

The results of the 36 interviews were analysed in China to arrive at consensus expert views on the likely future trends in the drivers. Consensus was considered to have been achieved where three out of four or all four of the experts agreed.

These consensus trends were then applied back to the matrices (Tables 3 and 4) to identify which of the animal and human diseases would be more likely to increase or decrease in the future in China.

After the work the authors held a meeting in Beijing and reviewed the experience to indicate lessons that should be taken into account in the future use of this methodology ('lessons learnt') planned in China.

Results

Expected trends in the drivers

Of the 96 drivers, consensus was achieved for 51 while for further three drivers the experts agreed the future was uncertain. For 23 of the 51 there was complete consensus between the four experts while for 28 there was only consensus between three out of four experts. These detailed results are shown in Table 5. The drivers for which there was consensus are listed in Table 3.

Table 3

Example of the relationship between drivers and infections – Human Immunodeficiency Virus (HIV). Foresight Infectious Diseases China Project.

Factors likely to be associated with...	... increased HIV transmission	... reduced HIV transmission
Governance and social cohesion	Decreasing social cohesion Increasing illegal practices Marginalisation of some groups	Increasing political leadership on health issues Increasing openness with the public
Demography and population change	Increasing urbanisation and use of migrant labour Increasing population movements (e.g. from rural to urban or from developing countries to China)	Ageing population
Conflict	Movement of refugees spreading diseases Internal conflict	
Technology and innovation and their governance	Emergence of drug resistant strains Longer survival of patients with chronic diseases	Impact of innovation on disease identification and treatments Dissemination of information New, faster identification of organisms Development of new antivirals and vaccines Improved diagnostics, Greater information dissemination
Economic factors	Greater income disparity Increased poverty Unemployment	Increased overall wealth Improved education levels
Transport and tourism	International movement of drug-resistant strains Increases in the rates of internal movements of people More tourism to and from China	
Human activity and social pressures	Changes in sexual practices to more unsafe sex More injecting drug use	Public demands for greater levels of safety Public perceptions of risk and willingness to change behaviours (if unsafe sex) Media reporting as a driver of how governments react to disease

Table 4

Areas of expert consensus on future trends in drivers. Foresight Infectious Diseases China Project.

A. Governance and social cohesion
• Social cohesion will increase
• International/national/regional interactions will increase
• Government openness with the public will increase
• Political leadership on health issues will increase
B. Demography and population change
• Use of migrant labour will increase
• Human population movements will increase
• Animal immigration into the country will increase

• Urbanisation of animals will increase
• Internal animal population movements will increase
• Movement of animals around the country will increase
C. Conflict
• Stress on administrative systems will increase and with it will there will be loss of effective identification and surveillance systems
• There will be some loss of effective identification and surveillance systems for animals
D. Technology and innovation and their governance
• There will be more innovation in disease identification and treatments for humans
• The potential to control human infections will generally increase
• The emergence of drug or pesticide resistant strains of infectious organisms will increase
• There will be more opportunities for innovative disease surveillance systems (detecting new, emerging diseases or monitoring movements of existing pathogens)
• The ability to disseminate information will increase
• The ability to identify organisms will increase as will the speed of identification
• Numbers of new antivirals and vaccines will become available
• Diagnostic ability will improve , leading to more accurate, less costly and more rapid detection of diseases
• High technology medicine will increase
• Use of antimicrobials for human infections will increase
• Identification and treatment of human diseases will increase
• Ability to control infections in animals will increase
• Drug or pesticide resistant strains appear more often in animals
• There will be more opportunities for developing surveillance systems for animal diseases
• Information dissemination about animal disease will increase
• Infections in animals will be identified more rapidly and easily
• There will be more use of antimicrobials in animals
• There will be improved diagnostics for animal infections
E. Changes in agriculture and land use
• The genetic uniformity in animal and plant populations will increase
• There will be developments in production economics involving greater movement of animals and hence more exposure to diseases such as foot and mouth disease
F. Economic factors (income, prosperity, employment)
• Overall wealth will increase
• Education levels in the general population will improve
• The availability of oil and other energy supplies will worsen
• Quality of sanitation and water supplies will improve for humans
• Poverty and malnutrition will decline
• Waste disposal as a source of human disease spread will improve
• The availability of a pool of experts to detect and identify human infectious diseases will improve
• The available pool of experts in animal health will enlarge
G. Trade and market related factors
• The behaviour and structure of markets as affecting infections will improve
H. Transport and tourism
• More internal movement of people, food, other goods live animals and microorganisms
• Future levels of tourism to and from China will increase
• Levels of internal tourism will increase
I. Human activity and social pressures
• Sexual practices will become more risky
• There will be other changes in lifestyle increasing risk of infection
• Public tolerance of infection risk will decline and the willingness to change behaviours to reduce such risk will increase
• Public demands for greater levels of safety will increase
• Ecological awareness in the public will increase
• Media reporting as a driver of how governments react to infectious disease will increase
• Crowding in hospitals will increase
• Farmers and producers will become more aware of infection risk and biosecurity
• Media reporting on animal health issues will increase

Notable areas of consensus on expected trends in the drivers were as follows: There would probably be greater social cohesion and more transparency in Chinese governance with greater leadership shown by government on human health issues. Movements of animals around the country and internationally (meaning into and out of China) would be likely to increase and there would probably be more animals in urban areas. Similarly, there would most likely be more and larger scale internal human migrations and movements of people and more use of migrant labour within China. Tourism within, and to and from China was also considered likely to increase.

It was expected that because of growth and urbanisation additional stress would be placed on administrative systems which could threaten some surveillance for animal and human diseases. Conversely technological developments would provide more opportunities for surveillance, better detection of organisms and there would probably be more dissemination and sharing of information.

The production of waste from animals was considered likely to increase substantially and, with it, problems of waste disposal but there was no consensus that the same would happen for human waste. Genetic uniformity was expected to increase in crops and animals. In human healthcare, high technology medicine, the development of new medicines and vaccines would all increase. In the additional remarks the experts in the relevant areas said that in their opinion, this would take place because of technological change, growing number of older people and people with chronic conditions and increasing healthcare expectations in the population. However the experts were not sure whether or not China's population would age overall. It was felt that the use of hospitals and overcrowding in hospitals would probably increase, as would the use of antimicrobials in humans and in animals.

Overall individual wealth and levels of education were expected to rise, though there was no consensus on what would happen concerning income disparities. It was felt that sexual behaviour would change in ways that overall would increase the risk of acquiring and passing on sexually transmitted infections including HIV and other blood-borne viruses. However, it was also expected that the population would become less accepting of risks from infection and that there would be greater demands for safety, more ecological awareness of the importance of the environment and greater media reporting of health and environmental issues. Human sanitation was expected to improve but the availability of energy sources would probably worsen. The intellectual capacity of China was expected to rise with more experts in animal and human health.

Possible consequent trends in the infections

The application of the changes in the drivers (Table 1) against the matrices (Appendix) indicated that if the predicted trends materialised, and no countermeasures were applied, adverse changes (rises) in the rates of the following groups of infections would be expected:

Table 5

Analysis of expert opinions as to whether the selected drivers would improve or worsen in the coming two decades

Drivers	Expert 1	Expert 2	Expert 3	Expert 4	Consensus (or not)
A. GOVERNANCE AND SOCIAL COHESION					
1. Biosecurity governance (currently there is little biosecurity governance or regulation in China)	A	A	C	C	no consensus
2. Social cohesion	C	C	C	C	Social cohesion will increase
3. Illegal practices	D	C+	C	A	no consensus
4. International/Regional interactions	C	A&C	C+	C	International and regional interactions will increase
5. Lack of interaction between policy and regulatory agencies	D	C	A	A	no consensus
6. Inter-ministerial agencies: will these become more common?	C	D	C	D	no consensus
7. Problems across international agencies (sharing of information with international agencies)	C	D	D	C	no consensus
8. Openness with the public (government transparency)	C+	C+	C	C	Government transparency will increase
9. Marginalisation of some groups	D	C	A	A	no consensus
10. Political leadership	C+	C+	C	C	More political leadership relating to health issues
B. DEMOGRAPHY AND POPULATION CHANGE					
11. Immigration	D	D	A	D	no consensus
12. Urbanisation	D	A	A	D	no consensus
13. Migrant labour	D	A	A	A	More use of migrant labour
14. Overall population (specify detailed changes if possible)	B	D	D	D	no consensus
15. Elderly population	B	A	C	D	no consensus
16. Dietary and occupational changes	B	A	C	C&D	no consensus
17. Population movements	A	A	A	D	More population movement
18. Animal immigration	A	A+	D	A	More animal movements
19. Animal populations (increase or reduce)	B	A	A	D	no consensus

20. Urbanisation of animals	A	A+	A	A	More animals in urban areas
21. Animal population movements	A	A	A	A	More movements of animals
22. Movement of animals around the country	A	A	B	A	More movements of animals
C. CONFLICT					
23. Difficulties in maintaining administrative systems so loss of effective identification and surveillance systems	A	A	A+	D	Stress on administrative systems
24. Movement of refugees	C	B	B	D	no consensus
25. Internal conflict	A+	D	B	D	no consensus
26. Loss of effective identification and surveillance systems (for animals)	A or B	A	A	A	More stress on animal surveillance systems
27. Unrestricted movement of animals around the country	B or C	A	B	A	no consensus
D. TECHNOLOGY AND INNOVATION AND THEIR GOVERNANCE					
28. Impact of innovation on human disease identification and treatments	C+	C	C	C	More innovation in human disease diagnosis and treatment
29. Ability to control human infections and control strategies	C	C	C	C	Improved infection control strategies
30. Use of genetically modified crops	D	B	D	D	no consensus
31. Drug- and pesticide-resistant organisms	A	A	A+	C	More drug- or pesticide-resistant organisms
32. New surveillance opportunities (e.g. web-based and remote systems)	C	C	C	C	Increased opportunities for surveillance in animals
33. Information dissemination	C	C+	C	C	Better information dissemination
34. Faster identification of organisms	C	C+	C	C	Faster organism identification
35. Antiviral, antimicrobial and vaccine development	C	C	D	C	More antimicrobials and vaccines becoming available
36. Improved diagnostics	C	C	C	C	Improved diagnostics
37. Transplant surgery	B	D	D	B	no consensus
38. Other high technology medicine	C	C	C	C	More high technology medicine
39. Use of antimicrobials for humans	C	C	C	A	More use of antimicrobials in humans
40. Longer survival of patients with chronic diseases	D	B	A	B	no consensus
41. Impact of innovation on human disease, (identification and treatments)	C	C	C	C	More identification of human disease and more treatment
42. Ability to control infections, control strategies in animals	C	C	C	C	Greater ability to control animal infections
43. Drug- or pesticide-resistant strains in animals	A	A	A	A	More drug resistant strains in animals
44. New surveillance systems for animal diseases	C	C	A	C	More surveillance systems for animal diseases
45. Information dissemination concerning animals	C	C+	C	C	Better information dissemination concerning animals
46. Faster identification of infections in animals	C	C+	B	C	Faster identification of infection in animals
47. Use of antimicrobials in animals	A	C	C	C	More use of antimicrobials in animals
48. Improved diagnostics for diseases in animals	C	C	C	C	Better identification of infection in animals
E. AGRICULTURE AND LAND USE CHANGE					
49. Changes in animal husbandry methods	D	D	A	D	Future unclear
50. Greater genetic uniformity in crops and animals	A	A	A	D	Greater genetic uniformity in crops and animals
51. Intensive farming	D	A	D	B	no consensus
52. New crops	D	B	A	D	no consensus
53. More attention to economics	C+	C	A	C	More movements of animals for economic reasons
54. Proximity of different farming systems	D	D	A	D	Future unclear
55. Changing patterns of land use	A+ or D	C	A	D	no consensus
F. ECONOMIC FACTORS (INCOME PROSPERITY AND EMPLOYMENT)					
56. Overall wealth	C	D	C	C	Wealth increasing overall
57. Income disparity	C	A	D	C	no consensus

58. Education levels in the general population	B	C	C	C	Education levels will improve
59. Future oil and other energy supplies	A+	D	A	A	Availability of energy sources will worsen
60. Quality of sanitation and water supplies	C	C	C	A	Sanitation will improve
61. Background pollution levels	B	C	A+	A	no consensus
62. Poverty and malnutrition	C	C	C	C	Poverty will decline
63. Waste disposal	C	A	C	C	Waste disposal will improve
64. Pool of experts in human disease	A	C+	C	C	Numbers of experts in human health will increase
65. Unemployment	A	C	C	A	no consensus
66. Waste production and disposal (from animals)	A	A	D	C	no consensus
67. Pool of experts in animal health	C	C	C	C	Numbers of experts in animal health will increase
G. TRADE AND MARKET RELATED FACTORS					
68. Changing pattern of trade	C	D	A	C	no consensus
69. Behavior and structure of markets	C	C	A	C	Behaviour of markets will improve
70. Future diets and demands for exotic products	D	D	A	D	no consensus
71. Illegal trade	D	A	A	D	no consensus
72. Food preservation technology (please specify changes)	C	C	A	D	
73. Trade barriers	A	D	D	D	Future unclear
74. Changing patterns of trade in animals	C	A	D	A	no consensus
75. Illegal trade in animals	C	A	A	C	no consensus
76. Trade barriers for trade in animals	B	C	C	D	no consensus
H. TRANSPORT AND TOURISM					
77. International movement of people, foods, other goods, live animals, microorganisms	A	A	C	D	no consensus
78. Changes in the rates of internal movement of people, food, other goods, live animals, microorganisms	A	A	A	D or A	More movement of all
79. Future levels of international tourism a) from China, b) to China	A	A	A	D	Increased tourism to and from China
80. Internal tourism (inside China)	A	A	A	D or A	Increased internal tourism
81. Emergence of 'just in time' stockkeeping (shops and industry having low levels of stock and relying on new supplies arriving at the right time)	A	D	B	D	no consensus
82. Internal migration	A	A	A	D or A	no consensus
I. HUMAN ACTITIVY AND SOCIAL PRESSURES					
83. Demands for more healthy food	B	B	C	C	no consensus
84. Demands for more sustainable production	D	D	D	C	no consensus
85. Changes in sexual practices	A	A	A+	A	More risky sexual behaviours
86. Changing lifestyles	A	A	D	A	Changes in lifestyles making more liable to risk of infections
87. Public perceptions of and acceptance of risk	C	C+	C	C	Less public tolerance of risk
88. Demands for greater levels of safety	C	C	C	C	More public demands for more safety
89. Demands for lower levels of pollution	A	C	C	D	no consensus
90. Ecological awareness	D	C	C	C	More awareness of ecological factors
91. Willingness to change	C	D	D	C	no consensus
92. Media reporting on human diseases	C	D	C	C	Greater media reporting
93. Overcrowding in hospitals	A	A	A	A	More overcrowding in hospitals
94. Farmers and producers perception of risk and biosecurity	C or D	C	C	C	Farmers more aware of risk and biosecurity issues
95. Willingness to change farming practices	D	D	D	C	no consensus
96. Media reporting on animal health issues	C	C	D	C	More media reporting on animal health issues

Legend
A = Intensify (getting worse) B = Stay the same C = Become less intensive (getting better) D = Future unclear
A+ or C+ were used if the expert said that large change was anticipated.
Consensus was reached if at least three of the four experts agreed.

- Animal infections (e.g. foot and mouth disease, avian influenza and classical swine fever) as a result of animal movements;
- Infections acquired as a result of receiving healthcare (nosocomial or healthcare-associated infections);
- Infections caused by drug-resistant organisms in animals and humans;
- Human sexually transmitted infections, including HIV;
- Human blood-borne viral infections associated with high-technology care (such as hepatitis B and C);
- Food-borne infections affecting humans and zoonoses in humans and animals including emerging infections;
- Imported and exotic infections.

Discussion and lessons learnt

Historically China has been a potent source of infections that have come to affect or threaten Europe. The influenza pandemics of 1957 and 1968, the avian influenza A(H5N1) ('bird flu') and severe acute respiratory syndrome (SARS) all appeared first in China [6-7]. The Foresight China Project has identified a number of likely future trends for drivers of infectious diseases in China that could potentially lead to increases in rates of healthcare-associated infections, drug-resistant organisms, sexually transmitted infections and zoonoses as well as other novel infections and variants of previously identified infections. The results identifying the probable changes in drivers in China can be compared to those obtained in the main Foresight project for the UK and Africa even if only limited predictions can be made as to their impact on actual diseases. These comparisons reveal some broad similarities in the trends in the drivers thus recognising the universality of some international changes [5].

Lessons from this application

The China Project also revealed a number of methodological issues that need addressing. The selection of drivers used in this study and the relationship between the drivers and infections were probably not sufficiently evidence-based and need to be supported by a literature review. The questions put to the experts were probably too open-ended and there were difficulties in the analysis of their additional comments. Because the subject of the project was known, there were difficulties in getting the experts to focus on the trends in the drivers without considering the trends in the infections that might result from these changes. Also, it was notable how the recent Chinese experience with SARS in 2003 influenced some of the expert opinions which tended to hark back to that event. The number of experts (only four per family of drivers) was perhaps too limited and for some of the areas it was felt that if the experts could have met together rather than individually, a more useful consensus would have been achieved.

It is important not to over-interpret the suggested trends indicated here. Aside from this being a limited initial application, there are difficulties in drawing any conclusions from this form of qualitative predictions. What should be concluded when two drivers are running contrary to each other? For example, it was suggested that sexual behaviours will become more risky while at the same time the public will generally become more aware of risks. An additional point is whether such a unitary approach can be undertaken for countries that are as large and diverse as China. Trends that might apply in the richer east and semi-tropical south of China might be quite different in the less well resourced western provinces and the temperate and continental north of China. In a way these considerations do not matter as long as the predictions are not seen as what will certainly happen. What are being suggested are the more likely changes in disease risks and possible threats that the authorities should be aware of and prepare for. These changes are not inevitable as future trends also depend on countermeasures deployed either against the infections or to offset the underlying drivers. The real conclusion is to suggest priorities for surveillance and development of countermeasures. The results suggest these priorities should include animal infections associated with animal movements, and, in humans, zoonoses, sexually transmitted infections, healthcare-associated infections and antimicrobial resistance. Equally, the authorities could consider whether to take a precautionary approach and implementation of countermeasures at an early stage, for example by giving more priority to hygiene in hospitals and rational approaches to antimicrobial prescribing. However, historical events including developments like SARS and highly pathogenic avian influenza in China indicate that to some extent future events in infectious diseases can never be entirely anticipated [7,10]. Hence it is crucial to establish basic surveillance and response mechanisms in a strong modern public health framework that can detect and respond to whatever threats should appear in the future.

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*The appendix with examples from the two matrices with the presumed relationship between animal and human infections and the drivers are available online: http://www.eurosurveillance.org/public/public_pdf/Foresight_China_Appendix.pdf Full matrices are available on application to the corresponding author.

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Sequence-based and monoclonal antibody typing of Legionella pneumophila isolated from patients in Portugal during 1987-2008

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The monoclonal antibodies and the sequence-based typing (SBT) are two methodologies widely used to characterise *Legionella pneumophila* strains serogroup 1 (sg1). In this study, we analysed the clinical strains received in two Portuguese laboratories since 1987, including the strains isolated in Portugal during the four years of the surveillance scheme for Legionnaires' disease implemented in 2004. In total, 63 clinical isolates of *L. pneumophila* sg1 were differentiated by SBT into 19 different sequence types. Ten of them were new in the SBT database of the European Working Group for Legionella Infections (EWGLI). As a result of the combination of the two methodologies, these strains were discriminated into 25 different profiles. This study enabled, for the first time in Portugal, not only to characterise the *L. pneumophila* sg1 clinical isolates, but also to create a database of Portuguese profiles for use in epidemiological surveillance efforts.

Introduction

Legionella pneumophila is a Gram-negative facultative intracellular pathogen, which is responsible for Legionnaires' Disease. This microorganism has increasingly been recognised as an important cause of pneumonia since its first description in 1977 [1]. The characterisation of clinical isolates of *L. pneumophila* enables us to learn about its epidemiology in a certain geographic region, as well as to create a database of circulating profiles [2-7].

The combination of a genotypic method with monoclonal antibody (MAb) typing has been described as a useful approach for epidemiological typing of *L. pneumophila* isolates [7-10]. MAbs of the Dresden panel allow subdividing the serogroup 1 of *L. pneumophila* as having, or not having, the epitope recognised by the MAb 3/1. According to epidemiological studies, this epitope appears to be associated with virulence [11]. Sequence-based typing (SBT) is one of the genotypic methods that can be applied for this purpose. It was adopted as an international standard and is widely used by the members of the European Working Group for Legionella Infections (EWGLI), since it is a simple, rapid and discriminatory typing method. Furthermore, it also allows the exchange of data between laboratories [7,8].

In 1999, the Portuguese public health authorities implemented a surveillance scheme for Legionnaires' Disease based on clinical reports. Later, in 2004, a surveillance scheme based on laboratory notifications was added. The Legionella laboratory in the microbiology department of the Faculty of Medical Sciences in Lisbon and the National Institute of Health Dr Ricardo Jorge (INSA) are the two laboratories involved in this surveillance scheme.

The aim of this study was to investigate the distribution of sequence types (ST) and monoclonal antibody subtypes among clinical isolates of *L. pneumophila* in Portugal.

Material and methods

As far as the present study is concerned, the SBT methodology, using seven genes (*flaA, pilE, asd, mip, mompS, proA and neuA*), was applied to 63 clinical isolates of *L. pneumophila* serogroup 1 (sg1), and four from non-sg1 (one isolate was sg 10, another was sg 12 and the two remaining reacted with "*Legionella pneumophila* serogroups 2-14 Latex Test Reagent" (Oxoid), but the serogroup could not be determined using our MAbs protocol) (see Table). The *L. pneumophila* strains were typed with MAbs of the Dresden panel, by using an indirect immunofluorescence test [10,11].

We analysed the clinical strains received since 1987 by the laboratories of Santa Cruz Hospital and the Faculty of Medical Sciences, including the 19 strains isolated during the four years of the surveillance scheme for Legionnaires' disease. In total, 67 strains were sent for typing by 17 Portuguese hospitals. Thirty of them were isolated from patients with nosocomial infections and 20 from patients with community-acquired infections; the remaining 17 had an undetermined origin.

The genomic DNA used for the SBT method was extracted with the InstaGene Matrix kit (Bio-Rad), and the PCR amplification was performed by using puRe Taq Ready-to-Go beads (Amersham Biosciences). The primers and the PCR conditions were the same as those used by Gaia *et al.* and Ratzow *et al.* [7,8,12]. After purification with the Qiaquick PCR purification Kit (Qiagen), both strands of the amplicons were sequenced by StabVida on a 3700 ABI DNA sequencer (Applied Biosystems) using the Big Dye terminator DNA sequencing kit. The nucleotide sequences obtained were compared to those in EWGLI-SBT database [13]. All putative new sequences were confirmed before being sent to the curators of the database.

Results

In this study, all but three of the strains included were typable by SBT using the seven genes (see Table). The *neuA* primers failed to type these three strains, all of which were non-sg1 (one sg 10 and the other two could not be identified with MAbs of Dresden panel), suggesting that *neuA* primers described by Ratzow *et al*. [12] are not always suitable for serogroups other than sg1. Other teams have also reported amplification problems with the *neuA* primers [14].

Applying SBT, the sample was discriminated into 7, 7, 11, 8, 12, 7 and 7 types, based on the sequences of f*laA, pilE, asd, mip, mompS, proA* and *neuA*, respectively. As a consequence, the 67 isolates were divided into 23 STs in total. The distribution was as follows: the 63 *L. pneumophila* sg1 isolates were included into 19 ST, and the four *L. pneumophila* non-sg1 isolates into the remaining four ST.

Ten of the 19 STs from *L. pneumophila* sg1 and the four STs from *L. pneumophila* non-sg1 were different from the ones that already existed in the EWGLI-SBT database. In addition, six new allele numbers (22 and 29 for the *mip* gene, and 24, 20, 34 and 23 for the *pilE, asd, mompS* and *proA* genes, respectively) were assigned by the curators after our data were submitted to the database. It is interesting to notice that five of these new allele numbers were detected only in *L. pneumophila* non-sg1 strains that were non-typable with MAbs from the Dresden panel (see Table). The ST100 (3,8,1,10,14,12,2) was the most frequent allele

Table

Twenty-three SBT profiles of 67 Portuguese *L. pneumophila* clinical isolates, 1987-2008

ST	Allelic profile[a]	No. of strains	Dresden panel			Epidemiological relatedness
			Serogroup	MAb subgroup	No. of strains	
100[b]	3,8,1,10,14,12,2	32	1	Allentown/France	18	Related
				Philadelphia	14	
1	1,4,3,1,1,1,1	3	1	Philadelphia	2	Unrelated
				Olda	1	
23	2,3,9,10,2,1,6	3	1	Philadelphia	2	Unrelated
				Knoxville	1	
62	8,10,3,15,18,1,6	3	1	Allentown/France	2	Unrelated
				Philadelphia	1	
103[b]	1,4,3,**22**[b],1,1,1	3	1	Philadelphia	3	Unrelated
20	2,3,18,15,2,1,6	2	1	Knoxville	2	Unrelated
42	4,7,11,3,11,12,9	2	1	Knoxville	1	Unrelated
				Benidorm	1	
44	4,8,11,10,10,12,2	2	1	Allentown/France	1	Unrelated
				Philadelphia	1	
99[b]	4,8,11,5,29,12,10	2	1	Knoxville	2	Unrelated
101[b]	6,10,15,15,21,4,6	2	1	Philadelphia	1	Unrelated
				Knoxville	1	
16	2,10,18,10,2,1,9	1	1	Knoxville	1	
22	2,3,6,10,2,1,6	1	1	Philadelphia	1	
94	12,8,11,5,20,12,2	1	1	Knoxville	1	
98[b]	8,10,3,10,2,5,6	1	1	Philadelphia	1	
102[b]	8,19,5,15,18,5,10	1	1	Philadelphia	1	
146	2,10,18,10,2,1,6	1	1	Philadelphia	1	
172[b]	1,4,3,1,1,1,2	1	1	Philadelphia	1	
173[b]	6,10,14,15,21,4,6	1	1	Knoxville	1	
174[b]	4,8,11,5,10,12,15	1	1	Allentown/France	1	
153[b]	2,10,3,28,9,14,3	1	12	---		
[c,b]	6,10,21,28,4,14,0	1	10	---		
[c,b]	2,**24,20,29,34,23**,0	1	[d]	---		
[c,b]	3,**24**,1,**29,34,23**,0	1	[d]	---		

SBT: sequence-based typing; ST: sequence type.
a Sequence of genes flaA, pilE, asd, mip, mompS, proA, neuA.
b New profiles and allele numbers are in bold.
c Problems in amplifying the neuA gene.
d Strain reactive with "Legionella pneumophila serogroups 2-14 Latex Test Reagent" (Oxoid). Serogroup could not be determined using MAbs of Dresden panel.

(32/67). This is a new profile and all of the ST100 strains had been isolated in patients of the same hospital over a period of several years. Twenty-four of the 32 strains with this profile came from nosocomial infections and the remaining eight from undetermined origin. These eight patients had subjacent diseases and needed hospital care frequently, suggesting that some or even all of these sporadic cases could be hospital-acquired, too. The STs 1, 20, 23, 42, 44, 62, 99, 101 and 103 were found in more than one strain. The 22 strains belonging to these nine STs were unrelated according to their source origin. In this study, ST1 (1,4,3,1,1,1,1), the most frequent profile reported in the world, was found only in three isolates (see Table).

The 19 strains sent by the surveillance scheme during the past four years, showed high profile diversity. Eleven distinct STs were obtained, five of them for the first time in Portugal. These strains were isolated in 11 different hospitals, five, four and two, respectively, from the north, the centre and the south of Portugal. The majority of the isolates came from community-acquired infections (12/19).

Using the Dresden panel of MAbs, the 63 *L. pneumophila* sg1 strains had previously been divided into five different subgroups (unpublished data). All strains but one possessed the virulence-associated epitope recognised by MAb 3/1 [11], and the Philadelphia subgroup was the most frequent with 28 of the 63 strains (see Table). As a result of the combination of the two methodologies, MAbs and SBT, these strains were now differentiated into 26 different profiles. The results showed that the Philadelphia subgroup was the most heterogeneous as it was divided into 12 different STs. On the other hand, identical STs were found among strains reactive with different MAbs (see Table). These two facts support the idea that it is valuable to add genotyping methods to MAb typing when defining profiles within a phenotypic subgroup [7,9].

Discussion

As far as our experience is concerned, the SBT scheme is technically simple for a laboratory with basic molecular expertise and equipment, provided that there is access to a sequencing laboratory. Although this method proved to be a good genotypic method for epidemiological investigations, showing unambiguous results that are easy to interpret [4,6-8], one of the limitations of the epidemiological studies is the fact that most diagnoses are made by urinary antigen test, without strain isolation. The EWGLI 2008 database showed that culture was the methodology used in only 62 of the 866 reported cases in the 35 countries participating in EWGLINET [15]. In Portugal, the data were similar: in the past four years, the strain was isolated for only 19 of 237 *Legionella* notifications (unpublished data). Thus, *Legionella* isolates are not available for the majority of cases and therefore the results of this study may not entirely reflect the distribution of the *Legionella* strains responsible for the disease in Portugal. However, our collection contains the majority of the clinical isolates collected in Portugal since 1987; so it is possible that this sampling is representative of the profiles circulating in Portugal.

The significant profile diversity we observed is in accordance with reports from the other countries [4,14,16,17]. Due to the relatively low number of isolates in each ST, with the majority (13/23) of the STs being detected only once, it is not possible to establish a correlation between the ST and the infection origin.

To summarise, this study enabled us, for the first time in Portugal, to characterise the *L. pneumophila* clinical isolates with SBT methodology and MAbs, as well as to create a database of Portuguese *L. pneumophila* profiles for use in epidemiological surveillance efforts. It was also a contribution to the EWGLI-SBT database and to the knowledge of the European *L. pneumophila* diversity, owing to the high rate of new STs obtained.

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Validity of routine surveillance data: a case study on Swedish notifications of methicillin-resistant Staphylococcus aureus

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Surveillance of communicable diseases is a public health corner stone. Routine notification data on communicable diseases are used as a basis for public health action as well as for policy making. While there are agreed standards for evaluating the performance of surveillance systems, it is rarely possible to analyse the validity of the data entered into these systems. In this study we compared data on all Swedish cases of methicillin-resistant *Staphylococcus* aureus (MRSA) routinely notified between 2000 and 2003 with follow-up information collected for each of these cases as part of a public health project. The variables *Reason for testing* (clinical sample, contact tracing, screening of risk group), *Clinical presentation* (disease, colonisation), *Transmission setting* (healthcare-acquired, community-acquired), *Country of acquisition* (Sweden, abroad) and *Risk-occupation* (yes, no) were analysed for sensitivity, positive predictive value and completeness of answers. The sensitivity varied between 23% and 83%, the positive predictive values were generally higher (55% to 97%), while missing answers varied from 11% to 59%. The proportion of community-acquired cases was markedly higher when excluding either cases of MRSA colonisation or cases found through public health-initiated activities (contact tracing or screening of risk groups). We conclude that the quality of routine surveillance data may be inadequate for in-depth epidemiological analyses. This should be taken into account when interpreting routine surveillance figures. Whether or not the case definition includes cases of MRSA colonisation may have a significant impact on population-wide estimates of MRSA occurrence.

Background

The overall aim of disease surveillance is to collect information for public health action. Disease control measures are costly both from a public health and from a healthcare perspective. For the healthcare system, diseases that spread nosocomially are particularly expensive. Disease control actions become more efficiently focused when based on valid surveillance data. However, it is rarely possible to assess the validity of notification data [1,2], and to our knowledge such an assessment has never been reported for any methicillin-resistant *Staphylococcus aureus* (MRSA) surveillance system. The epidemiology of diseases, such as MRSA, that can be transmitted both by symptomless carriers and by individuals with clinical infection is complex and their analysis requires a level of detail that can rarely be obtained from routine surveillance data. In contrast to most other countries [3-7], a comparatively lower occurrence of MRSA has hitherto been reported from the Netherlands and the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) [8]. During the late 1990s, Sweden experienced a large regional outbreak of healthcare-associated MRSA cases, which was brought under control by resolute efforts [8,12,13]. The experience from this outbreak forms the basis for the active strategy against MRSA currently employed in Sweden, with extensive screening of risk groups and contact tracing around known cases (symptomatic cases as well as asymptomatic carriers), aiming at preventing further transmission of MRSA.

The low-endemic MRSA-situation in Sweden and the allocation of resources in the period from 2000 to 2003 to map the epidemiology of MRSA in Sweden in detail, made it possible to collect in-depth data on every case of MRSA notified in the country during that period. This was done in addition to the collection of routine surveillance data. The resulting detailed and unique dataset made it possible to fulfil the two aims of the present study, i.e. to analyse the quality of the data supplied within the routine surveillance system and to show how case finding activities and inclusion or exclusion of MRSA carriers in the case definition influenced the estimated occurrence of MRSA in the population.

Materials and methods

Material

In Sweden, cases of clinical MRSA infection as well as asymptomatic carriage are notifiable by law to the Swedish Institute for Infectious Disease Control (SMI). The notifications are made

in parallel by the clinicians who diagnosed the patients and the laboratories that identified the pathogens. All MRSA notifications referring to the same individual are merged into one case record at the SMI, using a unique personal identification number. Thus, only new cases of MRSA are counted in the notification system. In this study, we included all cases notified in the years from 2000 to 2003. MRSA isolates from these cases were also sent to the SMI, where the bacteriological diagnosis was confirmed using PCR for the nuc and the mecA genes and epidemiological typing was performed using pulsed-field gel electrophoresis (PFGE).

A prospective, active follow-up on the epidemiological investigation of each notified case was performed in addition to the routine passive surveillance. Once the epidemiological investigation of a case was completed, updated data were collected by MRSA contact persons in each of the 21 counties in Sweden, and entered into a national MRSA study database [8]. These contact persons were infection control and public health officers involved in the local public health work on MRSA, and as such had full access to all information on the MRSA cases.

Definitions for case data evaluation

We analysed a subset of the variables used in the notification forms. The variable *Reason for testing* defined the reason for taking the first bacteriological sample from which MRSA was isolated from a case, categorised as: a) clinical sample (sample taken for diagnostic purposes), b) contact tracing (sample taken from a contact of a diagnosed MRSA case in order to identify a transmission chain), or c) screening of risk groups (sample taken from a patient with an increased risk of having MRSA, e.g. with healthcare contacts abroad or clinical risk factors such as breakages of the skin barrier or urinary catheter). *Clinical presentation* was defined as a) disease or b) colonisation. *Transmission setting* was defined as a) healthcare-acquired (HA), b) community-acquired (CA) or c) unknown. To be considered as HA (including municipal care institutions such as nursing homes), a case would need to have been in contact with a healthcare setting where other MRSA cases with the same PFGE pattern had occurred. If MRSA cases had been in close contact with each other outside any healthcare setting (e.g. family members, child daycare, girl- or boyfriend, work colleagues, sport contacts) and the PFGE patterns did not contradict transmission, or if, in the absence of an epidemiological link, the PFGE pattern was known to occur in the community, the case was considered to be CA. When neither HA nor CA could be ruled out, the transmission setting was considered as unknown. For the purpose of this study, detailed information on *Country of acquisition* was broadly grouped as a) abroad (acquired outside Sweden), b) domestic (acquired in Sweden) or c) unknown. A notified case was considered as acquired abroad if the patient had been abroad within six months preceding diagnosis and had either an MRSA strain known to have occurred in that part of the world or a strain previously unknown in Sweden and a likely Swedish source could not be found. When neither domestic acquisition nor acquisition abroad could be ruled out, country of acquisition was entered as unknown. Work in healthcare institutions, municipal care facilities and day nurseries was considered a *Risk occupation* for acquisition of MRSA (answer categories a) yes or b) no).

Data analysis

We compared the information on the routine clinical notification form of each case, with the data in the study database. In case of several clinical notifications on the same case, the first one was used for the analysis. We calculated sensitivity (the percentage of information per variable in the validated study database that was supplied correctly on the clinical notification form) and positive predictive value (PPV, the percentage of information in the first

Table 1

Data from the notifications of MRSA cases in Sweden, 2000-2003 (n=1,733)

Variable	Variable category	Number of cases according to study database	Number of cases according to notifications	Percentage of cases where notification data were in accordance with study database (sensitivity)	Percentage of cases where notification data were contradictory to study database	Percentage of cases where notification data were missing	Positive predictive value of notification data
Risk occupation	Yes	140	198	83% (76–89)	–	17% (11–24)	59% (51–66)
Country of acquisition	Domestic	1,265	911	69% (66–72)	7% (6–9)	24% (21–26)	96% (94–97)
	Abroad	444	376	76% (72–80)	12% (9–16)	11% (8–15)	90% (87–93)
Clinical presentation	Disease	798	653	65% (62–68)	19% (16–22)	16% (13–19)	80% (76–83)
	Colonisation	915	757	66% (63–69)	14% (12–16)	20% (18–23)	79% (76–82)
Transmission setting	Community-acquired	561	355	41% (37–45)	40% (36–44)	19% (16–22)	65% (60–70)
	Healthcare-acquired	903	563	51% (48–54)	34% (31–37)	15% (13–18)	82% (79–85)
Reason for testing	Clinical sample	203	83	23% (17–29)	18% (13–24)	59% (52–66)	55% (44–66)
	Contact tracing	437	184	41% (36–46)	24% (20–29)	35% (30–39)	97% (94–99)
	Screening of risk groups	268	136	37% (25–37)	32% (27–38)	31% (25–37)	73% (65–80)

MRSA: methicillin-resistant *Staphylococcus aureus*.
All percentages are presented with 95% confidence intervals in parentheses.
Clinical notifications were missing for 176 cases. For the variable *Reason for testing* the analysis was restricted to the 915 cases of MRSA colonisation, since this information was required in the notification form only for those cases.

notification that was in accordance with the information in the study database), with exact 95% confidence intervals. We also analysed the completeness of information on the first clinical notification form. The statistical analyses were performed in Stata version 8.2.

Results

A total of 1,733 MRSA cases were reported during the study period. Table 1 provides detailed information on each of the variables in the first clinical notifications compared to the data in the study database. It shows the sensitivity, the completeness, and the predictive capacity of the information that public health officers received in the first clinical notification, i.e. of the information available for the initiation of public health actions.

Sensitivity of data in original notification

Of 140 cases with *Risk occupations* according to the study database, 83% were correctly identified as such in the clinical notification (Table 1). Sensitivity was also high for the variable *Country of acquisition*, with 76% of patients with acquisition abroad and 69% of patients with acquisition in Sweden correctly identified in the notification. The sensitivity was low for the variable *Reason for testing*, mainly due to missing information in the original notification forms (see below).

Missing information in original notifications

Missing information for a variable (Table 1) was either due to missing information for that question or due to the fact that the clinical notification form was missing altogether. The most complete variable category was *Country of acquisition* 'abroad': this information was lacking in only 11% of cases that had acquired MRSA abroad. Other categories for which the information given in the first notification to a large extent was present were: *Transmission setting* 'healthcare' (15% missing information), *Clinical presentation* 'disease' (16% missing information) and *Risk occupation* 'yes' (17% missing information). The most incomplete information was found for the variable *Reason for testing*.

Positive predictive value of the information in the original notification

The proportion of accurate information in the original notification (PPV) was highest for the variable *Country of acquisition*, with a PPV of 96% for domestic acquisition and of 90% for acquisition abroad (Table 1). Least predictive was the information on 'clinical sample' as *Reason for testing* with only 55% of the cases being verified. The *Transmission setting* 'community-acquired'also had a low PPV (65%).

Effect of case definition and method of case finding on estimated MRSA occurrence

In order to assess the impact of different case definitions on the distribution of reported MRSA cases, we analysed the variable *Transmission setting* within the variables *Clinical presentation* and *Reason for testing* according to the study database (Table 2). Overall, 32% of cases were CA and 52% HA. If only cases with MRSA-caused disease (and not carriage) had been reported, the proportion of CA and HA cases would have been 41% and 39%, respectively. A similar effect on the distribution of cases was seen when considering only cases diagnosed by cultures that had been taken on clinical indication: the proportion of HA cases decreased significantly (43%) and the proportion of CA cases increased (35%).

Of the 1,733 cases in the study, 45 were identified through the isolation of MRSA from blood cultures. Nine of these cases were CA (20%; 95% CI 9.6-35) and 25 were HA (56%; 95% CI 40-70). The proportion of CA cases among these was thus significantly lower than among all clinical MRSA cases (Table 2).

Discussion and conclusion

Far-reaching decisions on public health interventions and policy, as well as research studies, are based on routine surveillance data. Surveillance data are also used to compare the disease occurrence over time and between populations, e.g. when making international comparisons between countries. When using surveillance data for such purposes it is essential that the case definitions and measured variables are valid and comparable. The project with the national Swedish MRSA-database 2000-2003 provided us with a unique opportunity to analyse the validity of routine surveillance case-data in Sweden. There are accepted guidelines for the general evaluation of public health surveillance systems [14], but such guidelines do not cover the evaluation of the actual data entered into the system and their validity – presumably because high quality reference datasets rarely exist to compare routine surveillance data against. The validity of notification data has been investigated for other diagnoses such as tuberculosis and human immunodeficiency virus infections/acquired immunodeficiency syndrome (HIV/AIDS) [1,2], but we are not aware of any report on MRSA surveillance and data validity. The general sensitivity of the Swedish statutory surveillance system to detect patients diagnosed with a notifiable disease has recently been analysed and was found to be very high - well above 90% [15].

Table 2

MRSA cases notified in Sweden between 2000 and 2003, according to the validated case information, comparing the proportion of community- and healthcare-acquired cases within the variable categories of *Clinical presentation* and *Reason for testing* (n=1,733)

Variable	Variable category	Community aquired	Healthcare acquired
		Percentage of cases (95% CI)* Number of cases	
Clinical presentation	Disease (n=798)	41% (37–44) 326	39% (35–42) 308
	Colonization (n=915)	25% (23–28) 233	64% (60–67) 582
Reason for testing	Clinical sample	35% (32–38) 332	43% (40–46) 404
	Contact tracing (n=472)	43% (38–48) 203	51% (47–56) 243
	Screening of risk groups (n=302)	8% (5–12) 25	81% (76–85) 244
Total (n=1,733)		32% (30–35) 561	52% (50–54) 903

MRSA: methicillin-resistant Staphylococcus aureus
The total number of cases is given for each variable category. Where cell numbers do not add up to the total of rows or columns, the difference is due to cases that did not fall under any of the categories.
*Percentages per variable category with exact confidence intervals

Pathogens like MRSA, which are able to colonise individuals as well as cause clinical disease, are particularly challenging for a surveillance system. Patients with clinical disease are more likely to seek healthcare and consequently more likely to be diagnosed and notified. The probability that a colonised individual is diagnosed and notified depends on the vigour with which case finding activities (contact tracing and screening of risk groups) are carried out. The incidence figures presented for different populations would therefore not be comparable if the proportions of colonised individuals identified through case finding activities differed, unless information on clinical presentation and/or reason for testing is specified. It has earlier been noted that differences in reported MRSA incidences can be a result of differences in case finding methods in neighbouring health-districts in England [16] as well as between hospital and community populations in an area of Manhattan, New York [17]. Studies of MRSA occurrence often include MRSA carriers [4,8,9,16-18]. To make a comparison valid, investigators need to characterise the cases for the closely interrelated variables *Reason for testing* and *Clinical presentation* (disease or colonisation), but this information is often not presented [8,11,18]. Simor *et al.* suspected an association between screening and colonisation among older MRSA patients in the Canadian Nosocomial Surveillance Program (CNISP) [19], but our study is to our own knowledge the first one to systematically address the effect of case finding on the incidence estimates of MRSA within a complete population on a national level.

The problem presented by an unknown proportion of carriers can be avoided by restricting the case definition to clinical infections only, or even to blood isolates only. In our study, less than two thirds of cases with MRSA colonisation and of cases with MRSA disease were shown to be correctly classified with regards to Clinical presentation. These findings indicate that the MRSA incidence would have been severely biased, if only MRSA disease had been notifiable. If only blood isolates were reported, such misclassification would be less likely. The rationale behind such an approach is that cases found through blood isolates act as a marker for the overall burden of MRSA [20]. Restricting the case definition in this way might however result in a biased estimate of the MRSA occurrence in the general population, as several studies found an association of MRSA bacteraemia with healthcare exposure [21,22]. This is substantiated by our study, in which the proportion of CA cases was significantly lower among those identified by blood culture compared to all cases with MRSA disease. A further advantage of considering all available MRSA cases is the increased statistical power and precision that comes with a larger number of study subjects. In smaller populations, such as single hospitals, this approach may be advantageous even in a high-endemic country like the United Kingdom [23]. Moreover, both MRSA carriers and those infected with MRSA are possible sources for further transmission in the population. From a point of view of MRSA control, a surveillance system should therefore include carriers. Our view is that ideally, all cases of MRSA, colonisation or disease, should be accounted for (provided there is a systematic case finding for colonised cases), along with data on the clinical presentation and/or the reason for testing, so that the analysis and interpretation of the figures can be adjusted accordingly. Public health-initiated case finding is carried out in situations where transmission is known to be high. Not monitoring cases from these settings, which generate a considerable number of new cases, is to neglect an important part of MRSA occurrence. How the surveillance of MRSA and other organisms that both colonise and cause disease is organised also depends on a number of other factors, such as the scope and level of the surveillance (e.g. hospital, district, regional or national), whether it is done in a high-endemic or low-endemic setting, and the available resources.

In conclusion, the present study clearly showed how differences in case definitions can influence the estimated number of MRSA cases categorised as healthcare-acquired or community-acquired, as well as the overall reported MRSA incidence. If carriers are included in the case definition, the overall occurrence and distribution of cases between the categories will also depend on the extent of the efforts to control MRSA through contact tracing and screening. We could identify considerable flaws in the quality of case data from routine notifications, e.g. misclassification of cases as colonisation or disease. Consequently, restricting the case definition to clinical cases only, would not be a reliable way to estimate the occurrence and distribution of MRSA. Surveillance systems and population-based epidemiologic studies thus need to specify the proportion of carriers and the reason for testing. This will also increase comparability of figures between countries or regions and between different points in time. Data validity cannot be taken for granted in a surveillance system, but needs to be ensured. For data that ultimately rely on information about transmission chains and results of epidemiological typing, the information should ideally be collected after the completion of the epidemiologic investigation of the cases.

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European Antibiotic Awareness Day, 2008 – the first Europe-wide public information campaign on prudent antibiotic use: methods and survey of activities in participating countries

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Antibiotic resistance is a major European and global public health problem and is, for a large part, driven by misuse of antibiotics. Hence, reducing unnecessary antibiotic use, particularly for the treatment of certain respiratory tract infections where they are not needed, is a public health priority. The success of national awareness campaigns to educate the public and primary care prescribers about appropriate antibiotic use in Belgium and France stimulated a European initiative coordinated by the European Centre for Disease Prevention and Control (ECDC), and named "European Antibiotic Awareness Day" (EAAD), to take place each year on 18 November. Specific campaign materials, including key messages, logos, slogans and a media toolkit, were developed and made available for use in European countries. The focus of the first EAAD campaign was about not taking antibiotics for viral infections such as colds and flu. A post-campaign survey was conducted in January 2009. Thirty-two European countries participated in the first EAAD, producing information materials and implementing activities to mark EAAD. Media coverage peaked on 18 and 19 November. At EU level, EAAD was launched at a scientific meeting in the European Parliament, Strasbourg. The event received EU political engagement through support from the EU Commissioner for Health, the Slovenian and French EU Presidencies, and Members of the European Parliament. Critical factors that led to the success of the first EAAD were good cooperation and process for building the campaign, strong political and stakeholder support and development of campaign materials based on scientific evidence. Countries indicated wide support for another EAAD in 2009. For this purpose, ECDC is developing several TV spots as well as a second set of EAAD campaign materials targeting primary care prescribers.

Introduction

Antibiotic resistance is a major European and global public health problem, and international efforts are necessary to counteract the selection and spread of resistance. There are substantial geographical differences in the proportions of resistance to various classes of antibiotics in Europe [1], the reasons being, on the one hand, differences in selection pressure from antibiotic usage and, on the other hand, differences in infection control practices [2-4].

The largest volume of antibiotics for systemic use are prescribed to outpatients in primary care, with respiratory tract infections (RTIs) being the most common indication. In some European countries, patients suffering from a respiratory tract infection are able to obtain antibiotics over the counter, without a prescription. Hence, reducing unnecessary antibiotic use, particularly for treatment of certain RTIs is a clear public health priority.

In November 2001, the European Union (EU) Health Ministers adopted a Council Recommendation on the prudent use of antimicrobial agents in human medicine [5] which stated that EU Member States should inform the general public of the importance of prudent use of antimicrobial agents by, in particular, raising awareness of the problem of antimicrobial resistance and encouraging realistic public expectations for the prescription of antimicrobial agents. As a result, for example, in Belgium and France, national awareness campaigns to educate the public and primary care prescribers about appropriate outpatient antibiotic use have successfully resulted in a decrease in antibiotic prescriptions [6-9]. Additionally, in both countries, the savings from reductions in antibiotic expenses for the national insurance system as a result of the public campaign largely outweighed the cost of the public campaign itself [6-7,10]. Importantly, these campaigns have included strategies to address behavioural aspects of the problem (e.g. taking antibiotics for viral illnesses), targeting both the public and primary care prescribers [11]. The success of these campaigns stimulated a European initiative coordinated by the European Centre for Disease Prevention and Control (ECDC), and named "European Antibiotic Awareness Day" (EAAD), to take place each year on 18 November.

ECDC endeavoured throughout 2008 to provide countries with a core set of tools (including visuals, key messages, a dedicated website and campaign materials) for use at country level. We present here the various steps in preparation for the first EAAD that took place on 18 November 2008, together with a post-

campaign survey regarding the materials used, and the types of activities carried out at national level, as well as suggestions for future improvement, based on a questionnaire distributed to all the participating countries in January 2009.

Materials and methods

At the beginning of 2008, ECDC set up a Technical Advisory Committee for the EAAD, including representatives from Belgium (chair), France, Greece, Poland, Spain, Sweden and the United Kingdom, as well as the Standing Committee of European Doctors (CPME), the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), European Commission's Directorate-General for Health and Consumers (DG SANCO) and Directorate-General for Research (DG RTD) and World Health Organization Regional Office for Europe (WHO/Europe). The Technical Advisory Committee's terms of reference are to discuss in detail the strategy for EAAD, including campaign objectives, target audience, key messages and evaluation methodology.

Preparation of EAAD was achieved through a collaboration amongst ECDC, the Technical Advisory Committee and the Network of National Antimicrobial Resistance (AMR) Focal Points, which is a network of country AMR experts designated by their national authorities to support ECDC in information exchange, coordination, and strategic and scientific inputs on AMR issues. In some cases, members of the Technical Advisory Committee representing Member States were also members of the National AMR Focal Points. ECDC therefore took care to regularly report the work of the Technical Advisory Committee to the National AMR Focal Points.

A good working partnership among all these institutions and Member State representatives was achieved through regular meetings, as well as exchange of information and ideas, in preparation of EAAD. ECDC hosted two meetings of the National AMR Focal Points (in September 2007 and March 2008), where draft campaign materials were proposed and discussed, and feedback was given. The second National AMR Focal Points meeting was held in cooperation with the Slovenian EU Presidency, and included a joint meeting with the Chief Medical Officers from all EU Member States. In addition, regular electronic updates were circulated to the group for comments. The Technical Advisory Committee also met twice at ECDC (in January 2008 and June 2008).

Gaining political support for the campaign was identified early on as an important success factor. Therefore, a lunch seminar for Members of the European Parliament was held in the European Parliament, Brussels, in October 2007, where the concept of an EAAD was publicly launched. In June 2008, ECDC Director Zsuzsanna Jakab also presented plans for EAAD to EU Health Ministers at the Employment, Social Policy, Health and Consumer Affairs Council (EPSCO) under the EU Presidency of Slovenia.

In the development of the campaign, ECDC and its partners decided to apply a social marketing approach. Social marketing is a process based on the application of marketing principles and techniques to create, communicate and deliver social values designed to influence target audience behaviours so that both society and the target audience benefit, according to the ideological framework used [12]. Taking such an approach when developing key messages, logos and slogans of a campaign can provide a greater chance to achieve sustainable behaviour changes amongst the target population. Through the gathering of consumer insights, a social marketer is able to formulate / offer messages in a way that promotes new behaviours that are more appealing and rewarding than old ones [13]. For the EAAD, such an approach was achieved through the identification of a desired behavioural change, the

Figure 1

Campaign themes with the European Antibiotic Awareness Day logo, hedgehog mascot visual and key messages

A) Logo

B) First visual and key message

C) Second visual and key message

setting up of focus groups to test the key campaign messages and visuals, and deciding on the section of the general public that would be most receptive to these messages as the main target audience. In addition, a post-campaign survey was conducted to gather feedback on EAAD via a questionnaire distributed to participating countries.

ECDC agreed with its partners to initially target the general public with messages about rational antibiotic use, in particular about not taking antibiotics for viral infections such as colds and flu. Other target audiences, mainly primary care prescribers, will be addressed in subsequent years. As the general public is a very broad target group, it was agreed to focus the campaign on parents and carers of children aged one to six years, as this age group has the highest rates of antibiotic consumption [7,9].

The EAAD campaign materials were developed by ECDC in close consultation with the National AMR Focal Points and the Technical Advisory Committee, as well as ECDC's Advisory Forum. The challenge of creating key messages, logos, visuals and slogans meeting the needs of 32 different countries, with many varying cultures and languages, was great. The solution was to develop a generic pill and stethoscope logo and a name that would be so uncontroversial as to be accepted by all countries. For the visuals and slogans designed to illustrate key messages on rational antibiotic use, a catalogue was developed from which countries could select visuals and slogans and adapt them at national level. The visuals included a number of hedgehog and scarf logos animating the slogans "cold, flu, get well without antibiotics" and "cold, flu, take care, not antibiotics" (Figure 1). The hedgehog was chosen as a mascot for the campaign, as it illustrates a character that is recognised as a vulnerable animal that tries to protect itself, but is nonetheless all too often the victim of human carelessness, (rather like the antibiotics).

Focus groups were set up to pre-test the key messages, logos, visuals and slogans with members of the general public representative of the main target audience in seven countries (Belgium, France, Greece, Poland, Spain, Sweden and UK). Each focus group consisted of three to four unrelated parents of children aged one to six years, and one to two unrelated day care professionals or other trained child care professionals. The feedback received from the focus groups was presented at the second National AMR Focal Points meeting in March 2008 and taken into account in the refinement of the campaign materials.

With the exception of the name of the day, which was provided translated into all 25 official EU languages, final campaign materials (key messages, logos, visuals, slogans and template materials for posters and brochures) were provided in English and translated in participating countries. These final campaign materials were disseminated to the countries in June 2008, and in September 2008, ECDC launched a campaign website aimed at the general public, with links provided to national campaign websites. A few weeks before EAAD, a complete media toolkit was made available to the National AMR Focal Points and the ECDC network of communication contact points in Member States for use by countries in the launch of national campaigns for 18 November. The media toolkit included a summary of the most recently available European data on antibiotic resistance from the European Antimicrobial Resistance Surveillance System (EARSS) [1] and on antibiotic consumption from the European Surveillance of Antimicrobial Consumption (ESAC) [2]. It also contained template press materials, such as a press release, presentation slides, photographs and audiovisual materials, as well as individual country antibiotic resistance and consumption data reports. Data on antibiotic consumption rates from ESAC and on antibiotic resistance rates from EARSS were analysed and compiled by ECDC experts into country reports showing the current situation in comparison to previous years. In addition, an EU report on the data was included in the media toolkit to illustrate the differences in rates of antibiotic consumption and antibiotic resistance across Europe.

A European workshop on public awareness campaigns on the prudent use of antibiotics was organised by the French EU Presidency on 6-7 November 2008 [14]. Finally, two special issues of Eurosurveillance [8, 15-24], published in November, were devoted to the issue of antibiotic resistance, including previous successful campaigns in some Member States.

In addition to the 27 EU Member States, two EEA/EFTA countries (Iceland and Norway), and three candidate countries (Croatia, the Former Yugoslav Republic of Macedonia and Turkey) participated in the campaign. The campaign also received support from ten partnering pan-European organisations: CPME, European Federation of Nurses (EFN), Pharmacist Group of EU (PGEU), European Patients' Federation (EPF), European Respiratory Society (ERS), European Older People's Platform (AGE), European Public Health Alliance (EPHA), European Association of Bio Industries (Europabio), European Federation of Pharmaceutical Industries and Associations (EFPIA) and European Generics Association (EGA).

An EU-level launch event, with the participation of the European Health Commissioner Androulla Vassiliou, the French EU Presidency and eight Members of the European Parliament, was held in the European Parliament, Strasbourg, while activities were coordinated at national level in the 32 countries.

With regard to monitoring the impact of EAAD, ECDC contracted a media monitoring company to track media articles published during the period from 14 November to 14 December 2008 that specifically mentioned "European Antibiotic Awareness Day". Furthermore, ECDC conducted a post-campaign survey to gather feedback on EAAD. ECDC distributed electronically in January 2009 a questionnaire (see Appendix) to the National AMR Focal Points in all 32 participating countries, aiming at identifying the countries' use of the campaign materials, the types of activities carried out at national level, and the lessons learned. The questionnaire included questions on national activities, government support, stakeholders, ECDC support and EAAD campaign materials, as well as a call for information on campaign evaluation that was planned or ongoing at national level. The National AMR Focal Points were asked to coordinate with other persons involved in the campaign at national level, and produce one completed questionnaire per country. We asked for all of the questionnaires to be returned to ECDC for evaluation within a two-week deadline that was met by all countries. Finally, a score measuring the uptake of the EAAD campaign in each country was calculated as the sum of national activities, campaign materials and use of EAAD materials; giving one point for each activity/material/use listed in the Table. Association of this score with having previously had a national campaign on prudent use of antibiotics was assessed with the independent-sample t-test for equality of means. Correlation with overall outpatient antibiotic

use (ATC J01) in Defined Daily Doses per 1,000 inhabitants and per day in 2006 [2] and with the percentage of penicillin-non susceptible Streptococcus pneumoniae from bloodstream and cerebrospinal fluid in 2007 [1] was assessed with the two-tailed Spearman's coefficient.

Results

National activities

Thirty-two European countries participated in the first EAAD; all of these countries provided responses to ECDC's questionnaire. All countries produced information materials (summarised in Figure 2) and implemented at least two activities to mark the EAAD, with the exception of Turkey which organised a press conference (Table and Figure 3). Twenty countries reported the publication of scientific/technical articles and 18 countries had implemented public awareness campaigns. Other activities reported by different countries included television (TV) and radio interviews (Croatia, Lithuania, Belgium), an exhibition and posters campaign (Poland), the launch of a national AMR campaign (Germany), the publication of guidelines on the appropriate use of antibiotics and the launch of dedicated websites (Belgium), competitions in schools (England), a prevalence survey on antibiotic prescriptions in paediatric primary care (Slovenia) and the launch of pilot information campaigns at regional level (Greece).

Media coverage varied across the countries, with half reporting one to ten media articles, while 11 countries reported 11 to 50 articles. A survey of media articles published in the period from 14 November to 14 December 2008 tracked 355 news articles that specifically mentioned "European Antibiotic Awareness Day". Coverage peaked on 18 and 19 November, with 113 and 88 media articles, respectively. According to the survey, the regional press generated the highest number of EAAD references (146 articles), accounting for 42% of the overall coverage. The Internet and the national press followed with 103 (29%) and 67 (19%) items, respectively, ahead of the trade press with 23 (6%) items. The highest number of articles tracked originated in Finland (45 articles), the United Kingdom (41 articles) and Poland (37 articles), while the Polish, Belgian and Finnish media recorded the highest potential audience reach (2.4, 1.6 and 1.2 million persons, respectively).

Government support

Most respondents indicated that their governments supported the EAAD campaign politically and financially. Thus, 27 (84%) countries reported having political support from their governments, mainly through the endorsement of the national campaigns, the organisation of press events and scientific meetings. Twenty (63%) countries reported that senior Ministry of Health officials (minister, deputy minister, chief medical officer) attended events organised at national level. In most countries, the Ministry of Health was identified as the main contributor and supporter of the campaign.

In terms of financial support, 22 (69%) countries reported that the government allocated funds to the organisation of the EAAD at national level. Financial contributions were varied in terms of direct funding, ranging from organising a press conference and production of materials, to providing support of more than €500,000 for a national awareness campaign.

From the countries' responses it emerged that all country teams invested significant effort and time in the EAAD campaign, based on the human resources and budget available in their countries. Some of the responses pointed out that the teams involved in EAAD were handling this campaign in addition to their regular work.

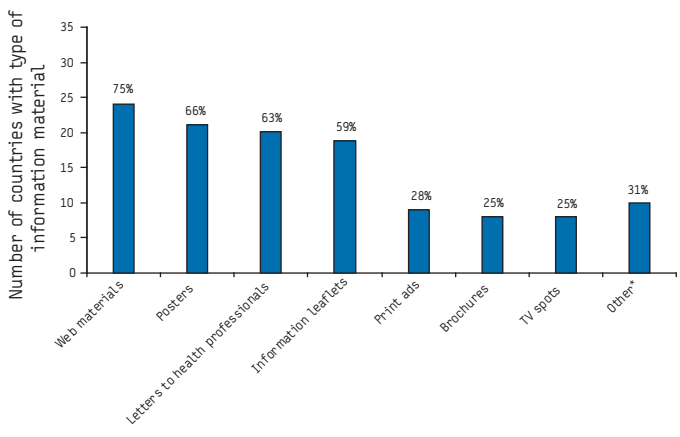
Twenty respondents reported that they had already secured political support for the organisation of the EAAD in 2009. However, only a few of the respondents have a clear picture of the funding that will be available to the organisation of the Day in their respective countries in 2009.

Non-governmental stakeholders

A significant number of national campaigns (72%) had support from health professionals' organisations. In 53% of the national

Figure 2

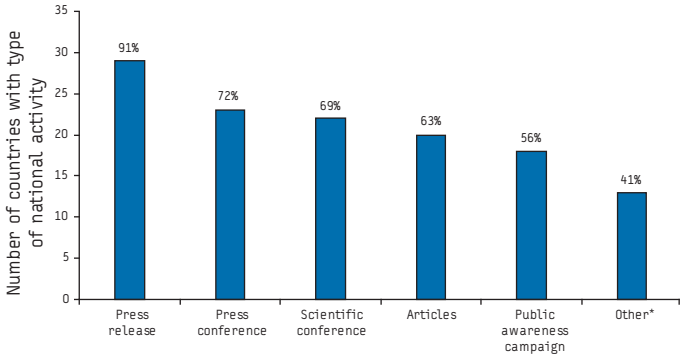
Information materials produced in participating countries for European Antibiotic Awareness Day in 2008



*Other: Treatment guidelines for primary care physicians (Greece), video spots in cinemas (Italy, Poland), radio spots (Luxembourg), emails to stakeholders (Spain), participation of antibiotic experts in TV game show (Bulgaria), DVD for professionals who care for children (France), and billboards outdoors (Ireland, Malta), on buses (Italy) and in the metro (Greece). Belgium reported that its awareness campaign included volunteer work to place swim armbands, the symbol of their campaign, on statues in some 30 cities across the country.

Figure 3

National activities on European Antibiotic Awareness Day in 2008)



*Other: TV and radio interviews, an exhibition and poster campaign, launch of a national antibiotic resistance campaign, publication of guidelines on appropriate use of antibiotics, launch of dedicated websites, an in-school competition and launch of a pilot information campaign at regional level.

campaigns, EAAD 2008 was supported by professional societies, and in 41% of the campaigns, pharmacies were identified as partners in the campaigns. Croatia and Cyprus reported financial support by pharmaceutical companies. None of the countries reported support from patient groups.

Table

Summary of national activities, type of campaign materials, governmental and stakeholder support and use of materials for European Antibiotic Awareness Day in 32 European countries

Country	National activities						Campaign materials								Govt. support		Stakeholder support								Use of EAAD materials					Received the media toolkit in time
	Awareness campaign	Scientific conference	Press conference	Press release	Articles	Other	Posters	Brochures	Leaflets	TV spot	Web materials	Print advertisements	Letters	Other	Political support	Financial support	Health professionals	Pharmacies	Patient groups	Non-governmental organisations	Non-pharmaceutical companies	Pharmaceutical companies	Professional societies	Insurance system	Other	Logo	Sitting Hedgehog	Kicking Hedgehog	Scarf	Film	
Austria		•	•	•	•	•	•		•		•				•	•	•	•		•	•	•	•	•		•	•			•	•
Belgium	•		•	•	•	•	•		•	•	•		•	•	•	•	•	•					•	•		•	•	•		•	•
Bulgaria		•	•	•							•		•		•		•	•		•	•		•			•	•	•			
Cyprus	•	•	•	•			•		•		•	•			•	•	•	•				•				•	•	•			•
Czech Republic		•	•	•	•		•		•		•	•	•		•								•	•	•		•				•
Denmark	•	•		•	•				•		•					•		•							•	•	•	•		•	
Estonia	•	•	•	•	•						•		•	•	•		•						•			•		•		•	•
Finland			•	•							•				•	•										•				•	•
France	•			•	•								•		•								•	•	•						
Germany		•		•	•	•					•			•	•	•									•	•					•
Greece	•		•	•	•	•	•	•	•	•				•	•	•							•			•				•	•
Hungary	•	•	•	•			•		•		•		•		•	•										•	•	•			
Ireland	•	•	•	•			•		•		•	•		•	•	•	•	•								•	•				•
Italy	•	•	•	•			•	•			•	•		•	•	•	•						•		•	•		•		•	•
Latvia		•	•	•	•						•		•				•			•			•			•			•	•	
Lithuania				•	•	•	•		•		•		•	•	•	•	•			•						•	•				
Luxembourg	•		•	•	•	•	•		•	•	•	•	•	•	•	•	•	•						•	•	•					•
Malta	•	•		•	•		•	•	•				•	•	•	•	•	•					•		•	•		•		•	•
Netherlands	•		•	•	•		•	•			•		•		•	•	•	•		•	•			•		•					
Poland	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•		•	•			•		•	•	•			
Portugal		•				•				•	•			•	•		•						•			•	•	•			•
Romania	•	•	•	•	•		•	•	•		•	•					•			•			•		•		•	•			•
Slovakia		•	•	•					•		•		•			•	•							•	•	•	•	•	•		•
Slovenia			•	•	•		•		•	•			•	•	•	•	•						•	•		•	•	•	•	•	•
Spain		•	•	•	•	•	•		•		•		•	•	•	•	•	•							•	•	•	•	•			•
Sweden		•	•	•	•	•	•	•					•		•		•						•		•	•	•	•			
United Kingdom	•	•			•	•	•		•		•	•	•		•	•	•	•					•			•		•		•	
Iceland	•			•							•				•											•					•
Norway	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•										•					•
Croatia	•	•	•	•		•	•		•				•			•	•	•				•	•			•	•	•		•	•
Former Yugoslav Republic of Macedonia		•	•	•			•	•							•	•	•			•						•		•			•
Turkey			•							•			•		•		•						•			•					

ECDC support

Thirty-one (97%) countries responded that they found ECDC's contribution helpful. Twenty-nine (91%) countries reported using the campaign logo. Furthermore, 18 (56%) countries used the "kicking hedgehog" visual and 17 (53%) used the "sitting hedgehog. Only four countries reported using the scarf. The visuals were used in a wide array of materials: posters (63%), web pages (53%), information leaflets (47%), letters (44%), advertisements (28%), brochures (19%) and TV spots (16%). Other ideas included a swimming armband (Belgium), drinks' coasters (England), presentation templates (Germany, Former Yugoslav Republic of Macedonia), an exhibition (Poland), billboards (Malta) and bookmarks distributed in schools (Cyprus). Twenty-one (66%) countries reported having received the media toolkit in time, and the use of materials was widespread among the different elements of the toolkit. The materials most used were the European and national data reports on antibiotic consumption and antibiotic resistance (50% and 38%, respectively), the template slides (34%), the press release (31%), the guidelines (22%), the photos and the template media invitation (19%). The audiovisual A-roll and B-roll (both narrated film and loosely edited film) were only used by three countries. Finally, 12 (38%) countries used the EAAD film.

The score measuring uptake of the first EAAD campaign in participating countries was not associated with either having previously had a national campaign on prudent use of antibiotics ($t=0.996$, $p>0.05$), or correlated with either overall outpatient antibiotic use ($r=0.164$, $p>0.05$) or the percentage of penicillin-non susceptible S. pneumoniae ($r=0.058$, $p>0.05$).

Suggestions for improvement

Many suggestions were received on ways to improve the EAAD website. Most countries (n=21, 66%) believe that more downloadable materials would be useful and multilingual versions of the website were requested by half of the respondents. A significant number (n=13, 40%) would also like to see more information on national campaigns available on the website. Many countries reported that evidence on the benefits of EAAD should be provided in order to secure support and funding of the future campaigns. Twenty-three (72%) countries stated that they would welcome a TV spot to illustrate the key messages of the campaign, e.g. "Cold? Flu? Take care, not antibiotics", developed by ECDC.

Discussion

The first EAAD was organised on 18 November 2008 in all 27 EU Member States, and five non-EU Member States. This event received EU political engagement through support from the EU Commissioner for Health, the Slovenian and French EU Presidencies, and Members of the European Parliament. The launch at EU level took place at a scientific meeting in the European Parliament, Strasbourg, gathering Members of the European Parliament, European Commission and Member State officials, representatives of professional organisations, leading European non-governmental organisations (NGOs) and media. Making use of the catalogue of materials developed for the campaign including key messages, visuals, logos, slogans, surveillance data, press and audiovisual materials, as well as a public website, the countries were able to develop a repertoire of approaches.

From the countries' responses to the survey questionnaire it is clear that all country teams invested significant effort and time into the EAAD campaign, based on the human resources and budget available at national level and the resources provided by ECDC. The fact that all 27 EU Member States, Norway and Iceland, as well as the three EU candidate countries planned and implemented activities for 18 November 2008 was a key indicator that the campaign was broadly well adopted. Clearly, the cost of the campaign varied significantly from country to country, with a large campaign including TV spots costing considerably more than a lower impact campaign with a single press conference and press release. Interestingly, however, some countries were able to activate partnerships to secure support in kind for their public service campaigns, including the development by an advertising agency of TV spots for free in one country.

We believe that a number of critical factors led to the EAAD's wide implementation in its first year:

• Good cooperation and processes for building the campaign:
- Planning well ahead – in this case, one and a half years – of the events
- Early establishment of a group of enthusiastic and committed experts representing countries and stakeholder groups in the Technical Advisory Committee;
- Working closely with a strong network of National AMR Focal Points meeting regularly to share information and best practice;
- Briefing of national communication contact points prior to the campaign and sharing contact information of the National AMR Focal Points with their communications counterparts.

• Strong political and stakeholder support:
- Strong political support and commitment at European and national level, secured at an early stage;
- Initiation of a broad stakeholder contact programme to inform interest groups and invite contributions;
- Good support from professional organisations

• Development of campaign materials based on a clear and rigorous approach:
- Drafting key messages based on scientific evidence from published studies to provide a basis for the development of all campaign materials;
- Building on existing success stories from a few countries;
- Allowing countries to choose from a catalogue of campaign materials and take ownership of local look and feel of the campaign;
- Pre-testing of campaign messages and visuals through focus groups.

Some aspects of a social marketing approach, which aims to achieve behavioural change considered to benefit society as a whole through the application of marketing principles and techniques, were difficult to develop at European level, given the great diversity in antibiotic consumption across Europe. In order that the campaign materials could be adapted and made appropriate for use at national level, it was agreed that the objectives of EAAD would be limited to the development of generic campaign materials, based on key messages rigorously backed up by data, that could be adapted for use by experts working at national level and delivered to the target audiences as part of national campaigns. This meant that at European level it was not possible to apply marketing principles and techniques, such as understanding the target market profile, the barriers to the desired behaviour in the target market and developing the marketing mix (product, price, place, promotion) in a way that would be fully consistent with a social marketing approach. Instead, the Technical Advisory Committee developed the

key messages and proposed campaign materials for EAAD, based on successes already achieved by existing national campaigns. For the future, it may be worthwhile to also take into account educational and/or psychological models upon which the campaign may be based.

A number of suggestions were received from the countries to improve the campaign in 2009. Of particular note, countries called for more campaign materials, more multi-lingual content in campaign materials, particularly the website, and earlier dissemination of template materials and toolkits. We also noted that whereas there was wide use of web-based materials, this was low for visual and audiovisual materials, such as high-resolution photographs and audiovisual A-roll and B-roll (only used in three countries) produced for the media toolkit to support selling in stories to TV news. For future campaigns, it will therefore be critical to develop and enrich the campaign website further, as well as develop more detailed guidance for using the visual and audiovisual campaign materials.

The lack of engagement of patient groups was identified as a missed opportunity. Although there are no groups dedicated to the problem of antibiotic resistance, it is a relevant issue for a number of disease-related (e.g. asthma, chronic obstructive pulmonary disease), as well as other health-focused NGOs. Therefore, engaging with patient group representatives at EU and national levels in order to disseminate EAAD messages and campaign materials should be addressed by future campaigns.

While organising public awareness activities in a multicultural and multilingual Europe will always remain a challenge, we believe that EAAD provides an example of how coordinated action may help to rapidly set up a European campaign. ECDC succeeded in creating a European scope and single identity for EAAD and provided support, while simultaneously allowing and enabling countries to adapt the materials to their own needs.

Reports have suggested an effect of public awareness activity on antibiotic use [6-9, 25], as well as an impact on antibiotic resistance [8,16]. However, these reports only used longitudinal surveillance data and lacked external controls. It is too early to determine if EAAD was successful in supporting behavioural change and a meaningful reduction in unnecessary antibiotic use, in particular for colds and flu, in the participating countries, and whether the campaign had an effect on antibiotic resistance. Evaluation of the EAAD campaign will require integration of longitudinal antibiotic consumption and resistance surveillance data, integrated with demographic and clinical data. Countries should be encouraged to plan prospective evaluation studies of the effect of their public awareness campaign. Several countries have already set up such evaluation studies, including the use of baseline data, which should allow assessment of the campaign's impact in these countries. Countries that did not participate in the EAAD or another campaign could be used as external controls.

Experience shows that public awareness campaigns must be repeated to achieve sustainability of behavioural change and coincide with quality assurance projects aimed at healthcare professionals. The post-EAAD survey indicated wide support from the countries for a 2009 campaign. Most countries agreed to focus on primary care prescribers and supported ECDC's intention to develop further materials and a TV spot for the campaign, and to provide materials and website pages translated into all EU languages.

Responding to requests for campaign materials to be available earlier, ECDC will break down communications toolkits into materials that can be delivered earlier in the year and those which are dependent on data sources not available until shortly before 18 November. Because most countries demand a TV spot developed by ECDC, and because evidence from Belgium and France underscores the importance of TV advertising, ECDC will develop a European TV spot. ECDC will also further develop the campaign website and provide multi-lingual content in all EU languages. In 2009, ECDC will develop a set of campaign materials targeting primary care prescribers, including general practitioners, to complement the 2008 campaign materials targeting the general public. ECDC will continue to promote rational use of antibiotics, in particular through key messages about appropriate use of antibiotics, such as this first EAAD's message not to use antibiotics for colds and flu.

Appendix. European Antibiotic Awareness Day (EAAD) 2008 Evaluation Questionnaire (available in pdf): http://www.eurosurveillance.org/public/public_pdf/EAAD_2008_questionnaire.pdf

European Antibiotic Awareness Day Technical Advisory Committee:

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European Antibiotic Awareness Day Collaborative Group:

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Changes in the epidemiology of hepatitis B virus infection following the implementation of immunisation programmes in northeastern Greece

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The objective of this study was to investigate changes in the epidemiology of hepatitis B virus infection in the general population and selected groups of immigrants in the region of northeastern Greece over the last decade in relation to the introduction of hepatitis B vaccination programmes. Two population-based seroprevalence surveys were carried out during the years 1992-1994 and 1998-2006. In total, 25,105 individuals were tested for the presence of hepatitis B virus markers: HBsAg, anti-HBs and anti-HBc. Childhood/adolescence immunisation programmes began early in 1994 in selected groups of immigrants and were complemented by the national vaccination programme in 1998. Between 1992-1994 and 1998-2006, the HBsAg carrier rate declined from 5.4% [95% CI: 4.5-5.9] in adults (20-60 years old) and 1.9% [95% CI: 1.6-2.4] in children/adolescents (5-19 years old) of indigenous residents to 3.4% [95% CI: 2.9-3.8] and 0.6% [95% CI: 0.2-1.4] respectively ($p<0.05$). In spite of a decrease compared with 1992-1994, the percentage of HBsAg carriers was still relatively high in 1998-2006 among the Muslim religious minority group (8.2% [95% CI: 8.0-8.7] in adults and 2% [95% CI: 1.7-2.4] in children/adolescents) and in immigrants from the former Soviet Union (4.3% [95% CI: 3.6-4.7] in adults and 1.1% [95% CI: 0.8-2.4] in children/adolescents) ($p<0.05$ for both selected groups versus general population). The decline of the prevalence of HBsAg in the general population and selected groups of immigrants in northeastern Greece over the last decade supports the effectiveness of the ongoing immunisation programme although the information on the actual number of cases of acute HBV infection is not available.

Introduction

Although hepatitis B virus (HBV) infection is a major public health problem throughout the world, the geographic variation in the epidemiology of this infection is considerable.

The prevalence of hepatitis B surface antigen (HBsAg) in the general population varies widely between European countries with high to intermediate HBsAg carrier rates in Turkey (8%), Bulgaria (4%), and Greece (2%) [1]. Furthermore, the prevalence of HBV infection in the Russian Federation and Ukraine is classified as intermediate with prevalence of HBsAg ranging from 2% to 7% [2].

In Greece, several recent studies investigated the prevalence of hepatitis B virus markers in certain risk groups, such as blood donors, healthcare workers, injecting drug users, alcoholics, pregnant women and small size populations [3-8]. In addition, a few prevalence studies in the general population are available which show a geographic variation of HBsAg seropositivity from 1.9% to 5% [9-11].

In 1994, a comprehensive hepatitis B immunisation programme was implemented only in the region of Thrace in northeastern Greece which covers three prefectures: Xanthi, Rodopi and Evros. The programme included vaccination of first generation immigrants from former Soviet Union and of the Muslim religious minority group of all ages. In 1998, a national vaccination programme for hepatitis B was started, including vaccination of pregnant women, infants (0-2 years old), children 5-6 years old and adults at high-risk of infection such as hospital workers, sexually active heterosexuals (more than one partner in the past six months), men who have sex with men, individuals diagnosed with a sexually transmitted infection (STI), illicit drug users (injecting, inhaling, snorting, pill popping), sex contacts or close household members of an infected person, children adopted from countries where hepatitis B is common (in Asia, Eastern Europe, and the Middle East), families of children adopted from the countries listed above, immigrants from countries where hepatitis B is common (listed above), individuals born to parents who have emigrated from countries where hepatitis B is common (listed above), recipients of a blood transfusion before 1992, renal dialysis patients and those in early renal failure [12]. Both vaccination programmes (regional and national) are free of charge.

The aim of this study was to evaluate the effectiveness of the HBV vaccination strategies in Thrace by analysing the data from two large population sero-surveys that were conducted in this region, one before and one after the vaccination programmes. Another objective was to identify variables that were independently associated with HBV infection, such as age and origin of residents.

Material and methods

Study population

Two community-based sero-surveys were conducted during the years 1992-1994 and 1998-2006 in Thrace. The estimated population of this region is 368,993 inhabitants [13]. A total of 25,105 individuals were investigated by physicians of the Unit of Preventive Medicine of Social Security Institute in Alexandroupolis: 14,483 during the first survey of 1992-1994 and 10,622 in the second survey of 1998-2006. People counted twice in the same serosurvey, foreigners or Greek visitors and adults older than 60 years were excluded from the sample population. The mobile survey unit visited almost all areas of the three prefectures of Thrace: Xanthi, Rodopi and Evros, including urban and rural areas, hard to reach mountains and plains. The adult population consisted of interested volunteers who were informed about the study by the local media. Young people 5-19 years old were included through screening organised stepwise by visiting all schools in the region and stratified according to geographic region (stratified random sampling).

The population sample was divided into the following groups: 1) immigrants born in countries of the former Soviet Union ('immigrant group'), 2) indigenous residents of Greek origin ('majority population group' or 'indigenous residents') and, 3) Muslim Thracians of Turkish origin ('Muslim religious minority group'). The above groups were further divided according to age into two groups: 5-19 and 20-60 years old. Children younger than 5 years of age were not included because the screening for children was organised in schools, while adults older than 60 years did not show interest to participate in the screening program.

Ethical approval

The study protocol was approved by the Research and Ethics Committee of the Unit of Preventive Medicine of the Social Security Institute of Greece and the Research Committee of the Ministry of Health and Welfare. The purpose and the protocol of the study were clearly explained. Informed consent was requested before a blood specimen was collected from each participant. For children and young participants <18 years old, informed consent was obtained from the parents or guardians. Only consenting volunteers, or children and young participants of consenting parents/guardians were included in the study.

The study was carried out in accordance with guidelines of the Declaration of Helsinki and was approved by the Hellenic Center for Infectious Diseases Control. All participants remained anonymous throughout the survey.

Serological testing and interpretation

All serum samples were stored at –200C until testing for HBV markers. The number of samples collected from the study population at first and second survey period is shown in Tables 1, 2 and 3. All blood samples in both studies were tested for the presence of hepatitis B surface antigen (HBsAg). Testing for antibodies to HBsAg (anti-HBs) and antibodies to hepatitis B core antigen (anti-HBc) was performed on all samples in the second serosurvey and on samples from the age group 5-19 only in the first serosurvey. Adults in the first serosurvey were tested for the HBsAg marker only. The tools used were enzyme immunoassay (EIA, Abott Diagnostics, Germany) during the first survey period and a fully automated microparticle enzyme immunoassay (Abbott AxSYM System version 3.0, Abbott Diagnostics, Germany) during the second period.

Individuals with anti-HBs antibodies alone [HBsAg(-)/anti-HBc(-)/anti-HBs(+)] were considered to have evidence of post vaccination immunity whereas those with positive anti-HBc and anti-HBs antibodies [HBsAg(-)/anti-HBc(+)/anti-HBs(+)] were considered to have evidence of past infection. In case of individuals who tested only anti-HBc-positive it was assumed that HBsAg had disappeared in long-term virus-carriers and the titer of anti-HBs was very low (undetectable) or HBsAg seroconversion to anti-HBs would occur later. Finally, individuals with HBsAg (+) only were considered to have evidence of acute HBV infection.

Statistical analysis

The sample was considered representative of the general population of Thrace between 5-60 years of age, as compared with age and other parameters. Statistical comparisons were performed using chi-squared (X2) test with SPSS software (SPSS Inc.). The level of significance was set at 5%.

The method of log-linear models, a type of stepwise logistic regression for discrete variables, was used to identify variables that were independently associated with HBV infection, such as age and origin of residents. The risk of infection for each variable was investigated by measuring both the unadjusted and the adjusted relative risk (RR).

Results

First epidemiological survey (1992-1994): Prevalence of HBV markers in selected groups compared to general population

The results of the first survey are shown in Tables 1, 3, and 4. Among the indigenous residents, 205 of the 3,789 adults (5.4%) [95% CI: 4.5-5.9] and 152 of the 7,864 children/adolescents (1.9%) [95% CI: 1.6-2.4] were HBsAg (+). Seroprotection rate varied with age from 1.7% (58/3408) [95% CI: 1.4-2.2] in adolescents to 10% (45/4456) [95% CI: 8.5-12.6] in children (Table 4). Interestingly, the prevalence of HBsAg among adolescents was higher than among children - 2.9% (101/3408) [95% CI: 1.9-2.8] compared with 0.92% (41/4456) [95% CI: 0.7-1.4] (Table 4).

Among the Muslim religious minority group, the prevalence of HBsAg was 9.9% (116/1165) [95% CI: 8.5-9.9] in adults and 5.1% [95% CI: 4.1-5.9] (33/643) in children/adolescents. In the group of immigrants from the former Soviet Union the rates were 5.3% (32/610) in adults [95% CI: 4.7-5.8] and 1.7% (7/412) in children/adolescents [95% CI: 1.1-2.4] (Table 1). The HBsAg prevalence was higher in adults and children/adolescents of

Table 1

Prevalence of hepatitis B surface antigen (HBsAg) before and after the introduction of vaccination programme: results of two seroprevalence studies in Thrace, northern Greece (period A: 1992-1994, period B: 1998-2006), according to ethnic origin and age (n=25,105)

HBsAg seroprevalence (%)				
Age group	5-19 years old		20-60 years old	
Period	A	B	A	B
Indigenous residents	1.9 (152/7,864)	0.6 (211/3,538)	5.4 (205/3,789)	3.4 (113/3,338)
Immigrants from the former Soviet Union	1.7 (7/412)	1.1 (4/363)	5.3 (32/610)	4.3 (20/463)
Muslim religious minority	5.1 (33/643)	2 (33/1,632)	9.9 (116/1,165)	8.2 (106/1,288)

Muslim religious minority group than in immigrants and indigenous residents (p<0.001) but the differences between the prevalence rates in immigrants and indigenous residents were not significant.

Seroprotection rate was low among children/adolescents of Muslim religious minority 7.7% (50/643) [95% CI: 6.0-8.4] and 4.6% (19/412) [95% CI: 4.1-5.4] among immigrants of the same age group (Table 3).

Second epidemiological survey (1998-2006): Prevalence of HBV markers in selected groups compared to general population

The findings of HBV markers of people screened in the second epidemiological survey are shown in Tables 1-4.

The prevalence of HBsAg was significantly higher in immigrants from the former Soviet Union both in adults and children/adolescents (p=0.03 in 5-19 and p=0.001 in 20-60 years age group) and in the Muslim religious minority group (p=0.0001 for adults and p=0.0002children/adolesdcents) compared to indigenous residents. In details, prevalence of HBsAg was 8.2 % [95% CI: 8.0-8.7] in adults and 2% [95% CI: 1.7-2.4] in children of Muslim religious minority group, 4.3% [95% CI: 3.6-4.7] and 1.1% [95% CI: 0.8-2.4] of immigrants from the former Soviet Union and, 3.4% [95% CI: 2.9-3.8] and 0.6% [95% CI: 0.2-1.4] of indigenous residents, respectively (Table 1). The prevalence of HBsAg was higher in adults and children/adolescents of Muslim religious minority group than those of indigenous residents and of immigrants from the former Soviet Union (p=0.0001 and p=0.0007 for adults and p=0.0002 and p=0.006 for children/adolescents, respectively).

The highest proportion of individuals with anti-HBs only positivity was found in the group of 5-19-year-old members of the Muslim religious minority group (73%) [95% CI: 69.5-78.3], followed by children of the indigenous residents (45%) [95% CI: 42.9-48.8] and of the immigrants from the former Soviet Union (38%) [95% CI: 34.9-41.2], (p=0.001) (Table 3). No significant differences were observed among immigrants and indigenous residents (p=0.26).

Risk factors for HBV infection

Multivariate analysis showed that older age and the origin of residents (immigrants from the former Soviet Union and residents from Muslim religious minority group) were independent risk factors for HBV infection (HBsAg positivity) (Table 5).

Effect of vaccination on pattern of HBV infection

In the indigenous residents the prevalence of HBsAg dropped significantly after the vaccination period, as shown from the two prevalence studies, from 5.4% (205/3789) [95% CI: 4.5-5.9] to 3.4% (113/3338) [95% CI: 2.9-3.8] in the adult group and from 1.9% (152/7864) [95% CI: 1.6-2.4] to 0.6% (211/3538) [95% CI: 0.2-1.4] in the 5-19 years age group (Table 1).

Table 2

Prevalence of hepatitis B virus (HBV) markers among adults aged 20-60 years, divided by ethnic origin, in the second seroprevalence study in Thrace, northern Greece, 1998-2006 (n=5,089)

	HBV markers							
Groups	HBsAg (+)		anti-HBc(+) only		anti-HBc (+) and anti-HBs (+)		anti-HBs (+)	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Immigrants from the former Soviet Union (n=463)	20	4.3	31	6.7	77	16.6	15	3.2
Muslim religious minority (n=1,288)	106	8.2	80	6.2	234	18.2	190	14.8
Indigenous residents (n=3,338)	113	3.4	200	6	551	16.5	213	6.4

Table 3

Prevalence of hepatitis B virus (HBV) markers in children and adolescents aged 5-19 years, divided by ethnic origin, in the first (period A: 1992-1994) and second (period B: 1998-2006) seroprevalence study in Thrace, northern Greece (n=14,452)

		HBV markers							
Study population		HBsAg (+)		anti-HBc+ only		anti-HBc (+) and anti-HBs (+)		anti-HBs (+)	
Groups	Period	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Immigrants from the former Soviet Union	A (n=412)	7	1.7	7	1.7	14	3.4	19	4.6
	B (n=363)	4	1.1	4	1.2	9	2.6	14	38
Muslim religious minority	A (n=643)	33	5.1	15	2.3	44	6.8	50	7.7
	B (n=1,632)	33	2.0	13	0.8	49	3	1,197	73.3
Indigenous residents	A (n=7,864)	152	1.9	102	1.3	197	2.5	103	1.3
	B (n=3,538)	21	0.6	30	0.85	68	1.9	1,581	44.7

Furthermore, despite the relatively low percentages of immunised immigrants of 5-19 years old the prevalence of HBsAg decreased from 1.7% (7/412) [95% CI: 1.1-2.4] to 1.1% (4/363) [95% CI: 0.8-2.4] with a concurrent increase in immunised children/adolescents (5-19 years old) from 4.6% (19/412) [95% CI: 4.1-5.4] up to 38% (14/363)[95% CI: 34.9-41.2] (Table 3).

Moreover, the percentage of immunised individuals in the 5-19 years old group of Muslim religious minority group has markedly increased from 7.7% (50/643) [95% CI: 6.4-8.4] to 73% (1197/1632) [95% CI: 69.5-78.3] while the prevalence of HBsAg decreased from 5.1% (33/643) [95% CI: 4.1-5.9] to 2% (33/1632) [95% CI: 1.7-2.4] (Table 3).

Discussion

This study describes the prevalence of HBV markers in the general population of northeastern Greece (Thrace) and in selected immigrant groups and investigates the impact of vaccination programmes – a regional one started in 1994 in northeastern Greece and a national one implemented in 1998. Two large population-based surveys involving in total 25,105 individuals were carried out, the first one in the period 1992-1994 preceding the

Table 4

Prevalence of hepatitis B virus (HBV) markers in different age groups of indigenous residents in the first (period A: 1992-1994) and second (period B: 1998-2006) seroprevalence study in Thrace, northern Greece (n=18,529)

			HBV markers							
Age groups (in years)	Period	(n)	HBsAg (+)		anti-HBc (+)		anti-HBc (+) and anti-HBs(+)		anti-HBs (+)	
			(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
5-12	A	4,456	41	0.92	43	0.9	48	1.1	45	10
	B	1,714	5	0.23	14	0.08	14	0.8	1,063	62
13-19	A	3,408	101	2.9	59	1.7	148	4.3	58	1.7
	B	1,824	16	0.9	16	0.8	54	3	518	28
20-30	A	930	17	1.8	*	*	*	*	*	*
	B	800	7	0.9	7	1.3	64	8	41	5.1
31-40	A	1,240	40	3.2	*	*	*	*	*	*
	B	1,010	15	1.4	60	5.9	158	15.6	77	7.6
41-50	A	912	85	9.3	*	*	*	*	*	*
	B	930	57	6.1	67	7.2	207	22.3	70	7.5
51-60	A	707	63	8.9	*	*	*	*	*	*
	B	598	34	5.5	66	11	122	20.4	25	4.1

*In the first seroprevalence study (period A: 1992-1994) the adult population was not tested for anti-HBc and anti-HBs

Table 5

Independent risk factors for HBsAg carrier state. Multivariate analysis of data from the second seroprevalence study in Thrace, northern Greece, 1998-2006

Risk factors	Sample	HBsAg(+)	RR [95% CI] Adjusted	*p*
Age groups				
5-19 years	3538	21 (0.6%)	1*	
20-30 years	800	7 (0.9%)	2.97 [0.19-13]	*>0.05*
31-40 years	1010	15 (1.4%)	5.19 [0.19-19.2]	*<0.01*
41-60 years	1528	91 (5.8%)	16.72 [2.34-57.3]	*<0.001*
Age group 20- 60 years by ethnic origin				
Indigenous residents	3338	113 (3.4%)	1*	
Muslim religious minority	1288	106 (8.2%)	10.82 [1.78-89.2]	*<0.0001*
Immigrants from the former Soviet Union	463	20 (4.3%)	1.98 [0.34-19.3]	*>0.05*
Age group 5-19 years by ethnic origin				
Indigenous residents	3538	21 (0.6%)	1*	
Muslim religious minority	1632	33 (2%)	23 [4.9-136.2]	*<0.0001*
Immigrants from the former Soviet Union	*363*	*4 (1.1%)*	*17.9 [2.1-98]*	*<0.001*

*Reference group

vaccination programmes and the second one in the period 1998-2006 following the implementation of vaccination programmes.

We have observed an impact of the immunisation programmes on the prevalence of HBsAg in all the study groups. Indeed, the HBsAg prevalence declined significantly in children and adolescents of all groups: indigenous residents (from 1.9% in 1992-1994 to 0.6% in 1998-2006), immigrants from the former Soviet Union (from 1.7% to 1.1%) and Muslim religious minority (from 5.1% to 2%). In the adult population, the HBsAg prevalence of indigenous residents also declined from 5.4% to 3.4%, of immigrants from 5.3% to 4.3% and of Muslim religious minority from 9.9% to 8.2%. However, this decline may also be related to factors other than vaccination, such as improvement of quality of life, use of disposable medical equipments and screening of blood donors and pregnant women.

The 3.4% prevalence of HBsAg in the adult indigenous residents (majority population group) obtained in the second survey was within the range reported by other studies (1.9%-5%) in Greece [9-11].

However, compared to other European countries, the burden of hepatitis B in northeastern Greece is higher. The prevalence of HBsAg in the general population varies widely between European countries with high to intermediate HBsAg carrier rates: in Turkey (8%), Romania (6%), Bulgaria (4%), Latvia (2%) and Greece (2%) [1]. In the Slovak Republic, Poland, Czech Republic, Belgium, Lithuania, Italy and Germany the HBsAg prevalence is 0.5%-1.5% and in the Netherlands, Estonia, Hungary, Slovenia and Norway below 0.5% [1,14]. Various studies in the past decade and recent years make comparisons difficult [1,2,14-19]. In France, the prevalence of anti-HBc and HBsAg in persons of French origin was 2.2% and 0.2% [16], in Belgium 6.9% and 0.7% [17], in Spain 10.2% and 0.9% [18], in Germany 8.71% and 0.62% [19], respectively. This might reflect differences in the epidemiology such as lower infection rates in newborns and infants corresponding to lower rates of chronicity.

The incidence of reported HBV cases in the European Union (EU) and European Economic Area / European Free Trade Association (EEA/EFTA) countries has declined over the past ten years from 6.7 cases per 100,000 population in 1995 to 1.5 cases per 100,000 population in 2006 [20]. However, although a notification system exists in Greece for acute and chronic HBV infection, there is significant underreporting because of the lack of compliance of doctors and unclear case definition and therefore changes in epidemiology of hepatitis B cannot rely on the reported incidence of acute hepatitis as in other countries.

In indigenous residents the HBsAg prevalence in age groups 5-19 and 20-60 years old (0.6% and 3.4% respectively) was somewhat higher than usually assumed (0.33-2.3%) in Greece [1]. However, a statistically higher prevalence of HBsAg was observed in age group 13-19 years (0.9%) compared with age group 5-12 years (0.23%), reflecting differences in the proportion of immunised children of these groups (28% and 62%, respectively). These findings support the fact that horizontal transmission from child to child and from mother to child has been eliminated due to vaccination and medical checks. Universal prenatal screening and infant immunisation will contribute to a further decline of HBV infection.

In many European countries immigrants from highly endemic regions are from 5 to 90 times more frequently affected by HBV than the general population [21-23]. Indeed, in our study, the prevalence of HBsAg among both the adult (4.3%) and the 5-19 years old (1.1%) groups of immigrants from the former Soviet Union was higher compared to the rates in the general population (3.4% and 0.6%, respectively). However, the prevalence of HBsAg among the immigrants of age group 5-19 (1.1%) was lower than that among children of the Muslim religious minority group (2%). Two Greek studies reported prevalence of HBsAg of 2.8% and 2.7% in groups of immigrants aged 12-18 years [22,24]. Higher rates of HBsAg positivity have been reported in other studies such as from the United States and Israel which reported prevalence of HBsAg of 4% in immigrants aged <20 years and 9% in 20-70 years age group [25,26]. Moreover, the children of first generation immigrants continue to have high prevalence of HBV infection as those of Muslim religious minority group. The overcrowded families may facilitate child to child transmission of HBV in the family.

We have also found a high rate of HBsAg prevalence (8.2%) in adults of Muslim religious minority. This high rate may be explained by higher risk of exposure such as poor adherence to standard control measures such as absence of screening pregnant women, childbirth at home, early weddings at the age of 12 years.

With respect to the strength of our study we should clarify that the sample adult population consisted of all interested adults whereas for the younger participants aged 5-19 years old samples stratified by age and geographic area were collected without selection bias from the participants at schools. We consider this study group to be representative of the general population of Thrace as compared with age and other parameters. Finally, this standardised methodology has been widely used and allows future comparative analyses to be performed [27].

Our study has several limitations. The results relied on serological data collected in the two surveys. The registration reporting system from the results recorded did not distinguish acute from chronic HBV infection for adults in the first survey since they were only screened for the marker HBsAg. Therefore, also some adult patients with a resolved or past infection could not be in the register. Incidence data and data regarding complications of chronic HBV infection are lacking. However other studies from this area provide such information [28,29].

In conclusion this study indicates the decline of the prevalence of HBsAg in the general population and selected groups of northeastern Greece over the last decade reflecting the effectiveness of HBV vaccination. Despite that, HBsAg prevalence remains high in certain communities such as immigrants and Muslim religious minority. Prevention programmes based on education and specific precautions for transmission along with vaccination are important.

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Genetic diversity of Streptococcus suis clinical isolates from pigs and humans in Italy (2003-2007)

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Streptococcus suis, a major porcine pathogen, is emerging as a zoonotic agent capable of causing severe invasive disease in humans exposed to pigs or pork products. *S. suis* infection is rare in industrialised countries and usually arises as sporadic cases, with meningitis the most common clinical presentation in humans. Recent reports of two cases of meningitis in Sardinia and north-eastern Italy prompted this first characterisation of Italian *S. suis* isolates. Fifty-nine *S. suis* strains, the two recent human strains and 57 swine clinical isolates collected between 2003 and 2007 from different Italian herds and regions, were tested for antimicrobial susceptibility, PCR-screened for virulence and antibiotic resistance genes, and subjected to molecular typing. Phenotypic and genotypic analysis demonstrated an overall high genetic diversity among isolates, the majority of which were resistant to macrolides (78%) and tetracyclines (90%). The *erm*(B), *tet*(O), mosaic *tet*(O/W/32/O), *tet*(W), and *tet*(M) genes were detected. The *tet*(O/W/32/O) gene, the most frequent *tet* gene after *tet*(O), had never been described in the genus *Streptococcus* before. In addition, a virulent *cps*2, *erm*(B) *tet*(O) clone, belonging to sequence type 1 (ST1) of the ST1 complex, was found to be prevalent and persistent in Italian swine herds. Finally, the two human isolates (both ST1) carrying *cps*2, *erm*(B) and *tet*(W) were seen to be closely related to each other.

Introduction

Streptococcus suis, a major porcine pathogen endemic in nearly all countries with a developed swine industry, causes meningitis, pneumonia, arthritis, endocarditis, and septicaemia in pigs [1]. *S. suis* is also emerging as a zoonotic agent capable of causing severe invasive disease in humans exposed to pigs or to pork products [2,3]. A carriage state has been documented in pigs, healthy carriers being a source of *S. suis* transmission in herds, mainly through the respiratory route [1]. As discussed in recent reports, the possibility cannot be excluded that humans may also be healthy carriers [1,3,4] and that *S. suis* may become an opportunistic pathogen under particular circumstances such as stress, immunodeficiency or cancer [1,5]. Meningitis with possible residual deafness is the most frequent clinical presentation of the infection in humans; septicaemia, pneumonia, endocarditis, arthritis and toxic shock syndrome have also been described. In industrialised countries, *S. suis* disease is rare, albeit probably underdiagnosed, and usually occurs as sporadic cases [2,3]. Most human cases reported so far originated from Southeast Asia, where the disease can be considered endemic and where some outbreaks have occurred [3]. Three major sequence type (ST) clonal complexes (ST1, ST27 and ST87) dominate the population [6]. The virulent ST1 complex, frequently associated with invasive infections, includes sequence type ST1, spread worldwide and recently detected for the first time in Italy [5], and ST7, responsible for several cases of toxic shock syndrome during a recent outbreak in China [7].

The antiphagocytic polysaccharide capsule (encoded by the cps gene) is the major virulence factor of *S. suis*. Thirty-three serotypes based on capsular antigens are currently recognised [8,9]. Serotype 2 is responsible for severe infections in swine [1] and is the most common serotype affecting humans worldwide [2]. The small number of human *S. suis* infections in North America has been linked to the low prevalence of serotype 2 among swine [1]. Serotypes 4, 14 and 16 have also been described in humans [1]. Proposed *S. suis* virulence factors [1], the significance of which is still unknown, include the muramidase released protein MRP (encoded by *mrp*), a peptidoglycan-associated protein probably acting as an adhesin and the extracellular protein factor EF (*epf*), both of which are suitable virulence markers of serotype 2 strains [10] and are also detected in other serotypes [11], a serum opacity factor OFS (*ofs*), proposed as a virulence trait of *cps*2 isolates [12,13], suilysin (*sly*), a haemolysin with a cytotoxic effect on various cell types [1], and arginine deiminase (*arc*A), a factor linked to survival in stress conditions [14]. Despite the lack of evidence for a critical role of one or more of these putative virulence factors in virulence, they may nonetheless serve as virulence markers, since MRP, EF, and suilysin are typical of Eurasian strains of the ST1 complex, while they are almost absent in less virulent North American strains [1]. An immune evasion strategy has recently been proposed to account for the allelic variability observed in *mrp*, *epf*, and *ofs* genes [11,13].

A trend toward mounting *S. suis* resistance to macrolides and tetracyclines has been reported worldwide [15-17]. Studies of genetic resistance traits have demonstrated *erm*(B) (ribosomal methylation) and *mef*(A) (active efflux) for macrolide resistance, and *tet*(M) and *tet*(O) (both ribosomal protection) for tetracycline

resistance [18-21]. The *tet*(W) gene, an emerging determinant commonly found in species inhabiting human and animal intestinal tracts [22], was first detected by our group in a human isolate of *S. suis* from a case of meningitis in Italy [5].

Overall, three human cases of *S. suis* meningitis have been reported in Italy, one in the 1990s [23] and two quite recently, in the course of little more than a year. The short interval between the last two cases and their arising in distant geographic areas, i.e. north-eastern Italy [24] and Sardinia [5], prompted this first characterisation of Italian *S. suis* isolates.

Methods

S. suis strains

A total of 59 *S. suis* isolates were studied, two of human and 57 of porcine origin (Table 1). The human isolates, one from Sardinia (SsCA-1: *cps*2 ST1 *erm*(B) *tet*(W)) [5] and the other from north-east Italy [24], here designated as SsUD, were from cerebrospinal fluid (CSF) of two patients with *S. suis* meningitis. All pig isolates were from clinical samples (23 brain, 22 lung and 12 spleen samples) collected in 24 herds in northern and central Italy from 2003 to 2007. They were divided into invasive (brain and spleen isolates: 35 strains) and non-invasive (lung isolates: 22 strains) according to the source of isolation. All strains were isolated on 5% sheep blood agar (Oxoid Ltd) and identified with ID 32 STREP kit (bioMérieux). Serotyping was performed by slide agglutination using specific antisera (Statens Serum Institute).

Susceptibility testing

Antimicrobial susceptibility testing by agar disk diffusion and minimal inhibitory concentration (MIC) was carried out according to standard procedures [25,26] (erythromycin and tetracycline antibiotics: Sigma Chemical Co, disks: Oxoid). *S. pneumoniae* ATCC 49619 was used for quality control. The erythromycin resistance phenotype was determined on the basis of the triple disk test (erythromycin plus clindamycin and josamycin) [27].

Genotyping

PCR amplification was carried out under published conditions using the oligonucleotide primer pairs and target genes listed in Table 2 [28-33].

Pulsed-Field Gel Electrophoresis (PFGE) was applied to study the genetic diversity of *S. suis* [19,34-36]. Macrorestriction with *Sma*I endonuclease (Roche) and PFGE analysis were performed essentially as described previously [35]. PFGE data were analysed considering each band as a separate putative locus and scoring it as present (1) or absent (0) in each accession. The dendrogram was constructed by use of the Dice coefficient and the unweighted pair group method with arithmetic averages. Genetic relatedness was interpreted according to the criteria of Tenover *et al.* [37].

A multilocus sequence typing (MLST) scheme for *S. suis* was developed in 2002 [6]. Primers for PCR amplification and sequencing of the housekeeping gene fragments of *aro*A (EPSP synthase), *cpn60* (60-kDa chaperonin), *dpr* (peroxide resistance), *gki* (glucose kinase), *mutS* (DNA mismatch repair enzyme), *recA* (homologous recombination) and *thrA* (aspartokinase) were

Table 1

***Streptococcus suis* isolates, Italy, 2003-2007 (n=59)**

Origin (no. of isolates)	Strain (herd*)	Area in Italy	Year
Pig (57)			
Brain (23)	v3 (PG/5), v20 (PG/1), v24 (PG/2)	Centre	2003
	v27 (PG/4), v28 (PG/2), v29 (MC/1), v31 (PG/1), v32 (PG/1), v34 (AR/1), v35 (AR/1), v40 (TR), v42 (PG/1), v36 (PG/1)	Centre	2004
	v54 (MC/2), v75 (PG/1), v76 (PG/1)	Centre	2005
	v96 (PG/1), v97 (PG/3)	Centre	2006
	170167 (RE), 188509 (RE), 219624 (RE), 202707 (RE)	North	2007
	v123 (PG/1)	Centre	2007
Spleen (12)	v73 (LT)	Centre	2005
	45445 (AP/1)	Centre	2006
	240370 (RE), 205206 (RE), 210671 (RE), 167757 (RE)	North	2007
	20801 (LI), 1303 (AP/1), 22583 (AP/1), 11683 (AP/1), 11707 (PG/7), 13469 (AP/1)	Centre	2007
Lung (22)	v21 (PU), v23 (IS), v25 (CH), v26 (PG/2)	Centre	2003
	3721 (AP/5), v38 (PG/8)	Centre	2004
	v92 (PG/3), (AP/1) 27894, (AP/1) 33421, (AP/3) 18237, 30676 (AP/1)	Centre	2006
	227794 (RE), 176414 (RE)	North	2007
	9649 (PG/6), 22919 (AP/2), 10432 (PG/6), 36774 (AP/1), 30203 (AP/4), 18315 (AN), 1227 (AP/4), 10584 (AP/1), 32457 (AP/1)	Centre	2007
Human (2)			
CSF (2)	SsUD	North	2006
	SsCA-1	Sardinia	2007

* AN: Ancona, AP: Ascoli Piceno (5 herds), AR: Arezzo, CH: Chieti, IS: Isernia, LI: Livorno, LT: Latina, MC: Macerata (2 herds), PG: Perugia (8 herds), PU: Pesaro/Urbino, RE: Reggio Emilia, TR: Terni.
CSF: cerebrospinal fluid.

synthesised according to the primer sequences on the *S. suis* MLST database website (http://ssuis.mlst.net). Sequences were compared with previously observed allelic sequences in the *S. suis* MLST database for identification of ST.

The nucleotide sequences reported here have been submitted to the GenBank/EMBL sequence database and assigned accession numbers FM201280 (*ofs* $^{\text{type 1S}}$), FN357200 (*epf* ^{915}), FN356743 (*tet*(W)) and FM164392 (*tet*(O/W/32/O)). Sequence similarity searches were carried out using BLAST, available online from the National Center for Biotechnology Information of the National Library of Medicine (http://www.ncbi.nlm.nih.gov).

Results

Capsular (cps) and virulence-associated genes

The 59 *S. suis* isolates were investigated by PCR using primer pairs specific for *cps*1, *cps*2, *cps*7, and *cps*9, and for virulence-associated genes *mrp*, *epf*, *ofs*, *sly*, and *arc*A. Size variants were detected by restriction analysis (*epf*: *Hin*dIII; *ofs*: *Mbo*I) and sequencing (*ofs*) of PCR products (Table 3). The distributions of *cps* and virulence-associated genes are reported in the Figure, and virulence profiles among invasive and non-invasive isolates are shown in Table 4.

Table 2

***Streptococcus suis* PCR primers and target genes**

Primers	Gene target	Primer sequence (5'-3')	Product length (bp)	Reference
Macrolide resistance genotype				
ERMB 1 ERMB 2	*erm*(B)	GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	639	[28]
III_{10} III_{8}	*erm*(TR)	AGGTTATAATGAAACAGA GCATGACATAAACCTTCA	208	[29]
MEFA 1 MEFA 2	*mef*(A)	AGTATCATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG	346	[28]
Tetracycline resistance genotype				
TETK-up TETK-rev	*tet*(K)	TATTTTGGCTTTGTATTCTTTCAT GCTATACCTGTTCCCTCTGATAA	1,159	[30]
TETL-up TETL-rev	*tet*(L)	ATAAATTGTTTCGGGTCGGTAAT AACCAGCCAACTAATGACAATGAT	1,077	[30]
TETM F TETM R	*tet*(M)	GAACTCGAACAAGAGGAAAGC ATGGAAGCCCAGAAAGGAT	740	[31]
TETO 1 TETO 2	*tet*(O)	AACTTAGGCATTCTGGCTCAC TCCCACTGTTCCATATCGTCA	519	[31]
TETOFF2 TETOFR3	*tet*(O)	TTGTTTTGGGGCTATTGGAG TATATGACTTTTGCAAGCTG	2,038	[32]
TETQ F TETQ R	*tet*(Q)	AGAATCTGCTGTTTGCCAGTG CGGAGTGTCAATGATATTGCA	167	[33]
TETS F TETS R	*tet*(S)	GAAAGCTTACTATACAGTAGC AGGAGTATCTACAATATTTAC	168	[33]
TETT F TETT R	*tet*(T)	AAGGTTTATTATATAAAAGTG AGGTGTATCTATGATATTTAC	167	[33]
TETWF F TETWF R	*tet*(W)	TTGGGGCTGTAAAGGGAGGAC CTTTACATTACCTTCTGA	1948	[32]
Virulence-associated factors				
CPS1F CPS1R	*cps*1J	TGGCTCTGTAGATGATTCTGCT TGATACGTCAAAATCCTCACCA	637	[11]
CPS2F CPS2R	*cps*2J	TTTGTCGGGAGGGTTACTTG TTTGGAAGCGATTCATCTCC	498	[11]
CPS7F CPS7R	*cps*7H	AATGCCCTCGTGGAATACAG TCCTGACACCAGGACACGTA	379	[11]
CPS9F CPS9R	*cps*9H	GGGATGATTGCTCGACAGAT CCGAAGTATCTGGGCTACTGA	303	[11]
MRP1 MRP2	*mrp*	ATTGCTCCACAAGAGGATGG TGAGCTTTACCTGAAGCGGT	188 [a]	[11]
EPF1 EPF2	*epf*	CGCAGACAACGAAAGATTGA AAGAATGTCTTTGGCGATGG	744[a]	[11]
OFS-F OFS-R2	*ofs*	GATGTGACTGTCCGCAGAGC AAAGTACCTGAGCTCCTACA	1,960 [b]	[13]
SLY1 SLY2	*sly*	GCTTGACTTACGAGCCACAA CCGCGCAATACTGATAAGC	248	[11]
ARC-A1 ARC-A2	*arc*A	TGATATGGTTGCTGCTGGTC GGACTCGAGGATAGCATTGG	118	[11]

[a] Reference strain D282; [b] Reference strain NIAH11433.

TABLE 3

The *mrp*, *epf*, and *ofs* gene size variants observed in *Streptococcus suis* isolates, Italy, 2003-2007

Target gene	Size variant	Amplicon size (bp)	References
mrp			
	mrp	1,148	[11]
	*mrp**	1,556	[11]
	mrp^{S}	747	[11]
epf			
	epf	744	[11]
	$epf^{\text{class I}}$	3,112	[40]
	epf^{915}	915	This study
ofs			
	$ofs^{\text{type 1}}$	1,960	[13]
	$ofs^{\text{type 1S}}$	1,636	This study
	$ofs^{\text{type 2}}$	2,113	[13]
	$ofs^{\text{type 3a}}$	1,627	[13]
	$ofs^{\text{type 3b}}$	1,786	[13]

Three *cps* genes were detected in 43 of the 59 isolates: *cps*1 (n=3 isolates, one invasive), *cps*2 (n=30, 23 invasive, including the two human CSF isolates) and *cps*9 (n=10, eight invasive). In agglutination tests, all *cps*2 strains showed agglutination with sera specific for serotype 2. The remaining 16 isolates, of which five were invasive, were negative and are referred to as non-typeable (NT).

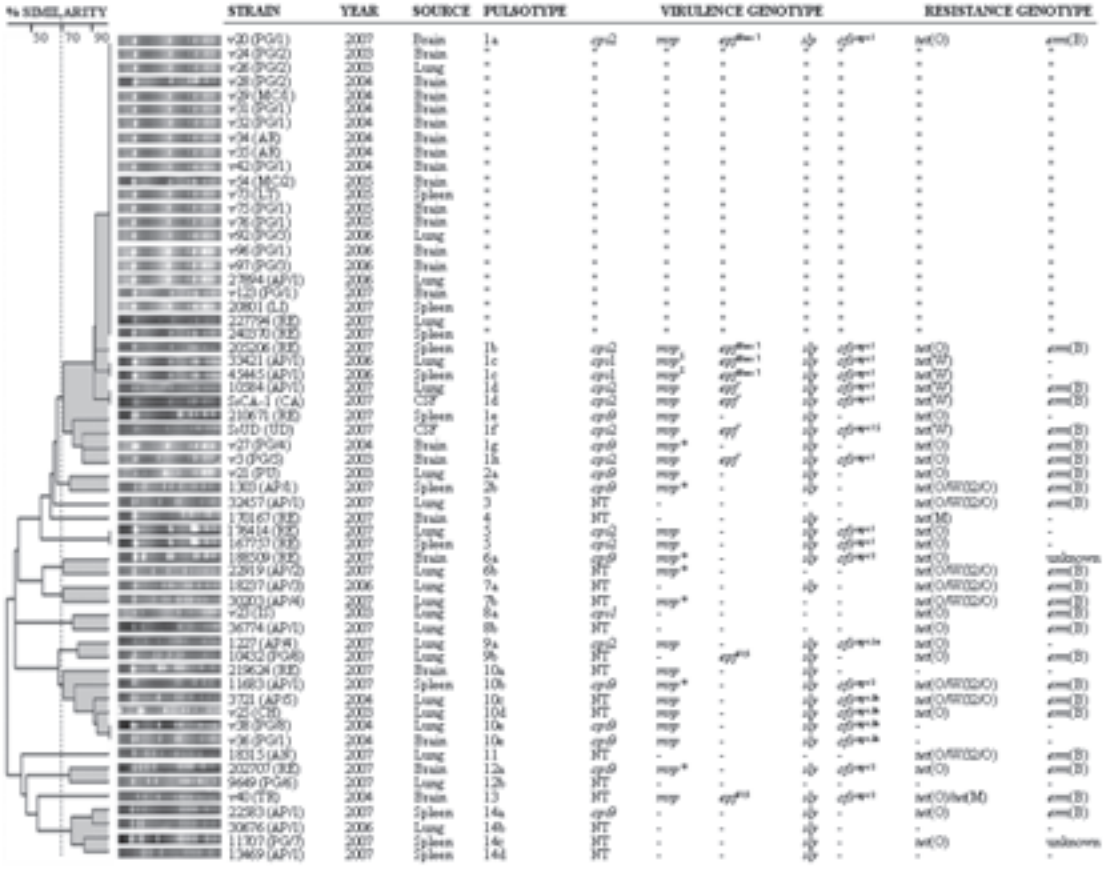
The *mrp* gene (three size variants: *mrp*; *mrp** and mrp^{S}) was detected in 47 strains (all 30 *cps*2 isolates, nine *cps*9, six NT, and two *cps*1 isolates); *epf* (three size variants: *epf*; $epf^{\text{class I}}$ and epf^{915}) was detected in 31 strains (27 *cps*2, two *cps*1 and two NT isolates); *ofs* (five size variants: $ofs^{\text{type 1}}$, $ofs^{\text{type 1S}}$, $ofs^{\text{type 2}}$, $ofs^{\text{type 3a}}$ and $ofs^{\text{type 3b}}$) was detected in 40 strains (all 30 *cps*2, five *cps*9, three NT and two *cps*1 isolates); sly was detected in 52 strains (all *cps*2 and *cps*9 isolates, two *cps*1 and 10 NT isolates), and *arc*A was found in all isolates.

Susceptibility testing and detection of resistance genes

The 59 strains were tested for susceptibility to tetracycline and erythromycin using phenotypic and genotypic methods. Fifty-three strains (90%) were resistant to tetracycline (MIC 8-64 mg/L) and

FIGURE

Similarity index of the 59 Streptococcus suis isolates, Italy, 2003-2007



For each isolate, the year and the source of isolation and the virulence and resistance genotypes are shown. Pulsed-field gel electrophoresis pulsotypes sharing >70% similarity were grouped into clusters (gray).
Unknown: neither *erm*(A) nor *erm*(B) nor *mef*(A).
ScCA-1 and SsUD are the two human isolates

46 (78%) were constitutively resistant to erythromycin (MIC >128 mg/L: n=44, including SsCA-1; MIC 4 mg/L: n=2, including SsUD). All erythromycin-resistant strains were also tetracycline-resistant. The *erm*(B) gene was the only erythromycin resistance determinant (Figure), found in 44 of 46 erythromycin-resistant strains. Neither *erm*(A) nor mef(A) were detected in the two erythromycin-resistant (MIC >128 mg/L) *erm*(B)-negative strains. Tetracycline resistance genes were distributed as follows: *tet*(O) (n=38), *tet*(O/W/32/O) (n=8), *tet*(W) (n=5); *tet*(M) (n=1), and *tet*(O)/*tet*(M) (n=1).

The presence of the mosaic gene was suspected from incongruent findings in PCR experiments, where a 519 bp amplicon was obtained in 38 strains using primers internal to *tet*(O) (TETO1 and TETO2), and a 2,038 bp amplicon was obtained in 46 strains (of which eight were negative when internal primers were used) using full-length *tet*(O) primers (TETOFF2 and TETOFR3). In the latter strains the presence of the mosaic gene *tet*(O/W/32/O) was confirmed by *Alu*I and *Hin*fI restriction analysis and sequencing of PCR products. Sequence analysis (FM164392) revealed that this gene was 99% identical to the tetracycline resistance gene *tet*(O/W/32/O) (EF065523.1) of an uncultured bacterium isolated from pig faeces [32]. The *tet*(W) gene was detected in three pig isolates and in both human isolates by *Hin*fI restriction analysis of the amplicons obtained with the tetWFF and tetWFR primer pair and sequencing. Sequence analysis (FN356743) disclosed that it was 99% identical to the tetracycline resistance gene *tet*(W) (DQ519395.1) of a porcine isolate of *Arcanobacterium pyogenes* [38].

TABLE 4

Virulence-associated gene profiles in *Streptococcus suis* isolates, Italy, 2003-2007 (n=59)

Profile	Invasive	Non-invasive
*cps*2 isolates (*n* = 30)	23	7
mrp epf$^{\text{class I}}$ *ofs* $^{\text{type 1}}$ *sly arcA*	19	4
mrp epf ofs $^{\text{type 1}}$ *sly arcA*	2*	1
mrp epf ofs $^{\text{type 1S}}$ *sly arcA*	1	-
mrp ofs $^{\text{type 1}}$ *sly arcA*	1	1
mrp ofs $^{\text{type 3a}}$ *arcA*	-	1
*cps*1 isolates (*n* = 3)	1	2
mrp^{S} *epf*$^{\text{class I}}$ *ofs* $^{\text{type I}}$ *sly arcA*	1	1
arcA	-	1
*cps*9 isolates (*n* = 10)	8	2
mrp ofs* $^{\text{type 2}}$ *sly arcA*	3	-
mrp ofs $^{\text{type 3b}}$ *sly arcA*	1	1
mrp sly arcA	1	1
mrp sly arcA*	2	-
sly arcA	1	-
[a]NT isolates (*n* = 16)	5	11
mrp epf^{915} *ofs* $^{\text{type 2}}$ *sly arcA*	1	-
mrp ofs $^{\text{type 3b}}$ *sly arcA*	-	2
epf^{915} *sly arcA*	-	1
mrp sly arcA	1	-
mrp arcA*	-	2
sly arcA	3	2
arcA	-	4

[a] NT: non-typeable (neither *cps*1, nor 2, 7 or 9).
* Human isolates

PFGE typing and MLST

All strains were PFGE-typed after *Sma*I digestion of total DNA. Thirty-four different pulsotypes were detected and grouped into 14 PFGE types (types 1 to 14) on the basis of a cut-off of 70% similarity (Figure). PFGE type 1 accounted for 52% of isolates and comprised eight pulsotypes (types a to h), of which pulsotype 1a was shared by 22 pig isolates collected from 10 different herds in northern and central Italy in the period from 2003 to 2007. Pulsotype 1d was shared by the human strain SsCA-1 (isolated in 2007) and the pig isolate 10584 (isolated in 2006), and pulsotype 1f was displayed by the human strain SsUD. Comparison of 1d with both pulsotypes 1a and 1f yielded a two-band difference, and comparison of 1a with 1f a three-band difference. MLST of strains v20 (chosen as representative of pulsotype 1a), SsCA-1 (1d), and SsUD (1f) identified the same allelic profile, corresponding to ST1.

Clones

The distribution of *cps* genes, virulence-associated genes, and tetracycline and erythromycin resistance determinants among the 59 *S. suis* strains subdivided by PFGE types and pulsotypes is detailed in the Figure. *S. suis* isolates with a unique combination of a given PFGE pulsotype, a given *cps* gene, a given virulence profile, and a given resistance genotype and phenotype were considered to represent a clone. According to this criterion, 34 different clones, corresponding to the 34 different pulsotypes, were recognised, 32 of which were found among the 57 pig isolates (Figure). A major *cps*2 swine clone (clone 1a: *mrp*, *epf*$^{\text{class I}}$; *ofstype 1*, *sly*, *arc*A; *tet*(O) *erm*(B)) accounted for 37% of the 59 isolates. Moreover, clones 1d (*mrp, epf; ofs*$^{\text{type 1}}$, *sly*, *arc*A; *tet*(W) *erm*(B)) and 1f (*mrp, epf; ofs*$^{\text{type 1S}}$, *sly*, *arc*A; *tet*(W) *erm*(B)), containing the two human isolates (SsCA-1 and SsUD, respectively), were seen to be closely related.

Discussion and conclusion

This is the first study of virulence and resistance traits in swine and human strains of *S. suis* in Italy. The cps genes coding for the capsular polysaccharide as well as *mrp, epf, ofs*, and *sly* genes were investigated. The most prevalent capsular gene was cps2, followed by *cps*9 and *cps*1. The *cps*2 and *cps*9 genes were detected more frequently among invasive isolates; NT isolates were more frequent among non-invasive isolates.

In the present study, virulence-associated genes *mrp, epf, sly,* and *ofs* were found in a large proportion of isolates, including NT isolates. The *arc*A gene was seen in all strains, confirming previous studies [1]. The *epf* gene was not detected in *cps*9 strains, in line with a previous report [11], whereas the recently described ofs gene [12,13] was detected not only in all *cps*2 but also in some *cps*1, *cps*9, and NT strains. Human and pig *cps*2 isolates carrying *mrp* and *epf*, were detected. Interestingly, strains carrying mrp and *epf* have been previously proved to induce meningitis and septicaemia in experimentally infected pigs [39]. Moreover, *cps*2 strains carrying *mrp epf*$^{\text{class I}}$ and *ofs*$^{\text{type 1}}$ were detected in pig isolates. The size variants *mrp* and *epf*$^{\text{class I}}$ have been described in human isolates in Europe [40] and recently found in invasive *cps*2 swine clones from Europe and Brazil [11,41]. The size variant *ofs*$^{\text{type 1}}$ has been found to be associated with the ST1 complex [13]. Other profiles, such as *cps*1 *mrp*S- and *cps*9 *mrp**- have also been described in isolates from diseased pigs in European countries [10,11].

The finding that invasive and non-invasive isolates share identical virulence profiles seems to support the hypothesis that other, as yet unknown virulence factors are involved in *S. suis* pathogenesis [1,3]. The high allele variability of these genes was confirmed by detection of several size variants of *mrp, epf*, and *ofs*, of which some had previously been described [10,11,13,40] and some were new (*epf*^{915}and *ofs*$^{\text{type 1S}}$).

High rates of resistance to macrolides and tetracyclines suggested widespread resistance to these antibiotics in Italy. In Europe, rising rates of resistance have been attributed to intensive use by swine breeders of the macrolide-class antibiotic tylosin as a growth promoter and of tetracycline as a therapeutic agent [15]. Co-resistance to macrolides and tetracyclines can be explained by the fact that tetracycline and erythromycin resistance determinants are often linked on mobile genetic elements [42].

All strains were PCR screened for *erm*(A), *erm*(B), and *mef*(A). Neither *erm*(A) nor *mef*(A) were detected. The *erm*(B) gene was found in all but two erythromycin-resistant pig strains, confirming its prevalence in *S. suis* in Europe [18,19]. A possible explanation for the erythromycin-resistant, *erm*(A)-, *erm*(B)- and *mef*(A)-negative strains could be an erythromycin resistance determinant previously unreported in *S. suis* [21]. The presence of *erm*(B) in both human isolates is consistent with its dissemination in the Italian swine population. The genetic basis of erythromycin resistance in human *S. suis* isolates has barely been investigated [5,21]. The very recent paper by Chu *et al.* [21] describes the prevalence of *mef*(A) in isolates from Hong Kong. Interestingly, all *mef*(A) isolates belonged to ST7 (endemic in Asia) whereas the only *erm*(B) strain belonged to ST1 (spread worldwide, including in Europe) [21].

The *tet*(M) and *tet*(O) genes are common resistance determinants in *S. suis*, found worldwide both in pig and in human isolates [19,20]. In this study, four *tet* genes, all coding for ribosomal protection proteins (http://faculty.washington.edu/marilynr/), were found in the Italian *S. suis* population. While *tet*(O) was prevalent, *tet*(M) was, inexplicably, almost absent. In addition *tet*(W), and the mosaic *tet*(O/W/32/O), the *tet* gene found most frequently in pig isolates after *tet*(O), were detected. The *tet*(W) gene is associated with tetracycline resistance in a wide range of bacterial species, including obligate anaerobic rumen bacteria and isolates from human gut and oral mucosa. *tet*(W) was first detected in *S. suis* by our group in the human isolate SsCA-1 [5], and then here in the other human strain (SSUD) and in some pig isolates. These data suggest that *tet*(W) could be widespread in *S. suis.*

The mosaic gene *tet*(O/W/32/O) has not been described in the genus Streptococcus before. Mosaic tet genes, originating from *tet*(O) and *tet*(W), were first detected in 2003 in anaerobic Gram-negative *Megasphaera elsdenii* from swine intestine [43,44]. Other mosaic genes, also comprising *tet*(32), were later detected in *Clostridium difficile* [45]. Initially thought to be confined to a small group of anaerobic bacteria [22], mosaic tet genes have now been found to be abundant in human and animal faecal samples [32] and have also been detected in *Bifidobacterium thermophilum* and *Lactobacillus johnsonii* isolates [46]. Further studies on the genetic elements carrying tet genes are warranted to explain the atypical tet distribution observed in Italian *S. suis* isolates.

Overall, the *S. suis* pig isolates demonstrated a high genetic diversity that correlates with a wide distribution of *S. suis* in Italy. In a heterogeneous background population, an identical virulence and resistance profile (*cps*2 *mrp epf*$^{\text{class I}}$ *ofs*$^{\text{type 1}}$ *sly erm*(B) *tet*(O)) and pulsotype were shared by more than a third of swine isolates, collected between 2003 and 2007 from different Italian herds and regions, demonstrating the presence and persistence of a dominant clone, 1a.

The results further revealed that the two human isolates shared a number of common or related features, i.e. both were serotype 2 and harboured *cps*2, both were resistant to erythromycin (MIC 4 µg/ml and >128 µg/ml, respectively) and contained the erm(B) gene, and both were resistant to tetracycline (MIC 16 µg/ml) and contained the *tet*(W) gene. Moreover, while sharing the same *mrp* and *epf* variants as well as *sly*, the two human isolates SsUD and SsCA-1 bore two different *ofs* variants, respectively *ofs*$^{\text{type 1}}$ and *ofs*$^{\text{type 1S}}$, a new variant with a 324 bp deletion in the *ofs*$^{\text{type 1}}$ coding sequence.

According to Tenover's criteria [37], a close relatedness between SsUD and SsCA-1 and between each human isolates and the dominant swine clone was documented by PFGE analysis which yielded pulsotypes with a difference in only two or three bands. MLST analysis assigned clones 1a and 1f (SsUD) to ST1 of the highly virulent ST1 complex, as previously demonstrated also for SsCA-1 (clone 1d) [5]. Overall, our data show that typical Eurasian strains, i.e. strains carrying genes coding for MRP, EF, and suilysin and belonging to the ST1 complex [1], are widespread in Italy.

In conclusion, this study demonstrated a high genetic diversity of Italian *S. suis* isolates, with a prevalent *cps*2, *erm*(B), *tet*(O) ST1 clone persistent in the swine population. It also demonstrated a close relatedness between two recently isolated *cps*2 *erm*(B) and *tet*(W) ST1 human strains and between human isolates and the dominant swine clone. Finally, it is the first report to demonstrate *tet*(O/W/32/O) in *S. suis* and suggests that mosaic tet genes should be sought in *S. suis* and in other streptococci.

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Repeated prevalence studies on antibiotic use in Latvia, 2003-2007

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Antibiotic resistance and nosocomial infections have recently been recognised as a growing threat in Latvian hospitals. We used a modified point prevalence study design to gain accurate information on the antibiotic prescription pattern and the prevalence of nosocomial infections in different hospital departments. A given department was observed on a given day in a given month (May) five years in a row. All antibiotic treatments, dose and route of administration were recorded, in addition to demographic data. The most commonly used antibiotic groups were first generation cephalosporins (35.6-38.9%), broad-spectrum penicillins (17.5-23.0%), fluoroquinolones (8.4-14.5%) and aminoglycosides (7.7-12.6%). Cefazolin was the most commonly used antibiotic. Antibiotics were predominantly used intravenously. The proportion of oral administration varied from 15.1% to 21.8%. A large proportion (13.3%) of the antibiotics was administered without clear reason. The crude prevalence rate of infection treated with antibiotics was 19.3%. The average prevalence of nosocomial infections was found to be 3.6%. These prevalence studies provided an opportunity to compare hospitals and outline variations and problem areas. They indicated the main problems in antibiotic prescription: large interhospital variations in the choice of an antibiotic for the most common infections, frequent antibiotic use without clear reason, and predominant intravenous administration.

Introduction

Antibiotics are one of the most frequently used drugs in outpatient and inpatient care and their use is considered to be an important risk factor for the development and spread of antimicrobial resistance [1]. During the past two decades, resistance to antibiotics has become a major public health concern due to the rapid spread of multiresistant bacterial clones and decreasing availability of new antibacterial drugs [2,3].

Consumption in hospital care accounts for only 5-15% of the total exposure to antibiotics in European countries [4,5]. Nevertheless, hospitals are considered to be the centre of antimicrobial resistance due to high density of broad-spectrum antibiotic use in a particularly vulnerable patient population. Therefore efforts to encourage prudent antibiotic use are a high priority. Benchmarking of antibiotic use is an important prerequisite for the control of antibiotic use.

Repeated point prevalence studies of nosocomial infections have been performed in several countries [6-11]. In spite of its shortcomings, this methodology is used as a tool for internal quality control and often preferred over prospective surveillance or aggregated data collection. In several recent studies, the point prevalence approach, simply selecting the patients that received an antibiotic therapy, was used to assess the prevalence of antibiotic use and to evaluate how appropriate the therapy was [12-15]. This simplified approach was less time consuming and, in addition, provided an opportunity to collect individual patient data on the prevalence of treated infections, dose of antibiotic, administration route, frequency, indication and main demographic data.

The aim of this study was to estimate the prevalence and pattern of antibiotic use in the largest Latvian hospitals. Internet-based software provided an opportunity for each hospital to get immediate feedback on their hospital data.

Methods

Five consecutive point prevalence studies were repeated annually from 2003 to 2007. We performed repeated point prevalence studies on antibiotic use in 16 selected Latvian hospitals. All hospitals participated on a voluntary basis and considered the study as an opportunity for quality control. In each hospital, the study was carried out by the same trained physician. Data were collected on Tuesdays, Wednesdays and Thursdays in May. Each department had to be surveyed on one day. All patients who were hospitalised at 8 am of the survey day and prescribed an antibiotic were included in the study. The patient charts were reviewed and anonymous data were collected using a standardised protocol which contained ward level and patient level data sheets. Ward level data included speciality of the ward, number of beds, the number of hospitalised patients and number of patients receiving antibiotics. Demographic data and duration of stay in hospital was collected for

each patient. The following prescription-related data were entered in the protocol: type of antibiotic, quantity (dose), frequency and route of administration, and indications or conditions for which antibiotics were given. If there was no evidence of infection or surgical prophylaxis was prolonged for more than 24 hours, the reason for antibiotic use was defined as unclear. The main source of information was the patient chart. If necessary, physicians and nurses where interviewed.

The percentage of antibiotic usage was calculated by the number of patients receiving an antibiotic per total number of hospitalised patients on the study day. Antibiotics were grouped according to the Anatomical Therapeutic Chemical (ATC) classification. Third and fourth generation cephalosporins, carbapenems, aminoglycosides and glycopeptides where additionally defined as hospital-specific antibiotics (HSA).

Infections were defined by the trained physician carrying out the survey according to clinical presentation and did not have specific definition criteria [12]. The prevalence of treated infections was calculated as a percentage of number of infections per total number of the hospitalised patients on the study day. Nosocomial infections were defined as infections that occurred more than 48 hours after hospitalisation. The study questionnaire and protocol were available on the study website (http://www.abresistance.lv/imed/login.jsp) and did not change over the study period.

Data from 2003 and 2004 were entered using EpiData 3.02 software. In 2005, a web-based database was designed. Since then all data have been entered online, and the hospital level results were available immediately after data entry. Each hospital was responsible for data entry themselves. Before complete analysis for

Table 1

Characteristics of the 16 hospitals participating in the study, Latvia, 2003-2007

Hospital	Participation in prevalence studies	Number of patients [mean ± (SD)]	Level	proportion of surgical patients [%]	proportion of intensive care patients [%]
A	2003-2007	914.4 (38.1)	Tertiary	40,3[c]	4.80[c]
B	2005-2007	656.3 (96.7)	Tertiary	39.2[c]	3.01[c]
C	2005	136	Regional	47.1[b]	ND
D	2003-2005	486 (23.6)	Regional	43.1	4.15
E	2003-2007	356 (24.2)	Regional	35.3[c]	2.60[c]
F	2004-2006	414.3 (104.6)	Specialised	5.7[b]	0.44[b]
G	2003-2005, 2007	272.5 (42.2)	Regional	38.1[c]	5.08[c]
H	2005	257	Regional	35.8[b]	3.9[b]
I	2005, 2007	130 (7,1)	Specialised	0[c]	1.60[c]
J	2003-2007	250.4 (31.4)	Specialised	99.2[c]	0.80[c]
K	2003-2005, 2007	504.5 (100.8)	Children	33.2[c]	10.05[b]
L	2003-2005, 2007	397 (83.0)	Specialised	0[c]	0[c]
M	2007	131	Specialised	20.6[c]	0.76[c]
N	2007	191	Specialised	45.0[c]	0.52[c]
O	2007	160	Children	0[c]	2.50[c]
P	2004	122	Regional	40.2[a]	1.6[a]

Data from year [a]2004,[b]2005, [#]2007.
ND: not determined; SD: standard deviation.

Table 2

Summary of antibiotic treatment and prevalence of infection for all study sites, Latvia, 2003-2007

	2003	2004	2005	2006	2007
No. of hospitals involved	7	9	12	5	11
No. of patients admitted	3,150	3,774	4,800	2,657	3,843
No. of patients with antibiotics (%) (95% CI)	845 (26.8) (25.3-28.4)	938 (24.8) (23.5-26.2)	1,385 (28.6) (27.3-29.9)	690 (26.0) (24.3-27.7)	1,038 (27.0) (25.6-28.4)
No. of antibiotics used per 100 patients (95% CI)	34.8 (33.2-36.5)	32.7 (31.2-34.2)	38.4 (37.0-39.8)	33.5 (31.7-35.3)	34.5 (33.0-36.1)
Prevalence of infections (95% CI)	17.3 (16.0-18.7)	19.8 (18.6-21.1)	22.0 (20.8-23.2)	16.4 (15.0-17.8)	18.8 (17.6-20.1)
Prevalence of community-acquired infections (95% CI)	13.4 (12.3-14.6)	15.9 (14.7-17.1)	18.8 (16.3-21.4)	12.7 (11.4-14.0)	15.3 (14.2-16.4)
Prevalence of nosocomial infections (95% CI)	3.9 (3.3-4.6)	4.0 (3.4-4.6)	3.1 (2.7-3.7)	3.7 (3.0-4.5)	3.5 (3.0-4.2)

CI: confidence interval.

scientific publication, a data check was done by an independent data manager.

Data were analysed using the SPSS 15.0 software package. Trends over time were examined using linear regression analysis. The study protocol was accepted by the local ethical committee.

Results

Five annual point prevalence studies where performed since 2003. The characteristics of the study hospitals are displayed in Table 1. The number of participating hospitals was not constant throughout the study period and varied from 7 hospitals in 2003 to 12 hospitals in 2006. A total of 18,226 patients were surveyed during the studies and their number varied from 2,657 to 4,800 by year (Table 2).

Across all study hospitals and all years, 6,389 antibiotic doses/courses were prescribed for 4,883 patients. The proportion of patients on antibiotics varied among all patients from 24.8% in 2004 to 28.6% in 2005 with high variability between hospitals (Table 1, Figure 1). On average 35.1 antibiotic treatments per 100 patients (median 38.0) were prescribed. Most patients received one antibiotic (72.7% in 2003, 71.2% in 2004, 69.1% in 2005, 71.3% in 2006, and 73.7% in 2007). The rest received a combination therapy of two or more antibiotics.

The pattern of antibiotic use

More than 40 different antibiotics were used. Twelve antibiotics in 2003, 15 in 2004, 14 in 2005, 11 in 2006 and 16 in 2007 constituted 90% of all antibiotic use.

The cephalosporins (35.6-38.9%), penicillins (17.5-23.0%) fluoroquinolones (8.4-14.5%) and aminoglycosides (7.7-12.6%) were the most commonly used antibiotic groups. The most common antibiotic subgroups were first generation cephalosporins (J01DB) (22% of all administered antibiotics), broad-spectrum penicillins (J01CA) (12.9%), other aminoglycosides (J01GB) (10.7%), third generation cephalosporins (J01DD) (10.6%), metronidazole (J01XD) (10.3%) and fluoroquinolones (J01MA) (10.2%). Cefazolin was the single most commonly used antibiotic in general. In some hospitals, ampicillin, co-amoxiclav or ceftriaxone were the most frequently prescribed drugs.

Use of hospital-specific antibiotics (HSA)

A total of 1,549 (24.2%, 95% confidence interval (CI): 23.2-25.2) prescriptions recorded during the study period were classified as prescriptions of HSA. There was a significant increase in consumption of over that period. The number of HSA prescribed per 100 patients increased from 7.4 in 2003 to 9.5 in 2007 ($p<0.05$). The proportion of HSA among all prescribed antibiotics increased from 21.4% in 2003 to 27.6% in 2007 ($p<0.05$).

Indications for antimicrobial therapy

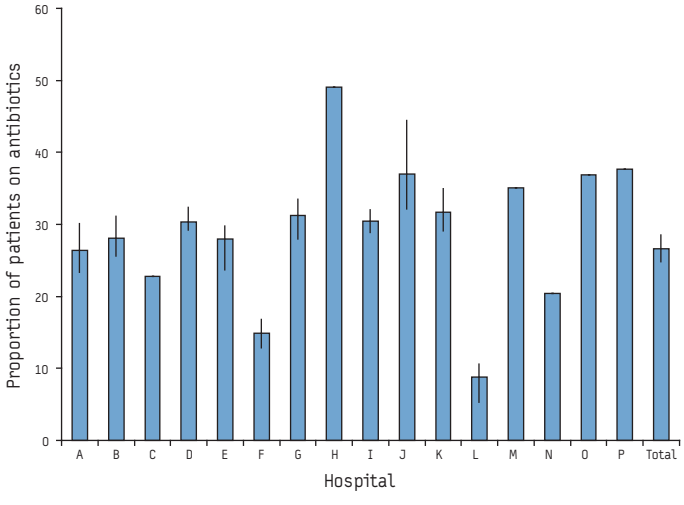
Infection

The most frequent indication for antibiotics was infection (69%). The prevalence of infections treated with antibiotics varied from 17.0% to 22.0% ($p<0.05$) across the study years, with the highest prevalence in 2005 (see Table 2).

The mean percentage of nosocomial infections treated with antibiotics was 3.6% (median 3.0%), but in five hospitals, the prevalence of nosocomial infections exceeded 6%. The highest mean prevalence of nosocomial infections were found in the large multidisciplinary teaching hospitals (4.5%, 95% CI: 4.0-5.0) and paediatric hospitals (4.0%, 95% CI: 3.4-4.5) (Figure 2). The most frequently reported nosocomial infections were lower respiratory tract infections 23.1% (20.3-30.0%) and surgical site infections 26.5% (19.1-32.0%). Fever of unknown origin with significantly increased C-reactive protein levels accounted for 13.9% of nosocomial infections. Nosocomial urinary tract infection, gastrointestinal infection and bacteriologically confirmed bloodstream infection were recorded in lower numbers (9%, 4% and 7%, respectively).

Figure 1

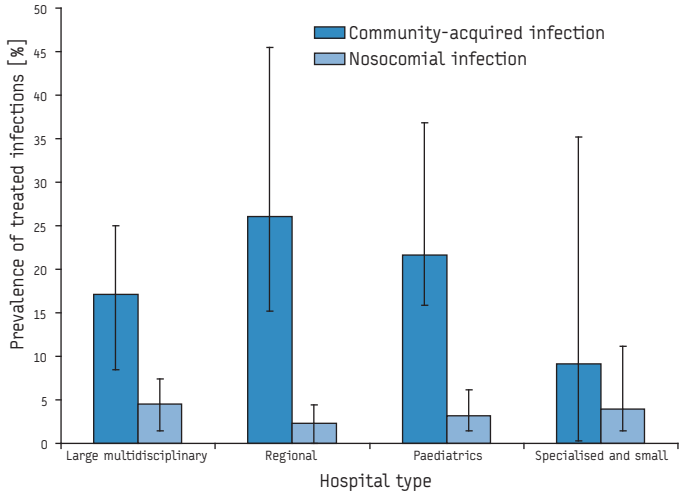
Mean number of patients receiving antibiotics with maximal and minimal annual variations, Latvia, 2003-2007



Hospitals C, H, M, N, O and P participated in the study only once.

Figure 2

Prevalence of treated infections and variations between hospitals according to hospital size and specialisation, Latvia, 2003-2007



Surgical prophylaxis

Of the total of 6,389 antibiotic courses, 785 (12.3%; 95% CI: 10.34-14.23) were prescribed for surgical prophylaxis. Cefazolin was the most commonly used drug and accounted for 58.6-80.5% of all prescriptions for surgical prophylaxis per year. Cefuroxime (5.2-11.7%), gentamicin (4.51-11.0%) and metronidazole (3.01-10.1%) were also used frequently.

Unclear use

Only a small proportion of antibiotics were used for medical prophylaxis. According to the investigators' observations, a large proportion, 13.3% (95% CI: 11.3;15.3), was administered without clear reason (16.9% in 2003, 9.9% in 2004, 9.9% in 2005, 19.4% n 2006, and 14.1% in 2007). Cefazolin was the antibiotic most often used without clear reason (mean 27.2%, 95% CI: 24.2-30.2). Metronidazole, ampicillin, and ceftriaxone were also often used without clear reason. In addition, an increase in the unclear use of ceftriaxone and metronidazole ($p<0.05$) was reported during the study period.

The route of administration

Antibiotics were most predominantly used intravenously (77.4%, 95% CI: 76.3-78.4) with a much smaller proportion of oral use (17.1%, 95% CI: 16.2-18,0). The proportion of oral use varied from 21.8% of all prescriptions in 2003 to 15.1% in 2006. The total intramuscular administration of antibiotics decreased from 8.2% in 2005 to 1.1% in 2007.

Discussion

Surveillance of antibiotic use and subsequent feedback to the staff could help to increase treatment quality, decrease the risk of antibacterial resistance and reduce unnecessary treatment costs.

The selection of the hospitals could be biased because the presence of a trained specialist in infectious diseases or clinical microbiology was defined as a precondition for participation. Many hospitals in Latvia did not employ such specialists. Nevertheless, nearly all largest regional hospitals participated in the study, and therefore, all regions of the country were represented in the study sample. The same protocol and data entry system was used in all hospitals and the study was performed by the same person over the years. It was therefore possible to compare the data longitudinally as suggested by earlier investigations [16-18].

In our study, 26.8% of hospitalised patients received antibiotics. This was less than reported in prevalence studies in Brazil [19], China [20], Greece [6], Italy [7,21], Malaysia [22], and Turkey [23] but significantly more than in German hospitals (17.7%) [18]. The proportion of patients on antibiotics in the study was similar to observed rates in Estonia [13], Lithuania [13] and the Netherlands [9] , Scotland [24] Sweden [12], Antibiotic consumption rates in hospitals in Latvia would therefore appear to be similar to what is observed in Northern and Central European countries.

There was a very high variability in the rates of antibiotic use between the hospitals investigated (see Figure 1). In 2007 for example, the proportion of patients on antibiotics varied from 5.3 to 44.4%. This variation could be due either to a different mixture of patients or to different treatment practices.

Cephalosporins were most commonly used antibiotic group in Latvian hospitals, with cefazolin being the most commonly used antibiotic. We could not find any clear explanation for its widespread use in Latvia because it did not provide any obvious cost benefit or treatment rationale.

The use of HSA was higher in Latvia than observed in other European countries (average 10%) [4] and increased from 21.4% in 2003 to 27.6% in 2007. Previously published studies indicate that extensive use of HSA may facilitate the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria and selected resistance in *Streptococcus pneumoniae* [2,25].

Almost 70% of all antibiotics were prescribed for treatment of infection, but 13.3% were used without defined reason. 12.3% of the antibiotics were used for surgical prophylaxis and that was similar to the proportion observed in other studies (14-42%) [12,13,22,26].

The crude percentage of infections treated with antibiotics was 19.3%. The prevalence of nosocomial infections was 3.6%, which is similar to other studies with comparable study design: In Swedish hospitals, the prevalence of all infections in 2003 and 2004 was found to be 17% and 18%, respectively [12], and in the Netherlands in 2004 it was 16,7% [9]. Nevertheless, the prevalence of nosocomial infections in those years was higher in Swedish studies (9.2% and 9.4%) than in our study. The overall prevalence of nosocomial infections was lower in our study than in most other studies [6,10,15,23,26]. This difference could be explained by differences in patient profile, length of hospitalisation and local health systems. We also observed significant variations of nosocomial infection rates over the years in some hospitals (Figure 2) for which we could not find an explanation.

Our study had several limitations regarding the detection of nosocomial infection. The approach of studying patients that receive antibiotics could have a relatively low sensitivity in finding nosocomial infections in certain patient populations [27,28]. Case definitions did not contain specific criteria and contained information only on what organs were affected. We relied only on the participating physician and his judgement. However, the study was performed by a well trained consultant specialist, and it was always the same person who collected the data over the years. Therefore, we believe in the consistency and good quality of their judgement. In addition, our first Latvian prevalence study for nosocomial infections that was performed on all hospitalised patients in 2001 using British National Survey definitions revealed very similar results [29].

Relatively low prevalence rates of nosocomial urinary tract and bloodstream infection compared with the high percentage of fever of unknown origin with significantly increased C reactive protein levels could indicate an insufficient clinical and laboratory capacity to identify these infections.

Oral use of antibiotics has been considered as a sufficient alternative even in hospitalised patients. It also reduces the risk of catheter-related infections, staff labour and costs. The proportion of intravenous use antibiotics found in Latvian hospitals was alarmingly high. Educational interventions to reduce intravenous and intramuscular use were taking place in several hospitals during the study period, but our data did not report any improvement except for a reduction in intramuscular use. Nevertheless, we can conclude that point prevalence studies can be used as simple approach to assess the efficacy of such educational interventions.

Each hospital could obtain an analysis of their data in the form of graphs immediately after the data entry was done. This option provided immediate feedback for the participants to plan educational activities based on the results of the study. Several interventions that were aimed at better antibiotic prescription and prevention of nosocomial infections were implemented during the study period in the participating hospitals. Nevertheless, our data did not reveal any significant improvements in our study endpoints after the period of five years. However, all participating hospitals achieved significant reductions in MRSA bacteraemia rates (EARSS, unpublished individual Latvian hospital data) over the study period, which might be partly related to the impact of repeated point prevalence surveys.

Point prevalence studies were considered mainly as a quality control exercise, but at the same time they provided useful information for further studies and targeted interventions. We consider point prevalence studies as an efficient, cheap and not very time-consuming measure for evaluation of antibiotic use in hospitals.

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Universal varicella vaccination in the Sicilian paediatric population: rapid uptake of the vaccination programme and morbidity trends over five years

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Following the licensure of the Oka/Merck varicella vaccine in Italy in January 2003, the Sicilian health authorities launched a universal vaccination programme in all nine Local Health Units. A two-cohort vaccination strategy was adopted to minimise the shift of the mean age of varicella occurrence to older age groups, with the goal of vaccinating with one dose at least 80% of children in their second year of life and 50% of susceptible adolescents in their 12th year of life. Two studies were implemented in parallel to closely monitor vaccination coverage as well as varicella incidence. Overall, the programme achieved its target, with 87.5% vaccine coverage for the birth cohort 2005 and 90.2% for adolescents born in 1995 and 1996. Varicella surveillance data obtained from a total of 28,188 children (0-14 years-old) monitored by family paediatricians showed a decline in incidence rates from 95.7 (95% confidence interval (CI): 72.2-126.8) for 1,000 person-years (PY) in 2004 to 9.0 (95% CI: 6.4-12.6) for 1,000 PY in 2007. In Europe, the only similar experience is the routine childhood varicella vaccination programme in Germany that started in 2004 with a single dose at the age of 11-14 months. The two-cohort universal vaccination programme implemented in Sicily, as well as the network for the surveillance study, can offer a model to other European countries that are considering introducing universal childhood varicella vaccination.

Introduction

Now that many vaccine preventable paediatric diseases have been eliminated or controlled, varicella remains one of the most common childhood diseases. Although varicella-zoster virus (VZV) infections are generally mild and self-limiting in the vast majority of children, complications such as secondary bacterial infections, pneumonia, encephalitis, cerebellar ataxia, transverse myelitis and death, can occur [1].

The incidence of varicella in Italy is believed to approximate the birth cohort, with over 5,300 annual estimated cases per 100,000 children under the age of 15 years [2], 3.5-5% of whom develop complications such as upper respiratory tract and cutaneous infections [3]. The figures presented in this report illustrate the significant burden of the disease in Sicily both for parents and for health services [4] and support the launch of a universal vaccination programme. Following the licensure of the Oka/Merck varicella vaccine (Varivax®) in Italy in 2001 for use in healthy children, the Sicilian health authorities launched a two-cohort universal vaccination programme. The impact of varicella vaccination was monitored in two studies conducted in parallel, one focusing on vaccination coverage and the other on varicella incidence.

Methods

Coverage study

Sicily (5,015,297 inhabitants in the national census of 1 January 2006) is one of twenty Regions in Italy. Public health policies are established autonomously in the Regions, based on recommendations from the Italian National Health Service. Compulsory and recommended vaccinations are actively offered free of charge to all Sicilian children against diphtheria, tetanus, poliomyelitis, hepatitis B, pertussis, *Haemophilus influenzae* type b, measles, mumps, and rubella. In Italy, childhood vaccinations are mostly performed in Vaccination Centres (VCs). Sicily counts 386 VCs that are part of Health Districts (HDs), themselves part of Local Health Units (LHUs).

Vaccination programme

Universal varicella vaccination was added to the standard childhood vaccination programme in January 2003 and was actively offered free of charge to all children in their second year of life (at about 15 months of age) and to all susceptible adolescents in their 12th year of age, at the time of the measles, mumps and rubella (MMR) vaccination in order to improve parents compliance. Although two vaccines were available, only Varivax® was licensed for universal vaccination at the time and thus selected for the programme. Once the parents consented to the vaccination, varicella vaccine was administered on the same occasion as MMR

vaccine, injected in the counter lateral arm. Following existing recommendations at the start of the programme, one dose of varicella vaccine was administered to every participating child and adolescent.

Public health physicians carried out most of the vaccinations, although paediatricians were the key contacts for counselling and in some cases vaccinated the children themselves. In addition, ad hoc information campaigns in secondary school were performed and susceptible adolescents could also be vaccinated at their own school surgery. Varicella vaccine was also offered free of charge to the siblings of all vaccinated children and to household contacts of varicella cases.

The vaccination target was set at ≥80% coverage for children in their second year of life and ≥50 % for susceptible adolescents. Vaccination coverage was analysed overall, by age group and by birth cohort.

Collection and recording of data

Demographic and vaccination data were collected by VCs and reported monthly to HDs. Data was entered in a protected internet database with varying levels of access, connecting HDs, LHUs and the Regional Public Health Office (RPHO), each of these entities having a different level of access for data entry, data monitoring and analysis. For the few vaccinations performed by family paediatricians (FPs) or other structures, the vaccination data was communicated to the public health system for entry into the database. Quality control of the database was monitored by an external agency through quarterly visits and audits.

Target population for data analysis

The target population for data analyses for the period 2003-2007 included:

all children aged 12–23 months (100% of the resident population in this age group),

all susceptible adolescents aged 11–12 years (18% of the resident population in this age group).

Susceptibility to varicella was based on self-reported negative history for the disease. Although there are limitations associated with parental reporting (e.g. under- or overestimation of disease occurrence), these limitations are usually accepted in observational epidemiological surveillance studies.

The denominator for coverage rate was calculated using resident population numbers according to the National Institute of Statistics (ISTAT, data as of 1 January 2006) and prevalence of VZV, extrapolated using known Italian VZV seroprevalence data by age range [5].

Surveillance study

Varicella surveillance was performed through a sentinel network of randomly selected FPs in order to describe age-specific varicella incidence rates among children 0-14 years after the introduction of the universal vaccination programme, as well as age-specific related complications. FPs offer a unique surveillance opportunity since every child in Italy is registered with an FP from birth until the age of 14 years. Thus, each FP has a precise paediatric population under their care (between 800 and 1,000 children) and their public health duty includes routine control visits that are perfect opportunities for offering vaccination, for disease control and surveillance. Of the 844 FPs operating in Sicily, 30 were randomly selected to participate in the study. The number of FPs from each LHU was balanced by resident population and geographical location (urban versus rural) with at least one FP from each of the nine LHUs. Computations of incident cases and person-year (PY) computation were recorded prospectively from March 2005 and retrospectively (based on physicians records) for the period from January 2003 to February 2005.This could result in some degree of underreporting for the retrospective period, although it is noteworthy that most of the physicians participating in the study had already been involved in active infectious diseases surveillance before the start of the study.

Table

Number of children vaccinated against varicella, by birth cohort, Sicily, 2003–2007

Birth cohort	1995–1996	1997–2004	2005
Vaccinated children	8,839	152,308	35,123
Resident children*	108,958	410,652	50,202
Susceptible children**	19,613	nd	50,202

nd: not done.
*National Institute of Statistics data (ISTAT) data as of 1 January 2006.
**Estimated based on published seroprevalence data [5].

Figure 1

Coverage rates for varicella vaccination of children, by birth cohort (2001-2005), Sicily

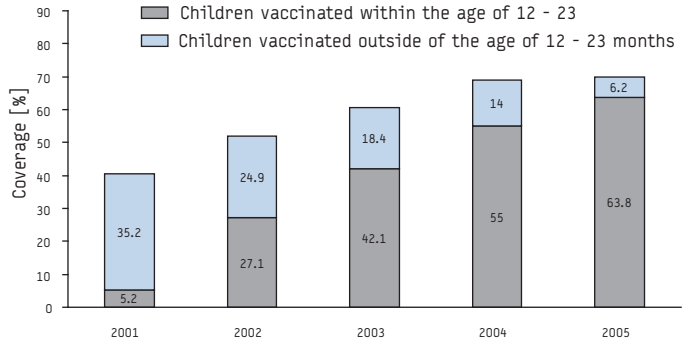
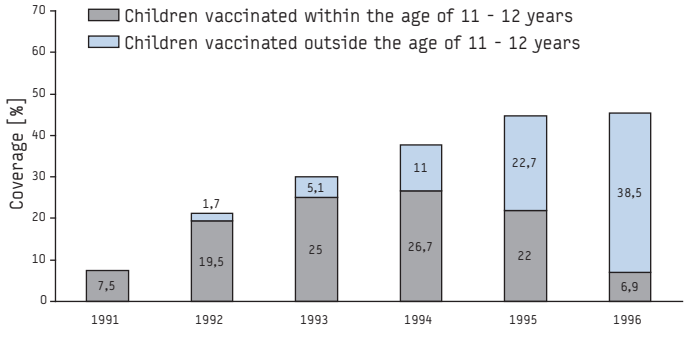


Figure 2

Coverage rates for varicella vaccination of adolescents, by birth cohort (1991-1996), Sicily



All children registered with the 30 sentinel FPs were proposed participation into the study. Informed parental consent was requested. The at-risk population (denominator) included all children susceptible to varicella who were followed by participating FPs. The PY contribution of each child followed up was calculated using information from the FPs' records. This was done for the active surveillance period of the study (2005 to 2007), as well as for the years 2003 and 2004 using information in the FPs' records to obtain 'historical' rates for the period between the introduction of the vaccine in Sicily (2003) and the implementation of the study (2005).

FIGURE 3

Overall coverage rates of varicella vaccination in children 12-23 months, by Local Health Unit, Sicily, 2003–2007

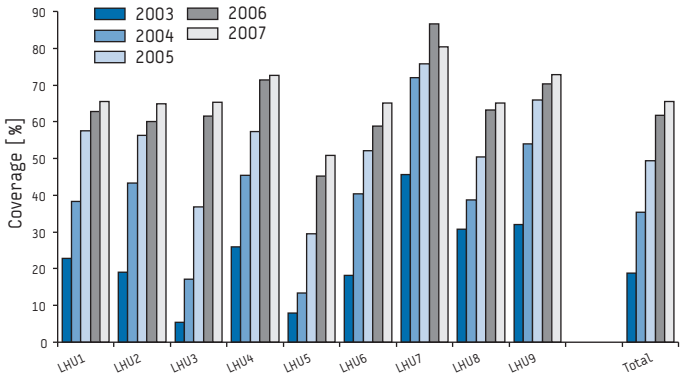


FIGURE 4

Annual varicella incidence rates per age group, sentinel FP network, Sicily, 2003-2007

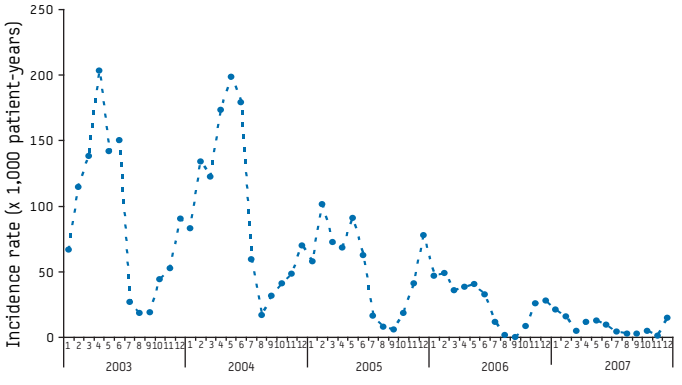
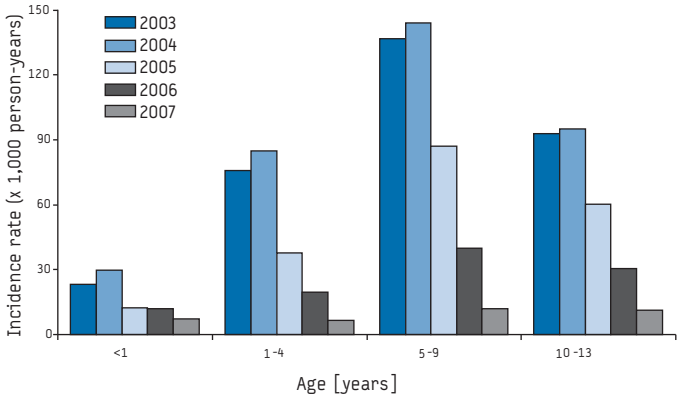


FIGURE 5

Annual varicella incidence rates per age group, sentinel FP network, Sicily, 2003-2007



Results

A total of 225,642 children vaccinated during the study period (1 January 2003 to 31 December 2007) were taken into consideration for the analysis, as presented in the Table.

The coverage rate for children born in 2005 was 70.0% (Figure 1), while that of susceptible adolescents born in 1995 and 1996 was 45.1% (Figure 2).

The overall coverage rate for 2007 was 65.5% in children 12-23 months (range 50.9-80.5%), as shown in Figure 3, and 12.1% in adolescents 11-12 years of age (range 5.1-40.9%).

Varicella surveillance data were obtained from a total of 28,188 children at the age of 0-14 years (the 86.7% of the registered children for whom informed parental consent was obtained). Of those, 21,568 susceptible children were taken into account for the calculation of varicella incidence. The varicella incidence rates per month in 0-14 year-old children are presented in Figure 4.

Annual incidence rates declined from 95.7 (95% confidence interval (CI): 72.2-126.8) for 1,000 PY in 2004 to 9.0 (95% CI: 6.4-12.6) for 1,000 PY in 2007. The incidence of varicella declined in all age groups (Figure 5).

A total of 22 cases of breakthrough varicella (occurring more than six weeks after vaccination) were reported. Ten cases occurred in 1-4 year-old children, nine cases in the age group of 5-9 year-olds and three cases in 10-13 year-old children. No case required hospitalisation. In addition, seven herpes zoster cases were reported among vaccinated children: three in 1-4 year-old children, three cases in 5-9 year-olds and one case in the age group of 10-13 year-olds.

Discussion

Varicella vaccination is not yet routine in Europe despite the availability of VZV vaccines in at least 14 European countries [6]. In general, selected high-risk groups, such as healthcare workers, susceptible adults, and immunocompromised patients are targeted for vaccination. Although the epidemiology of varicella in Europe is similar to that observed in the prevaccine aera in the United States, Germany remains the only country that has incorporated the VZV vaccine nationwide in the routine immunisation schedule as a single dose at the age of 11-14 months, starting from July 2004 [7].

Sicily was the first Italian region to introduce universal varicella vaccination in its childhood vaccination programme in 2003 and to date, only three other Italian regions have a similar programme for varicella. The model adopted by the Sicilian health authorities took into account the peculiarities of the age-specific varicella seroprevalence in Italy. Indeed, the reproduction numbers and herd immunity thresholds can have a profound impact on susceptibility patterns and disease transmission and thus have important implications for the design and implementation of varicella vaccination programmes in a given country [6]. Standardised serological surveillance established for eight-vaccine preventable

diseases [8,9] showed striking variations in the rate of VZV transmission in different European countries. While seroprevalence for varicella at the age of five years was high in some countries (97% in the Netherlands, 86% in Israel, 81% in Belgium), but very low in Italy, with only 38% of children seropositive for VZV antibodies [5]. Within Italy, however, VZV is circulating more intensely in the southern part of the country and affects people at an earlier age [4]. No clear explanations can be given for the relatively low seroprevalence of varicella antibodies across all age groups in the Italian population. Nevertheless, these data provide a good rationale for varicella vaccination in early childhood and adolescence in a population relatively less well protected by natural immunity compared to other European countries.

The programme Sicily was taken up rapidly, with increasing coverage rates in both cohorts over time. Although significant differences were initially observed between LHUs, the figures became more uniform over the years. The average coverage rates were 65.5% for children in their second year of life and 12.1% for adolescents at the age of 11-12 years. A steady uptake of the programme was observed between 2003 and 2007, and the programme's target was achieved, with 87.5% coverage for the birth cohort 2005 and 90.2% for adolescents born in 1995-1996. The introduction of the combined MMR-VZV vaccine is expected to modify the acceptance of varicella vaccination and could further increase coverage rates [10] in view of the MMR vaccination rates of up to 85% currently attained in all Sicilian LHUs.

Vaccination of young children before the peak age of varicella prevalence can have a significant impact on the incidence of the disease, as already demonstrated in the United States (US), where universal childhood vaccination was introduced in 1995. In Sicily, the main targeted cohort (children at the age of 15 months) was selected based on the fact that infection in Sicily occurs at an earlier age compared to the rest of the country. After the launch of the varicella vaccination programme, a steady decrease in varicella incidence was observed, reaching 9.0 for 1,000 PY during the last year of observation (2007), a number well below the national estimate of 70 for 1,000 PY. So far, this strategy has proven very effective and breakthrough disease has been rare (only 22 cases reported in the surveyed population). Low levels of circulating VZV in early childhood warrant better protection of susceptible adults and adolescents and can limit the potential shift of the disease towards older age that is generally put forward as a risk of universal varicella vaccination in childhood. The possible need for booster doses in adolescents and adults cannot be excluded, although the two-dose vaccination regimen currently proposed for all ages seems to lower the risk of breakthrough varicella in vaccinated children considerably. Good coverage rates in susceptible adolescents will be an additional barrier against the shift of the disease to older age. Clearly, the dynamics of disease epidemiology after the start of the vaccination programme will need further assessment and one key element will be the observed incidence of breakthrough disease.

Another potential risk that has limited the uptake of varicella vaccination in Europe is the possible increase in the incidence of herpes zoster. Our data show a very low number of herpes zoster cases in the surveyed population. Unfortunately, virological data were not available for these latter cases, although virological typing (differentiating between the Oka/Merck vaccine strain and the wildtype virus) had been made available to participating physicians for a number of complications or breakthrough cases. Longer follow-up is required, as well as more consistent data on the background rates of herpes zoster in the general paediatric population.

Overall, long-term surveillance is needed to evaluate the effectiveness of the programme over time, and the progressive introduction of the second dose of varicella vaccine in early childhood, as already recommended in the US [11], will have to be closely monitored. Nevertheless, very good results have already been obtained in the five years of universal varicella vaccination with one dose, as shown by the very low incidence of the disease in all age groups in 2007, including those not targeted by the vaccination programme. Close collaboration between public health services and family paediatricians also proved effective.

The two-cohort universal vaccination programme implemented in Sicily, as well as the network for the surveillance study, can offer a model to other European countries that are considering introducing universal childhood varicella vaccination.

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Conflict of interest statement:

LR Biasio is Medical and Development Director of Sanofi Pasteur MSD.

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Multidrug-resistant Neisseria gonorrhoeae with reduced cefotaxime susceptibility is increasingly common in men who have sex with men, Amsterdam, the Netherlands

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Antimicrobial resistance is an increasing problem in *Neisseria gonorrhoeae* (NG) treatment. Presently, third-generation parenteral cephalosporins, like ceftriaxone and cefotaxime, are the first option. Resistance to oral, but not to parenteral, third-generation cephalosporins has been reported previously. We analysed the microbial susceptibility (as minimum inhibitory concentration - MIC) of NG cultures obtained from high-risk visitors of the largest Dutch outpatient clinic for sexually transmitted infections (STI) in Amsterdam, the Netherlands. Among 1,596 visitors, we identified 102 patients with at least one NG isolate with reduced susceptibility to cefotaxime (0.125 µg/ml < MIC ≤ 0.5 µg/ml). The percentage of NG isolates with reduced susceptibility to cefotaxime rose from 4.8% in 2006 to 12.1% in 2008 (chi^2 17.5, p<0.001). With multivariate logistic regression, being a man who has sex with men (MSM) was significantly associated with reduced susceptibility to cefotaxime (p<0.001). Compared to susceptible NG isolates, those with decreased susceptiblity to cefotaxime were more often resistant also to penicillin (16.5% vs. 43.3%), tetracycline (21.5% vs. 68.9%) and ciprofloxacin (44.4% vs. 90.0%, all p<0.001). The increased prevalence of NG strains with reduced susceptibility to cefotaxime among MSM may herald resistance to third-generation parenteral cephalosporins. A considerable proportion of these strains show resistance to multiple antibiotics which could limit future NG treatment options.

Introduction

Gonorrhoea is a highly contagious sexually transmitted infection caused by *Neisseria gonorrhoeae* (NG). In the majority of cases NG urogenital infections in males cause symptoms like discharge or urethritis whereas anal and pharyngeal NG infections and urogenital NG infections in females are asymptomatic in a large proportion of cases. Uncomplicated urogenital NG infections can lead to salpingitis in females and epididymitis in males, conditions that are associated with infertility. In some cases localised NG infections can lead to haematogenic dissemination causing severe complications like sepsis, meningitis and endocarditis [1].

Previously we reported a rise in the proportion of fluoroquinolone-resistant *N. gonorrhoeae* (FRNG) isolates among NG isolates obtained from men who have sex with men (MSM) visiting the Amsterdam clinic for sexually transmitted infections (STI) from 0.2% in 2000 to 10.5% in 2003 and among those obtained from men who have sex with women from 0.7% to 3.2%, respectively [2]. A year later, in 2004, a prevalence of FRNG up to 15% was found among heterosexual visitors of STI clinics [3], and in 2008 the proportion of FRNG has risen to 45% among STI clinic visitors throughout the Netherlands [4].

Similar increases in circulating FRNG isolates among STI visitors were documented earlier in the United Kingdom around 2000 [5], and during the 1990s throughout Asia [6]. As soon as the prevalence of antibiotic–resistant strains of a circulating pathogen in a patient population exceeds 5%, both the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend to stop using this antibiotic for treatment of patients infected with this pathogen [1,6]. Therefore since 2004 fluoroquinolones have no longer been recommended as first line treatment for NG in the Netherlands and in many other countries. In the national clinical guidelines issued by the Dutch Dermatological and Venereological Society (Nederlandse Vereniging voor Dermatologie en Venereologie, NVDV) and the Dutch General Practitioners Society (Nederlands Huisartsen Genootschap, NHG), both parenteral third-generation cephalosporins - ceftriaxone and cefotaxime - are the first line treatment option for patients infected with NG [7]. Before fluoroquinolones were abandoned as the first treatment option, penicillin and tetracycline had already been discontinued as preferred treatment option for NG infections due

to unacceptable high prevalence of circulating NG strains resistant to these antibiotics [6].

In collaboration with the Municipal Health Service Public Health Laboratory, we have been closely monitoring the resistance to antibiotics of NG isolates found in the visitors to the STI clinic in Amsterdam. In addition to penicillin (both chromosomal and plasmid mediated resistance), tetracycline and ciprofloxacin, since 2004 we have also monitored the resistance to cefotaxime.

In this article we report an alarming increase in the proportion of multidrug-resistant NG strains with reduced susceptibility to cefotaxime among isolates obtained from visitors frequenting the Amsterdam STI outpatient clinic in 2008 compared to 2006-2007. These NG strains with reduced susceptibility to cefotaxime are found for the larger part among MSM with high-risk behaviour for other STI's. Evolvement of true resistance to third-generation cephalosporins would seriously hamper effective control of NG infections.

Methods

Time frame and study population

The Amsterdam STI outpatient clinic is the largest setting of its kind in the Netherlands, with nearly 28,000 new consultations in 2008 [8]. Upon arrival at the clinic, visitors are prioritised based on a short questionnaire to estimate the risk for having an STI. The prioritising system is described in more detail elsewhere by Heijman et al. [9]. In short, all visitors that are either referred by a healthcare professional, have a sex partner with an STI, had STI-related complaints, or are MSM, are considered high-risk patients and get a full STI check-up including, for the largest part, the collection of swabs for NG cultivation from the pharynx, urethra, cervix and/or rectum depending on the sex technique practiced in the previous six months. Those with negative answers to the questionnaire, are considered low-risk visitors. From these no NG isolates are available since only a nucleic acid amplification test is used to perform NG diagnostics in this group.

All demographic and clinical characteristics used in this study were recorded in an electronic patient database as described earlier [10]. Patients diagnosed with an NG infection (urogenital, anal or pharyngeal) were treated with 500 mg ceftriaxone i.m. according to the national guidelines of the Dutch Dermatological and Venereological Society (NVDV) [7]. In case symptoms persisted one week after treatment, visitors were requested to return to the clinic and additional swabs for NG cultivation were obtained to see if the treatment had been successful ("test for cure"). Moreover, all visitors were screened for *C. trachomatis* infections (including lymphogranuloma venereum in MSM), syphilis, hepatitis B and upon consent HIV, as described elsewhere [10]. All data and samples for this study were collected as part of the routine clinical procedure; therefore no Ethical Committee approval was needed. Care was provided in accordance with the Helsinki Declaration of 1975, as revised in 1983 [11].

All NG isolates with available MIC information collected between October 2006 and December 2008 from high-risk patients were included in the analysis. We examined whether there was a trend in the proportion of NG isolates with reduced susceptibility per quarter. From patients with more than one isolate with a MIC value, the isolate with the highest MIC value was used in the analysis, for all antibiotics tested. In a sub-analysis we examined whether susceptibility to cefotaxim was associated with the anatomical site from which the isolate was originating. Statistical analysis was performed using SPSS version 15.0 (SPSS, Inc., Chicago, IL, US) and Stata version 9.2 (Stata Corporation, College Station, TX, US). We examined the association between reduced susceptibility to cefotaxime and age, sexual orientation, nationality, previous and current STI diagnoses, including HIV status and result of *Treponema pallidum* haemagglutination (TPHA) test. Factors associated with reduced susceptibility in univariate analysis at $p<0.20$, were included in a logistic regression model. Factors that were not significantly associated with the outcome were one by one omitted from that model (level of significance set at $p=0.05$).

N. gonorrhoeae susceptibility testing

All swabs for NG cultivation were swiped on selective feeder plates (GC-LECT; Becton Dickinson, Franklin Lakes, NJ, United States) as described previously [2]. In short, the plates were incubated immediately at 37 ºC in CO2 enriched atmosphere before and after transportation in "candle jars" to the laboratory. After 40-48 hrs the plates were inspected for colony formation. Determination of NG isolates was based on Gram-staining, oxidase-, sugar fermentation-, and aminopeptidase reactions and hybridisation with a DNA probe (Accuprobe, Biomerieux). In cultured NG isolates, the minimum inhibitory concentration (MIC) of penicillin, tetracycline, ciprofloxacin, and cefotaxime was measured using E-tests (AB Biodisk, Solna, Sweden). Moreover, plasmid-mediated penicillin resistance was tested with the help of a beta-lactamase test.

For MIC validation and quality control, the public health laboratory of the Municipal Health Service Amsterdam participated in the European Surveillance of STI (ESSTI) NG isolate panel exchange collaboration programme in 2008. This panel included WHO strains K and L, which both display a reduced susceptibility to third-generation cephalosporins due to a pen A mosaic allele (K) or an A501 mutation in the penA gene (L) [12]. These strains had an MIC for cefotaxime of 0.5 and 0.25 µg/ml, respectively.

Before 2006, ceftriaxone was not available in the Netherlands in acceptable dosages for treating patients with NG infections. Therefore, cefotaxime was the nationally recommended first treatment option and, consequently, the Dutch health authorities (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) provided cefotaxime tests to NG reference laboratories throughout the country for the monitoring of parenteral third-generation cephalosporin susceptibility. From 2006 onwards, the 500 mg ceftriaxone i.m. dosage became available and was then recommended as the first treatment option for NG infections, but the government continued to provide the cefotaxime susceptibility test. Therefore during the study period we treated NG infections with ceftriaxone while testing susceptibility for cefotaxime. Since structural homologies between both molecules are high, we consider cefotaxime susceptibility an appropriate marker for susceptibility to all third-generation cephalosporins. This was confirmed by our finding that genetically well-described WHO reference strains with diminished susceptibility to cefixime and ceftriaxone had also increased MICs for cefotaxime, whereas all other ESSTI control strains were fully susceptible. According to the guidelines and recommendations of the Clinical and Laboratory Standards Institute (CLSI) the following MIC cut-off values were used to define antibiotic susceptibility [13]:

- For cefotaxime: susceptible ≤0.125 µg/ml; 0.125 µg/ml < reduced susceptibility ≤0.5 µg/ml; resistant >0.5 µg/ml.

- For penicillin: susceptible ≤0.06 µg/ml; 0.06 µg/ml < reduced susceptibility < 2.0 µg/ml; resistant ≥2.0 µg/ml.
- For tetracycline: susceptible ≤0.25; 0.25 µg/ml < reduced susceptibility <2.0 µg/ml; resistant ≥2.0 µg/ml.
- For ciprofloxacin: susceptible ≤0.06 µg/ml; 0.06 µg/ml < reduced susceptibility <1.0 µg/ml; resistant ≥1.0 µg/ml.

Results

From October 2006 until December 2008, gonorrhoea was diagnosed in 1,821 high-risk patients (out of the total number of 35,411 high-risk patients who visited the clinic in this period, which gives 5.1% gonorrhoea prevalence in this group) and in 115 low-risk patients (out of 25,304 low-risk patients in total, which gives 0.5% gonorrhoea prevalence in this group). In 225 of the high-risk patients NG isolates were not available, in most cases because the gonorrhoea diagnosis was based on a nucleic acid amplification test or because the gonorrhoea culture did not grow. From the remaining 1,596 patients a total of 1,883 NG isolates obtained from various locations were available for which MIC testing was performed. We compared the demographic and clinical characteristics of the high-risk patients with MIC information versus those without MIC information. There was no significant difference between the two groups regarding concurrent syphilis (i.e. stage 1, 2 or early latent syphilis) or lymphogranuloma venereum infection at the time of consultation, past syphilis infection (i.e. TPHA seropositivity), HIV seropositivity and age distribution. However, the proportion of MSM was significantly higher in the group without MIC information ($p<0.001$, chi^2 test).

Among the 1,596 patients with MIC data, we identified 102 with at least one NG isolate with reduced susceptibility to cefotaxime (0.125 µg/ml < MIC <0.5 µg/ml, Table 1). No NG isolates resistant to cefotaxime were identified and isolates obtained from the remaining 1,494 patients were all susceptible to cefotaxime (MIC ≤ 0.125 µg/ml). Between October 2006 and December 2008 an important and significant rise in both absolute and relative terms was observed in the number of patients with NG isolates with reduced cefotaxime susceptibility: from 8 (4.8%) in the fourth quarter of 2006 to 23 (12.1%) in the fourth quarter of 2008 (chi^2 for trend = 17.5, $p<0.0001$, Figure).

Demographic and clinical characteristics of patients with reduced cefotaxime susceptibility

The following patient characteristics were significantly associated with having an NG isolate with reduced susceptibility to cefotaxime (Table 1): age >35 years (p=0.004), MSM (p<0.001), a concurrent lymphogranuloma venereum infection at the time of consultation (p=0.04), positive HIV serology, either as a new diagnosis or known HIV seropositivity (p=0.023), positive TPHA serology (p=0.01, for all comparisons a chi^2 test was used). In a multivariate logistic regression model, only being MSM was significantly associated with reduced susceptibility to cefotaxime (OR=2.9, 95% CI 1.4-5.8, p<0.001, adjusted for age).

Table 1

Demographic and clinical characteristics of 1,596 patients with at least one *Neisseria gonorrhoeae* isolate; STI outpatient clinic, Amsterdam, the Netherlands, 2006-2008

Patient characteristics	cefotaxime MIC ≤ 0.125 µg/ml (n= 1,494)	cefotaxime MIC > 0.125 µg/ml, (n=102)	OR (95%CI)	Overall p value[1]
Age				
≤35 years	847 (56.7%)	43 (42.2%)	1 (ref)	0.004
>35 years	647 (43.3%)	59 (57.8%)	1.8 (1.2-2.7)	
Sexual preference				
Men who have sex with women (exclusively)	317 (21.2%)	9 (8.8%)	1 (ref)	<0.001
Women who have sex with men	192 (12.9%)	3 (2.9%)	0.6 (0.1-2.1)	
Men who have sex with men (and/or women)	985 (65.9%)	90 (88.2%)	3.2 (1.6-6.5)	
HIV serology				
Positive, new diagnosis	39 (2.6%)	7 (6.9%)	3.1 (1.3-7.3)	0.023
Known positive	337 (22.6%)	30 (29.4%)	1.5 (1.0-2.4)	
Negative	952 (63.7%)	55 (53.9%)	1 (ref)	
Not tested	166 (11.1%)	10 (9.8%)	1.0 (0.5-2.1)	
Concurrent infectious syphilis[2]				
No infectious syphilis	1,441 (96.5%)	101 (99%)	1 (ref)	0.165
Infectious syphilis	53 (3.5%)	1 (1.0%)	0.3 (0.04-1.96)	
Syphilis serology[3]				
TPHA-negative	1,145 (76.9%)	67 (65.7%)	1 (ref)	0.011
TPHA-positive	345 (23.2%)	35 (34.3%)	1.7 (1.1-2.7)	
Concurrent lymphogranuloma venereum				
No lymphogranuloma venereum	1,466 (98.1%)	97 (95.1%)	1 (ref)	0.04
Lymphogranuloma venereum	28 (1.9%)	5 (4.9%)	2.7 (1.02-7.1)	

Data are number of patients (% of total).
1p values were calculated with chi^2 test.
2Infectious syphilis infections are stage 1, 2 or early latent stages diagnosed at the date of visit.
3Data on TPHA (Treponema pallidum haemagglutination) test missing for n=4.

NG isolates from MSM

In total 1,231 isolates with MIC information were obtained from 1,075 MSM patients (Table 2). Of these, 1,134 isolates from 985 patients showed good susceptibility to cefotaxime (MIC ≤ 0.125 µg/ml) and 97 isolates from 90 patients showed decreased cefotaxime susceptibility (MIC >0.125 µg/ml). A considerable number of these isolates originated from rectal location (respectively 534 [47.1%] and 48 [49.5%]). Site of infection was not significantly associated with reduced susceptibility (p=0.61).

We analysed the susceptibility to antibiotics other than cefotaxime of all 1,231 NG isolates from 1,075 MSM patients (Table 3). Isolates with decreased susceptibility to cefotaxime showed significantly more often resistance to penicillin (43.3% vs. 16.5%), tetracycline (68.9% vs. 21.5%) and ciprofloxacin (90.0% vs. 44.4%, for all antibiotics separately p<0.001) compared to NG strains susceptible to cefotaxime.

Moreover, multiple resistance to the additionally tested antibiotics (penicillin, tetracycline and ciprofloxacin) was significantly more frequent among isolates with decreased susceptibility to cefotaxime compared to those susceptible (77.5% and 22.9% for at least two of the three additionally tested antibiotics, respectively, and 30.3% and 4.7% for all three additionally tested antibiotics, both comparisons p<0.001).

Discussion

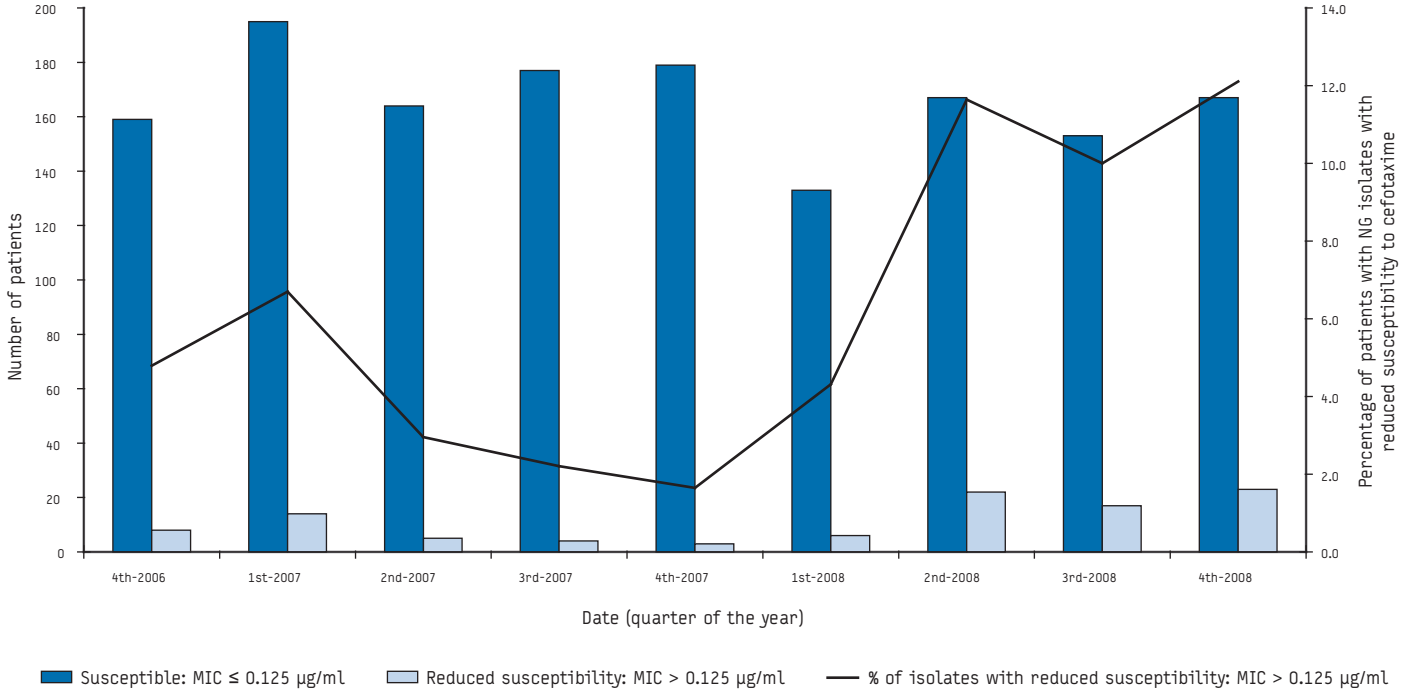
We report on an alarming significant increase of NG isolates with reduced susceptibility for cefotaxime, a parenteral third-generation cephalosporin, among STI clinic visitors in Amsterdam, the Netherlands from 2006 to 2008. Following the resistance to sulfanilamide in the 1940s, penicillin and tetracycline in the 1980s and, lastly, fluorochinolones in the early 1990s, third-generation cephalosporins have nowadays become the first option of treatment in most countries [1,6].

N. gonorrhoeae strains with reduced susceptibility to oral third generation cephalosporins have been described in Japan [14,15], Sweden [16], Australia [17] and Greece [18]. Mosaic patterns of the *penA* gene, encoding penicillin binding protein 2, partly originating from the pharyngeal commensal species *N. cinerea* and *N. perflava* have been reported in such strains [14,16]. In addition, alterations in *mtrR*, resulting in increased expression of efflux pumps, *porB1b*, resulting in altered permeability of the porin *por1B*, and *ponA*, leading to decreased affinity of penicillin binding protein 1 to beta-lactam antibiotics have been reported [16]. In contrast to susceptible strains, these strains cannot always be eradicated using two 200 mg doses of oral cefixime [19]. For this reason, the European Committee on Antimicrobial Susceptibility Testing (EUCAST), recommends to use only a susceptible/resistant breakpoint for all cephalosporins at > 0.12 [20]. According to EUCAST guidelines, all 102 strains with an MIC > 0.125 µg/ml found in our study would have been considered resistant against third-generation cephalosporins.

It is feasible that a decrease in cefotaxime susceptibility among circulating NG strains will necessitate the use of larger doses of third-generation cephalosporins for effective elimination of gonorrhoea in infected individuals, a phenomenon already experienced in the treatment of gonorrhoea patients with penicillin in the past. For uncomplicated gonorrhoea infections, the CDC recommends 125 mg ceftriaxone i.m. [21] and the International

Figure

Numbers and percentages of patients with *Neisseria gonorrhoeae* isolates susceptible and with reduced susceptibility to cefotaxime, by quarter of the year, 2006-2008, STI outpatient clinic, Amsterdam, the Netherlands



Union against sexually transmitted infections (IUSTI)/WHO guidelines a dose of 250 mg ceftriaxone i.m. [22]. Possibly because in the Netherlands we already use higher than recommended doses of 500 mg ceftriaxone i.m., we have not experienced treatment failure in patients treated with ceftriaxone yet (although we do not systematically test all patients treated for gonorrhoea in our clinic to see if the treatment has been successful – "test for cure"). Finally, it is likely that decreasing susceptibility to third-generation cephalosporins will lead to the loss of this class of antimicrobials for the treatment of gonorrhoea [23].

In the present paper, we show that the number of *N. gonorrhoeae* strains with reduced susceptibility to cefotaxime is sharply increasing. The increase is mainly found among MSM patients and is associated with high-risk behaviour as indicated by increased prevalence of other STIs. This differs from the recently published outbreak in Greece, in which 17 patients were infected with NG strains of reduced susceptibility to cephalosporins after casual male-to-female sexual contacts [18].

N. gonorrhoeae with reduced cefotaxime susceptibility found mainly among MSM

A sharp increase in the percentage of NG isolates with decreased susceptibility to cefotaxime has been observed since the last quarter of 2007 and the rising trend was significant for the whole study period. During the last three quarters of 2008 the prevalence of NG isolates with reduced susceptibility to cefotaxime was continuously above 10% which indicates sustained circulation of these strains.

Patients bearing NG strains with reduced cefotaxime susceptibility (MIC >0.125 µg/ml) were significantly more often 35 years old or older, MSM, HIV–positive, and had concurrent STI, compared to those with cefotaxime-susceptible NG strains. Similar characteristics (MSM, >=35 years, multiple concurrent and previously documented STI, especially HIV) were also identified in patients with emerging STIs like lymphogranuloma venereum and sexually acquired hepatitis C [24,25].

The present finding of NG strains with reduced susceptibility to cefotaxime and multiple resistances to other antibiotics circulating in this MSM core group once again underlines the importance of tailored and intensified STI care for high-risk MSM patients focused on multiple concurrent chronic and incident STI infections. This is important for the individual patient but also for the population at large because emerging STIs circulating within a core group can easily spread to the population at large as experienced with ciprofloxacin-resistant NG strains in the Netherlands.

The incidence of NG isolates with reduced susceptibility to cefotaxime was highly associated with MSM patients, also in multivariate analysis. For this reason we focused the second part of our analysis on the characteristics of NG strains collected from MSM patients only. We did not find an association between antibiotic susceptibility to cefotaxime and the various collection sites. Almost half of the NG strains originated from rectal swabs, both among NG strains susceptible to cefotaxime (47.1%) and among those with reduced cefotaxime susceptibility (49.5%) (Table 2). Rectal gonorrhoea infections are an increasing problem among MSM as reported earlier, and should always be considered since many of these infections are asymptomatic [26].

Multidrug-resistance common in NG strains with reduced susceptibility for cefotaxime

Among the NG isolates with reduced susceptibility to cefotaxime obtained from MSM patients, a considerable number was found resistant to multiple antibiotics such as penicillin, tetracycline and ciprofloxacin. This implies that if the trend of reduced susceptibility to cefotaxime progresses towards resistance to all third-generation

Table 2

Collection site of 1,231 isolates obtained from 1,075 men who have sex with men; STI outpatient clinic, Amsterdam, the Netherlands 2006-2008

Site	cefotaxime MIC ≤ 0.125 µg/ml (n=1,134)	cefotaxime MIC > 0.125 µg/ml (n=97)	Overall p value[1]
Urethra	469 (41.4%)	41 (42.3%)	0.61
Rectum	534 (47.1%)	48 (49.5%)	
Pharynx	131 (11.6%)	8 (8.2%)	

Data are the number of isolates (% of total) including 1,134 isolates from 985 patients with a cefotaxime MIC value ≤ 0.125 µg/ml and 97 isolates from 90 patients with a cefotaxime MIC value > 0.125 µg/ml.
1p value based on chi^2 test.

Table 3

Other antibiotic resistance characteristics of 1,231 isolates obtained from 1,075 men who have sex with men; STI outpatient clinic, Amsterdam, the Netherlands, 2006-2008

Antibiotic resistance	cefotaxime MIC ≤ 0.125 µg/ml, (n= 985)	cefotaxime MIC > 0.125 µg/ml, (n=90)	Overall p value[1]
Penicillin resistance[2]	162 (16.5%)	39 (43.3%)	<0.001
Tetracycline resistance[3]	211 (21.5%)	62 (68.9%)	<0.001
Ciprofloxacin resistance[4]	437 (44.4%)	81 (90.0%)	<0.001
Resistance to at least two of the following: penicillin, tetracycline and ciprofloxacin[5]	224 (22.9%)	69 (77.5%)	<0.001
Resistance to all three antibiotics: penicillin, tetracycline and ciprofloxacin[5]	46 (4.7%)	27 (30.3%)	<0.001

Data are the number of isolates (% of total).
1p values are based on chi^2 test.
2For penicillin either chromosomal or plasmid-mediated resistance, missing data on penicillin resistance (excluded from analysis) n=4.
3Missing data on tetracycline resistance (excluded) n=2.
4Missing data on ciprofloxacin resistance (excluded) n=2.
5Missing data (excluded) n=6.

cephalosporins, a switch to previously recommended antibiotics for gonorrhoea will not be an option. It has been suggested that spectinomycin, which is structurally unrelated to third-generation cephalosporins, should be used as the primary therapy for gonorrhoea but this drug is not available everywhere, at least not in the Netherlands [14]. Other treatment options are gentamycin, carbapenems and dual antibiotic therapy [6].

Although we only included high-risk patients in our analysis and excluded the low-risk group we do not think that this could have led to significant bias since the prevalence of NG among low-risk visitors was only 0.5%. Moreover, we compared the selection of patients diagnosed with gonorrhoea with available MIC values to those without MIC data. Being MSM was the only characteristic overrepresented in the group without MIC data. Since the compositions of the two groups were similar, the group with MIC info can be considered representative of the whole.

At present we are working on the molecular typing of the NG isolates with decreased susceptibility to ceftriaxone to investigate if the cefixime-associated mosaic patterns of the *penA* gene are also associated with our findings. The increased prevalence of NG strains with reduced susceptibility to cefotaxime among MSM may herald the evolution towards third-generation cephalosporin-resistant NG strains and this trend needs to be closely monitored. Gonorrhoea can still be treated effectively with third-generation parenteral cephalosporins, however, previous experience has demonstrated that the use of increased doses of antimicrobials only postpones the development of resistance, but does not prevent the eventual demise of the drug.

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Biogeographical origin and varicella risk in the adult immigration population in Catalonia, Spain (2004-2006)

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Immigrants to the European Union may have a higher susceptibility to varicella-zoster virus primo-infection than the indigenous population. There is no evidence as yet that this is caused by genetic or social factors. Therefore, susceptibility could be due to a lesser transmission of the virus in their ecosystems of origin. A multicentre observational study was performed from July 2004 to June 2006 in four primary healthcare centres in Catalonia, Spain, monitoring varicella incidences and comparing standardised incidence rates and standardised rate ratios among different populations classified according to their biogeographical origin (holarctic, Asian paleotropical, African paleotropical or neotropical). Overall, 516 varicella cases were recorded. The standardised incidence rates per 1,000 inhabitants per year were: holarctic: 2.17 (95% confidence interval (CI): 1.95-2.39); autochthonous 2.26 (95% CI: 2.03-2.49); immigrants 3.59 (95% CI: 2.92-4.26); neotropical 4.50 (95% CI: 3.28-5.71); non-holarctic 5.38 (95% CI: 4.27-6.14); Asian paleotropical 7.03 (95% CI: 4.77-9.28); and African paleotropical 7.05 (95% CI: 1.12-23.58). The difference to the autochthonous population was greatest in immigrants of neotropical origin (standardised rate ratio = 2.07 (95% CI: 1.61-2.64) or 4.5 excess cases per 1,000 inhabitants per year) and Asian paleotropical origin (standardised rate ratio = 3.24 (95% CI: 2.47-4.11) or 9.6 excess cases per 1,000 inhabitants per year). Biogeographical origin may therefore account for the vulnerability of certain immigrant populations to varicella, in particular those from Asian paleotropical (Indostan and Southest Asia) and neotropical (South America and the Caribbean) ecosystems. Vaccination of immigrants at high risk (fertile women, healthcare workers) could be recommendable.

Introduction

Varicella is typically a childhood disease (87% of the cases in Spain between 1997 and 2004 were reported in children under the age of 15 years) caused by varicella-zoster virus (VZV)-induced primo-infection [1]. Although the disease is usually self-limiting, some cases can be serious, with 2-6% of them resulting in complications and a hospitalisation rate in the European Union ranging from 1.3 to 4.5 per 100,000 population per year [2]. While the disease is universally distributed, some studies (based on comparison of seroprevalence) point to a greater incidence in cold or temperate northern hemisphere countries – holarctic ecosystem – particularly in winter and spring. There is no explanation to date for the possible lower incidence in Asian, African and South American climates – Asian paleotropical, African paleotropical and neotropical ecosystems, respectively [3]. No hypothesis points to an explanation based on genetics; consequently, it has generally been attributed to a mixture of underreporting and ecological reasons, i.e. VZV would be less transmissible in those ecosystems, although the mechanisms responsible have never been accurately defined [4,5].

However, if these ecological factors do exist, more varicella cases should be seen in the adult immigrant population from tropical-subtropical climates compared to the autochthonous population in Spain who would have a greater degree of immunity from childhood. Furthermore, significant differences should exist between populations of holarctic origin and those of other origin, but not between holarctic immigrants and the autochthonous population.

After centuries of net emigration, Spain has experienced large-scale immigration since the year 2000. Currently, the country has the second highest immigration rate of Western Europe just after Cyprus [6]. According to official statistics, 4,144,000 immigrant residents were recorded in Spain in 2005, of whom over 1,500,000 were from Morocco, Ecuador and Romania [6]. Therefore, Spain represents an excellent platform to carry out such a study since the country received a large number of immigrants over a relatively short period of time, which may allow verifying epidemiologically whether or not there was an increase in the incidence of varicella within the immigrant population. Such an analysis of varicella

incidence should take into account the ecological (biogeographical) and not the geopolitical (according to nationality) origin of the subjects [7-9].

The aim of the present study was to ascertain and compare varicella incidence rates in autochthonous and immigrant populations in Spain according to their biogeographical origin using case registration analysis in primary healthcare centres. It further aimed at discussing the value of the findings with regard to prevention policies to be employed in a country which, in the medium term, is going to receive many more immigrants.

Methods

This was a multicentre, longitudinal study based on new varicella cases in adults (>14 years, i.e. the unvaccinated population, as vaccination was included in the official vaccination calendar in 2004) from four primary healthcare centres (Centre d'Atenció Primaria, CAP) with a substantial immigrant population, registered between July 2004 and June 2006. Two of these centres are located in Santa Coloma de Gramenet, one in the city of Barcelona and one in Sant Adrià del Besòs. All belong to the Catalan Health Institute (Institut Català de la Salut). The 59 general practitioners (GPs) working in these centres were all blinded to the study question.

The study population consisted of all patients assigned to the four primary healthcare centres. According to data from the Primary Health Informatics System, this comprised 103,902 patients in June 2008. This is a local registration under the primary healthcare system and therefore may reflect more complete and reliable data than census or other general records.

All individuals born in the World Health Oganization (WHO) European Region were defined as autochthonous, and those born outside as immigrants. People born in WHO European Region to immigrant parents were considered to be autochthonous. Classification of the study population according to their ecological zone of origin was based on classical bioregion mapping, which divides the emerged land surfaces into seven zones (Figure) [10]:

- the holarctic region (North America, Europe, Maghreb, Near East, Central Asia, Siberia, China, Korea and Japan),
- the African paleotropical region (sub-Sahara Africa except the western half of South Africa),
- the Asian paleotropical (Indian sub-continent and Southeast Asia),
- the neotropical region (Central America, Caribbean islands and South America),
- and three other regions (South Africa, Antarctica and Oceania) that were excluded since no immigrants from these regions were registered during the study period.

For example, the autochthonous population and immigrants from Morocco or China were considered 'holarctic', while immigrants from Ecuador were classified as 'neotropical'.

A varicella case was defined as a patient registered by the GP as varicella infection in the 'conditions and problems' (diagnosis) section of the computerised clinical records. Cases labelled as 'suspected' or 'probable' were excluded. The variables of age, sex and biogeographical origin were collected.

Age-specific and crude incidence rates were calculated using the population of the four healthcare facilities in the study area as the denominator. Rates were stratified by sex and biogeographical origin, and standardised by the direct method, using the population of Catalonia as a reference according to data available from the 2005 Catalan Statistics Institute [11]. Confidence intervals (CI) for the age-specific and age-standardised rates were calculated using the normal distribution in all groups except for the group of African paleotropical immigrants that contained such a small number of cases that the exact approximation method was used.

Age-standardised rate ratios (ASRR) were defined as the incidence rates of varicella in each group divided by the corresponding incidence rate in the reference group. CIs of ASRR were calculated using the median unbiased estimation method; the CIs which did not include the value '1' were considered significant ($p<0.05$) [12]. Subtraction among standardised rate ratios (SRR) represented the excess or deficit of cases per 1,000 inhabitants per year compared to the incidence of the autochthonous population once the differences attributable to population structure had been eliminated.

The EPIDAT 3.0 and R programmes were used for calculating the incidence rates and their respective CIs.

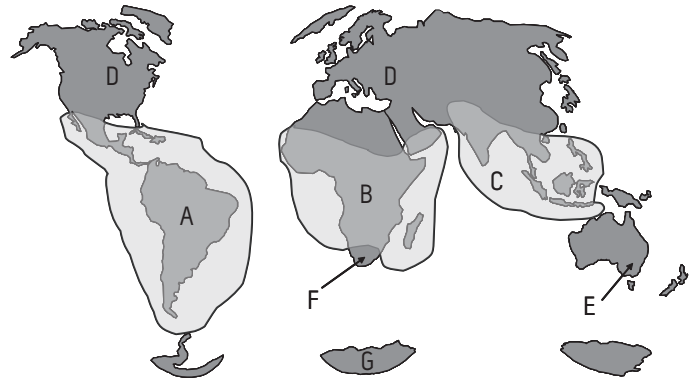
Results

The study population comprised 103,902 individuals with medical records in their healthcare centres. Of these, 14,387 (13.8%) were immigrants. According to the biogeographical origin of those immigrants, 5,470 (5.3%) belonged to the holarctic, 2,806 (2.7%) to the Asian paleotropical, 338 (0.3%) to the African paleotropical, and 5,773 (5.6%) to the neotropical ecosystem.

There were 516 recorded cases of varicella: 296 (57.4%) men and 220 (42.6%) women with a mean age of 28.1 (standard deviation (SD) 10.5) years. The total incidence of the disease in the adult population, standardised according to age and sex, was 2.44 (95% CI: 2.23-2.65) cases per 1,000 inhabitants per year. According to biogeographical origin, the incidence rates standardised per 1,000 persons per year were, in ascending order: holarctic 2.17 (95% CI: 1.95-2.39), autochthonous 2.25 (95% CI:

Figure

Biogeographical regions of the world



A: Latin America (neotropical); B Africa (African paleotropical); C: Tropical Asia (Asian paleotropical); D: Holoarctic; E: Oceania; F: South Africa; G: Antarctica.

2.02-2.47), all immigrants 3.59 (95% CI: 2.92-4.26), neotropical immigrants 4.50 (95% CI: 3.28-5.91), non-holarctic immigrants 5.38 (95% CI: 4.27-6.14), Asian paleotropical immigrants 7.03 (95% CI: 4.77-9.28) and African paleotropical immigrants 7.05 (95% CI: 1.12-23.58).

The crude rates obtained during the study period and those standardised for age are presented in Tables 1-3. Crude and age-standardised rate ratios according to biogeographical origin are shown in Table 4. The resulting excess of varicella cases per 1,000 inhabitants is displayed in Table 5.

Table 1

Crude, age-specific and age-standardised incidence rates of varicella, by geographical origin (autochthonous/immigrants) and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex and age group	Autochtonous			Immigrants			Total		
	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)
Male									
15-34 years	147	12,768	5.75 (4.82-6.68)	73	4,642	7.86 (6.6-9.66)	220	17,410	6.32 (5.48-7.15)
35+ years	62	27,887	1.11 (0.83-1.37)	14	3,546	1.97 (0.94-3.01)	76	31,433	1.19 (0.92-1.46)
Total / crude rate	209	40,655	2.57 (2.23-2.93)	87	8,188	5.32 (4.25-6.55)	296	48,843	3.00 (2.65-3.34)
Age-standardised rate			2.66 (1.33-3.04)			3.94 (3.03-4.86)			2.91 (2.58-3.24)
Female									
15-34 years	134	14,422	4.64 (3.86-5.43)	40	3,637	5.50 (3.79-7.20)	174	18,059	4.82 (4.10-5.54)
35+ years	36	34,438	0.52 (0.35-0.68)	10	2,562	1.95 (0.74-3.16)	46	37,000	0.62 (0.44-0.80)
Total / crude rate	170	48,860	1.73 (1.48-2.01)	50	6,199	4.04 (2.99-5.31)	220	55,059	1,99 (1.73-2.26)
Age-standardised rate			1.90 (1.61-2.18)			3.14 (2.15-4.12)			2.02 (1.75-2.29)
Total									
15-34 years	281	27,190	5.16 (4.56-5.77)	113	8,279	6.82 (5.65-8.33)	394	35,469	5.55 (5.01-6.11)
35+ years	98	62,325	0.78 (0.62-0.93)	24	6,108	1.96 (1.18-2.75)	122	68,433	0.89 (073-1.1)
Total / crude rate	379	89,515	2.11 (1.90-2.34)	137	14,387	4.76 (3.99-5.63)	516	103,902	2.48 (2.26-270)
Age-standardised rate			2.26 (2.03-2.49)			3.59 (2.92-4.26)			2.45 (2.24-2.67)

Rates are per 1,000 inhabitants per year. The age-standardised rates were calculated using the age distribution of the 2005 Catalan population census and confidence intervals (CI) rates of 95%.

Table 2

Crude, age-specific and age-standardised incidence rates of varicella by biogeographical origin (holarctic/non-holarctic) and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex and age groups	Holarctic			Non-holarctic			Total		
	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)
Male									
15-34 years	152	13,950	5.45 (4.58-6.31)	68	3,079	11.04 (8.42-13.66)	220	17,029	6.46 (5.63-7.37)
35+ years	62	29,391	1.05 (0.79-1.32)	14	1,865	3.75 (1.78-5.72)	76	31,256	1.21 (0.96-1.52)
Total / crude rate	214	43,341	2.47 (2.15-2.82)	82	4,944	8.29 (6.59-10.29)	296	48,285	3.07 (2.72-3.43)
Age-standardised rate			2.52 (2.19-2.86)			6.19 (4.61-7.77)			2.97 (2.63-3.31)
Female									
15-34 years	139	15,300	4.54 (3.78-5.30)	35	2,504	6.99 (4.67-9.31)	174	17,804	4.88 (4.17-5.67)
35+ years	37	35,534	0.52 (0.36-0.69)	9	1,469	3.65 (1.06-5.06)	46	37,003	0.62 (0.46-0.88)
Total / crude rate	176	50,834	1.73 (1.48-4.00)	44	3,973	6.54 (4.03-7.43)	220	54,807	2.00 (1.75-2.29)
Age-standardised rate			1.86 (1.59-2.14)			4.37 (2.83-5.92)			2.05 (1.28-2.32)
Total									
15-34 years	291	29,250	4.97 (4.41-5.54)	103	5,583	9.22 (7.44-11.00)	394	34,833	5.65 (5.10-6.23)
35+ years	99	64,925	0.76 (0.61-0.91)	23	3,334	3.45 (2.04-4.86)	122	68,259	0.89 (0.74-1.07)
Total / crude rate	390	94,175	2.07 (1.87-2.28)	126	8,917	7.07 (5.88-8.41)	516	103,902	2.50 (2.28-2.70)
Age-standardised rate			2.17 (1.95-2.39)			5.38 (4.27-6.14)			2.49 (2.27-2.70)

Rates are per 1,000 inhabitants per year. Age-standardised rates were calculated using the age distribution of the 2005 Catalan population census and confidence intervals (CI) rates of 95%.

In summary, significant differences in the varicella incidence rates were found between the autochthonous and immigrant populations. These differences were accentuated when the group of holarctic populations was compared with the group of non-holarctic populations, and when the holarctic population was compared to the neotropical and, particularly, Asian paleotropical populations. In contrast, no statistically significant differences were observed when the autochthonous and holarctic populations were compared.

Discussion and conclusions

The possible epidemiological changes in the number of varicella infections due to the influx of immigrants had already been noted

Table 3

Crude, age-specific and age-standardised incidence rates of varicella by biogeographical origin and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex and age groups	Holarctic			Asian paleotropical			African paleotropical			Neotropical		
	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI)	Number of cases	Study population	Rate (95% CI) [1]	Number of cases	Study population	Rate (95% CI)
Male												
15-34 years	152	13,950	5.45 (4.58-6.31)	27	1,418	9.52 (6.28-13.58)	1	156	3.21 (0.8-17.86)	40	1,505	13.29 (9.49-18.09)
35+ years	62	29,391	1.05 (0.79-1.32)	9	922	4.88 (2.23-9.25)	0	90	0.00	5	853	2.93 (0.95-6.84)
Total / crude rate	214	43,341	2.47 (2.15-2.82)	36	2,340	7.69 (5.39-10.65)	1	246	2.04 (0.05-11.33)	45	2,358	9.53 (6.96-12.77)
Age-standardised rate			2.52 (2.19-2.86)			6.43 (3.99-8.87)			1.07 (0.003-1.47)			6.40 (4.20-8.59)
Female												
15-34 years	139	15,300	4.54 (3.78-5.30)	11	253	21.74 (10.85-38.89)	0	77	0.00	24	2,174	6.52 (3.53-8.21)
35+ years	37	35,534	0.52 (0.36-0.69)	2	213	4.69 (0.57-16.96)	2	15	66.66 (8.08-240.82)	5	1,241	2.02 (0.65-4.70)
Total / crude rate	176	50,834	1.73 (1.48-4.00)	13	466	13.95 (7.43-23.85)	2	92	10.87 (1.31.-39.26)	29	3,415	4.24 (2.84-6.10)
Age-standardised rate			1.86 (1.59-2.14)			10.40 (4.30-16.5)			44.36 (5.37-160.22)			3.19 (1.80-4.57)
Total												
15-34 years	291	29,250	4.97 (4.41-5.54)	38	1,671	11.37 (8.04-15.60)	1	233	1.14 (0.06-11.95)	64	3,679	8.7 (6.70-9.81)
35+ years	99	64,925	0.76 (0.61-0.91)	11	1,135	4.84 (2.42-8.67)	2	105	9.52 (1.15-34.40)	10	2,094	2.39 (1.14-4.39)
Total / crude rate	390	94,175	2.07 (1.87-2.28)	49	2,806	8.73 (6.46-11.54)	3	338	4.44 (0.91-12.97)	74	5,773	6.41 (5.04-8.05)
Age-standardised rate			2.17 (1.95-2.39)			7.03 (4.77-9.28)			7.05 (1.12-23.58)			4.50 (3.28-5.71)

Rates are per 1,000 inhabitants per year. Age-standardised rates were calculated using the age distribution of the 2005 Catalan population census and confidence intervals (CI) rates of 95 %.
1 Because of the small number of cases for the African paleotropical group, 95% CIs were calculated using exact methods.

Table 4

Crude rate ratio (CRR) and age-standardised rate ratio (ASRR) for varicella by geographical and biogeographical origin and sex in the urban health area of Barcelonès nord-Maresme, Catalonia, Spain, 2004-2006

Sex	Male		Female		Total	
Geographical and biogeographical origin	CRR (95% CI)	ASRR (95% CI)	CRR (95% CI)	ASRR (95% CI)	CRR (95% CI)	ASRR (95% CI)
Autochtonous	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Immigrants	2.09 (1.61-2.67)	1.48 (1.14-1.87)	2.33 (1.69-3.18)	1.65 (1.19-2.24)	2.27 (1.86-2.75)	1.60 (1.25-1.96)
Holarctic	1(reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Asian paleotropical	3.12 (2.16-4.39)	2.55 (1.79-3.60)	8.15 (4.40-13.75)	5.57 (3.21-9.51)	4.22 (3.10-5.63)	3.24 (2.47-4.11)
African paleotropical	0.94 (0.04-4.10)	0.42 (0.01-3.89)	6.76 (1.05-21.02)	23.76 (5.94-69.2)	2.25 (0.54-5.88)	3.25 (0.8-5.84)
Neotropical	3.87 (2.77-5.29)	2.53 (1.83-3.44)	2.45 (1.63-3.59)	1.71 (1.26-2.03)	3.10 (2.40-3.95)	2.07 (1.61-2.64)
Non-holarctic [1]	3.36 (2.59-4.32)	2.45 (1.88-3.11)	3.20 (2.28-4.42)	2.34 (1.68-3.24)	3.41 (2.78-4.16)	2.48 (2.03-3.02)

Rate ratio is statistically significant (p<0.05) if non-holarctic group is not included.
1 Non-holarctic represent Asian paleotropical, African paleotropical and neotropical grouped together.

in the mid-twentieth century. Causes of the changes have been assumed to be: the presence of adult immigrants susceptible to the disease, and the introduction of new strains of VZV [13,14]. Those studies were based on seroprevalence – generally on samples from blood banks – and on genetic analyses of the isolated viruses. Subsequently, a substantial series of articles was published that warned of epidemic outbreaks in immigrant communities and their immunological vulnerability. Articles cited in PubMed between 1997 and 2007 defined as risk communities immigrants from the Indian subcontinent [15-17], South America [18,19], Africa [20] or a combination of two of them [21]. Only one study (in Oceania, a non-holarctic ecosystem) did not report differences between immigrants and autochthonous individuals [22]. Although the evidence provided in these studies is based solely on the greater seronegativity in immigrants, they clearly suggest that this vulnerability is due to factors related to the transmissibility of VZV in their respective ecosystems of origin. No hypothesis regarding genetic or cultural causes was raised.

The present study is consistent with the approaches above in that it confirms – from a purely epidemiological point of view – higher incidence rates in individuals from ecological environments that are very different from the European or holarctic region. Furthermore, the fact that no differences were found between the autochthonous European population and immigrants of other holarctic biogeographical origin (i.e. people from the Maghreb, Near East, central Asia, China and North America) is not only in line with the ecological hypothesis but an argument against a role of genetic factors. One of the limitations of this study is the lack of a comparative analysis of varicella incidence rates in children and further research in that direction should be encouraged. One would expect the differences between the groups to disappear when comparing rates in children of autochthonous families and children of immigrant families born in Europe.

Two further limitations of the study should be noted: firstly, the possible bias due to different social situations in the study groups (e.g. housing conditions or the proportion of women of childbearing age) that could facilitate the spread of the infection in a given group, and secondly, the strictly epidemiological approach of the study which did not take into account seroprevalence. Also, immigrants from certain areas, particularly Africa, could be under-registred in Spain and therefore cause a bias. Nevertheless, the local demographic records used are considered trustworthy and, thus, any bias was likely small.

Table 5

Excess of cases per 1,000 inhabitants by biogeographical origin with respect to the autochthonous population, Catalonia, Spain, 2004-2006

Biogeographical origin	Excess of cases per 1,000 inhabitants
Autochthonous	0 (reference)
African paleotropical	-1.25
Holarctic	0.16
All immigrants	2.68*
Neotropical	4.5*
Non-holarctic	6.26*
Asian paleotropical	9.56*

*p< 0.05

The observed differences were most marked in male individuals under the age of 35 years. However, this may be a coincidental observation and due to the limited number of cases in people over 35 years of age. Nevertheless, the differences between the sexes could be due to possible inequalities in the access to healthcare for female immigrants who may be discriminated against. Other epidemiology-based studies also raised this possibility [23,24].

Similarly, the African paleotropical population was not strongly represented which makes it difficult to draw definitive conclusions. The incidence CI in this group compared with the autochthonous and holarctic populations was not significant for the men, but significant for the women. Only one earlier study has described an African paleotropical population with a seroprevalence of antibodies against VZV that was significantly lower than that in the European population [18], but these were immigrants from East Africa (refugees from Somalia) who are very rare in Spain and therefore do not allow a comparison with our study. From our data, we can only conclude that significant differences do not appear to exist between the sub-Saharan African population from West Africa, the largest in Spain and the local autochthonous population.

The vulnerability to varicella in immigrants from the holarctic ecosystem (i.e. China or Turkey) appeared to follow a similar age pattern as in the autochthonous population. Both groups had fewer cases in adults than in children. This probably reflects a higher VZV transmission in childhood and the development of permanent immunity after infection. In contrast, immigrants from the neotropical and, particularly, Asian paleotropical ecosystems show an epidemiological pattern typical of an adult population with greater susceptibility to the disease [25].

All varicella cases were identified according to the diagnosis made by 59 GPs. Considering their experience, this purely clinical ascertainment, albeit subjective, is likely to be reliable. There is no reason to suppose that the diagnosis should be more or less accurate for immigrants than for autochthonous groups.

Preventive measures for these populations with lower immunity should be considered. Although determining the cost-effectiveness of a vaccination against VZV was not the aim of this study, it should be emphasised that some institutions do recommend (United States Army) [26] or consider it (Catalan Autonomous Government) [27]. Studies that supported the inclusion of the chickenpox vaccine in the childhood vaccination schedule defined an incidence threshold for cost-effectiveness at much lower rates than those found in vulnerable immigrant communities [28]. Selective vaccination of the young adult population from South America or the Indian subcontinent without a history of chickenpox in childhood would very likely be cost-effective as far as direct and particularly indirect expenses (loss of productivity) are concerned [29]. Within these populations, two high-risk groups can be defined: women of childbearing age, in order to protect them against varicella infection during pregnancy and to ensure the transplacental diffusion of maternal antibodies to the foetus [30,31], and immigrant healthcare personnel, who are at a high risk of acquiring the infection [32]. An additional argument in favour of selective vaccination is the possibility that immigrants might introduce holarctic strains of VZV to their countries of origin when visiting their families. Such transcontinental spreads caused the measles epidemic in Ecuador in 2008 [33] and, probably, the outbreak of German measles in Brazil in 2007 [34].

Those high-risk target populations could be vaccinated without the need for a serological study [35]. In countries with limited resources such as Spain (where patients must purchase a vaccine at prices ranging from EUR 45 to 60), a possible strategy aimed at increasing social acceptance would be to test healthy immigrant women for varicella antibodies (usually accounting for less than EUR 15) in primary care screening programmes, which usually include the routine test for German measles [36].

Varicella vaccination would surely be indicated for adult immigrants living together with child index cases, since epidemic outbreaks with a high attack rate within families have been described [37]. Thereby, cases of chickenpox in pregnant women and neonates could be avoided, the two groups in whom severe cases occur most frequently. This would require an improved coordination of GPs and epidemiologists, a faster response to an increase in the number of cases, and easier access to vaccination without costs for the individuals [38].

In conclusion, certain immigrant populations, particularly from neotropical and Asian paleotropical biogeographic origin, present a higher incidence rate of varicella than autochthonous inhabitants of Spain and other holarctic populations. The reasons for this elevated vulnerability probably depend on ecological factors that limit transmissibility of the virus in their ecosystems of origin. Thus, the implementation of preventive measures using biogeographical origin as a criterion could be effective. Analysis of the influence of different biogeographical origins on the epidemiology of infectious microorganisms should be completed with further studies.

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Molecular characterisation of PFGE non-typable methicillin-resistant Staphylococcus aureus in the Netherlands, 2007

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In 2007 in the Netherlands, 30% of all human isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) sent to the National Institute for Public Health and the Environment could not be typed by pulsed-field gel electrophoresis (non-typable (NT)-MRSA). Molecular characterisation of the NT-MRSA isolates revealed 27 different spa types and two distinct SCC*mec* types, type IV and V. All NT-MRSA isolates were closely related based on spa and multi-locus sequence typing and belonged to the ST398 lineage. The rapid increase of NT-MRSA (ST398) isolates over the last years shows the importance of this relatively new clonal lineage.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen causing not only infections in the hospital but also in the community. Lately, the epidemiology of MRSA is changing, focusing more and more on community-associated (CA-) MRSA. CA-MRSA are often Panton-Valentine leukocidin (PVL)-positive and can cause serious skin and soft-tissue infections, but also conditions such as necrotising pneumonia [1].

Since 2005, pigs have been identified as a possible new reservoir for MRSA [2,3]. Although the pig-related MRSA isolates are almost always PVL-negative, they could be considered as CA-MRSA. Pig-related MRSA strains can easily be identified by *Sma*I pulsed-field gel electrophoresis (PFGE). These strains possess a methylation enzyme that methylates the *Sma*I restriction sites. Consequently, no banding pattern is obtained in the PFGE and no PFGE type can be assigned [4]. Furthermore, staphylococcal protein A (*spa*) typing has shown that the pig-related strains belong to specific spa types, indicating a clonal structure. This clonality was also supported by multi-locus sequence typing (MLST), since the pig-related strains were all sequence type (ST) 398 or single locus variants of ST398 (ST752 and ST753) [5]. To date, the pig-related MRSA has also been found in other farm animals such as

Table 1

Incidence of *spa* types of PFGE NT-MRSA isolates in the Netherlands in 2007 (n=793)

Spa type	No. of isolates*	% of NT-MRSA*	% of all isolates*
t011	370	46.7	14.1
t108	268	33.8	10.2
t567	42	5.3	1.6
t034	20	2.5	0.8
t899	19	2.4	0.7
t571	15	1.9	0.6
t2330	11	1.4	0.4
t2123	8	1.0	0.3
t1456, 2383	5 each	0.6	0.2
t1255, t3013	4 each	0.5	0.2
t588, t1184, t1457	3 each	0.4	0.1
t2582	2	0.3	0.1
t779, t943, t1451, t2287, t2329, t2748, t2971, t3014, t3053, t3146, t3208	1 each	0.1	0.0
Total	**793**	**100**	**30.3**

* Data per *spa* type
NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*; PFGE: pulsed-field gel electrophoresis.

horses [6], poultry [7], and is associated with cattle [5]. Therefore, it is no longer considered a pig-related MRSA clone but a livestock-related clonal lineage.

In January 2003, the first MRSA was found in the Netherlands which could not be typed by PFGE. From then on it was referred to as PFGE non-typable (NT)-MRSA. In the following years, the number of NT-MRSA increased rapidly and due to the relation of NT-MRSA with pigs, several studies were performed in order to get an idea about the MRSA carriage rate of pigs and pig farmers [2,5,8-10]. Upon the first results it became clear that pigs could be considered as a reservoir for MRSA and pig-to-human transmission had occurred [11]. Although rarely seen to date, human infections due to ST398 MRSA have occurred [12-14]. Based on the results of the NT-MRSA studies, the Dutch national MRSA guidelines were adjusted in July 2006 and November 2007 to the effect that all individuals working or living in close contact with living pigs or cattle are isolated and screened for MRSA upon admission to a hospital. As a result of this new guideline the number of detected NT-MRSA isolates increased.

In the Netherlands, all MRSA isolates are sent to the national MRSA reference centre. The present paper gives an overview of NT-MRSA in the Netherlands in 2007, the molecular characteristics of NT-MRSA and the clonal structure of these isolates. The data will show the importance of this relatively new CA-MRSA clonal lineage.

Methods

Bacterial isolates

In the Netherlands, the National Institute for Public Health and the Environment (RIVM) serves as the national reference centre for surveillance of MRSA. All first MRSA isolates of newly identified carriers, one per patient, are sent to the RIVM for typing. All MRSA isolates submitted in 2007 were used for typing.

Typing

All MRSA isolates were typed by PFGE using *Sma*I as the restriction enzyme according to the HARMONY PFGE protocol [15]. The isolates that could not be typed by PFGE were selected and used for *spa* typing [16]. New spa types were assigned with Ridom Staphtype software version 1.5.13 (Ridom GmbH). For the analysis of *spa* types and the creation of a minimum spanning tree, Bionumerics software version 5.1 (Applied Maths) was used. The PVL genes were detected by PCR according to the method described by Lina *et al.* [1]. The first 300 NT-MRSA isolates of 2007 were used to determine the SCCmec type by multiplex PCR according to Kondo et al. [17]. In case the *spa* type of an isolate was different from those found among the first 300 NT-MRSA isolates, it was also subjected to SCC*mec* typing. Thus, a total of 308 isolates were analysed for SCC*mec* type. Furthermore, at least one isolate per spa type was subjected to MLST [18].

Results

In 2007, the RIVM received 2,619 unique MRSA isolates, of which 793 (30.3%) were non-typable by PFGE. *Spa* typing of these NT-MRSA isolates revealed 27 different *spa* types. Table 1 shows the incidence of the different *spa* types.

Two dominant *spa* types, t011 and t108, accounted for 80% of all NT-MRSA isolates and eleven *spa* types were only found once. In order to determine whether the 27 different *spa* types were related to each other, the repeats of each *spa* type were aligned (Table 2), and a minimum spanning tree was made based on the *spa* types of the isolates (Figure).

All *spa* types were closely related, as confirmed by the MLST results. All isolates belonged to ST398. Two different SCC*mec* types were found among the 308 NT-MRSA that were tested: SCC*mec* type IV (n= 78) and type V (n=198) (Table 3).

For 32 isolates the SCC*mec* type could not be determined. For *spa* type t011, 139 NT-MRSA isolates were tested and were of SCC*mec* type IV (n=62) and type V (n=64), or could not be typed (n=13). Surprisingly, 98 isolates with *spa* type t108 were either SCC*mec* type V (n=93) or could not be typed (n=5). No SCC*mec* type IV was found among these 98.

Only one NT-MRSA isolate was PVL positive. This isolate had *spa* type t034 and the SCC*mec* PCR resulted in SCC*mec* type V. The PVL PCR was retested on this isolate and PVL was confirmed.

Table 2

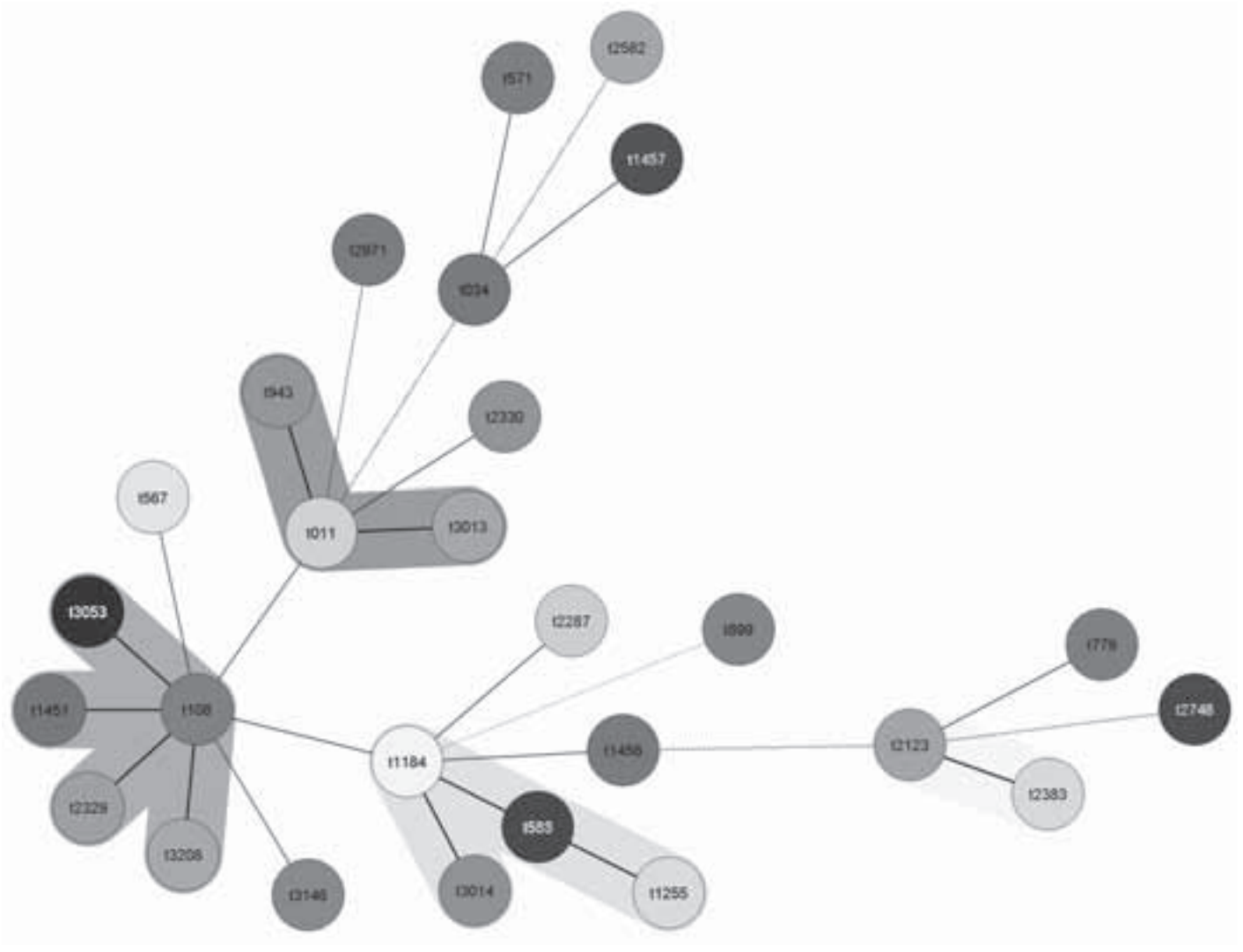
Alignment of tandem repeats* of NT-MRSA *spa* types, Netherlands, 2007

spa	repeats													
t011	8	16	-	2	25	-	-	-	-	-	34	24	25	-
t034	8	16	-	2	25	-	2	25	-	-	34	24	25	-
t108	8	16	-	2	25	-	-	-	-	-	-	24	25	-
t567	8	-	-	2	25	-	-	-	-	-	-	24	25	-
t571	8	16	-	2	25	-	2	25	-	-	34	-	25	-
t588	8	16	-	2	-	-	-	-	-	-	-	24	25	-
t779	8	-	-	-	-	-	-	-	-	-	-	-	-	-
t899	7	16	23	2	-	-	-	-	-	-	34	-	-	-
t943	8	16	-	2	25	-	-	25	-	-	-	24	25	-
t1184	8	16	-	2	25	-	-	-	-	-	-	-	25	-
t1255	8	16	-	-	-	-	-	-	-	-	34	24	25	-
t1451	8	16	-	2	25	-	-	-	-	-	34	-	25	-
t1456	8	16	-	2	25	-	-	-	-	-	-	-	-	-
t1457	8	16	-	2	25	34	2	25	-	-	34	24	25	-
t2123	8	-	-	-	25	-	-	-	-	-	-	-	-	-
t2287	8	-	-	2	25	-	-	25	-	-	-	-	-	-
t2329	8	16	-	159	25	-	-	-	-	-	-	24	25	-
t2330	8	16	-	2	25	-	-	-	-	-	34	24	25	25
t2383	8	16	-	-	-	-	-	-	-	-	-	-	-	-
t2582	8	16	-	2	25	-	2	25	2	25	34	24	25	-
t2748	26	-	-	-	-	-	-	-	-	-	-	24	25	-
t2971	8	-	23	-	25	-	-	-	-	-	34	24	25	-
t3013	8	16	-	-	-	34	-	25	-	-	34	24	25	-
t3014	8	16	-	2	65	-	-	25	-	-	-	-	-	-
t3053	8	16	-	2	65	-	-	-	-	-	-	24	25	-
t3146	8	16	-	2	-	-	-	-	-	-	-	24	25	25
t3208	8	16	-	2	25	-	-	-	-	-	-	24	24	-

* Repeats with only one base difference are mentioned in the same column, e.g. repeat 7, 8, and 26. Repeat 16 and 23 also differ in one base but because they were are also found together in *spa* type t899 they were put into a separate column.
NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*.

Figure

Genetic relatedness of 793 NT-MRSA isolates, represented as a minimum spanning tree based on *spa* types



The *spa* types are displayed as circles. Clustering was performed using the Bionumerics *spa*-plugin with a conservative alignment setting of 100% and 0% maximum duplication length. The halos surrounding the circles indicate related *spa* types.
NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*.

Table 3

SCCmec type of PFGE NT-MRSA isolates in the Netherlands in 2007 (n=308)

	spa type (n)
SCC*mec* type IV (n=78)	t011 (62), t034 (1), t571 (1), t899 (5), t1451 (1), t2123 (4), t2383 (1), t2748 (1), t2971 (1), t3013 (1)
SCC*mec* type V (n=198)	t011 (64), t034 (5), t108 (93), t567 (5), t571 (5), t588 (2), t779 (1), t899 (8), t943 (1), t1184 (1), t1255 (1), t1456 (1), t1457 (2), t2123 (1), t2329 (1), t2330 (3), t3014 (1), t3053 (1), t3146 (1), t3208 (1)
unknown SCC*mec* type (n=32)	t011 (13), t034 (4), t108 (5), t567 (8), t2287 (1), t2582 (1)
total (n=308)	

NT-MRSA: non-typable methicillin-resistant *Staphylococcus aureus*; PFGE: pulsed-field gel electrophoresis.

Discussion

In the year 2003 the first MRSA was found in the Dutch national MRSA surveillance which could not be typed by PFGE. At that time the correlation between animals and NT-MRSA was unknown. All MRSA isolates in 2002 had been typable by PFGE. The importance of NT-MRSA became clear when an increasing number of these isolates were observed. After the MRSA screening guidelines were adjusted to include screening of all hospitalised patients who had close contact with living pigs or cattle, even more NT-MRSA isolates were recorded. In 2007, the NT-MRSA clone accounted for 30% of all MRSA isolates sent to the Dutch national reference centre (see Table 1). The MRSA isolates included in this study were those found through screening of patients in healthcare settings. NT-MRSA isolates found in research programmes were excluded.

Molecular characterisation of all NT-MRSA isolates from 2007 (n=793) showed 27 different *spa* types and two different SCC*mec* types. Two dominant *spa* types, t011 and t108, accounted for 80% of all NT-MRSA isolates. Eleven *spa* types were found only once. All *spa* types formed one clonal *spa* cluster (see Table 2 and Figure). MLST was performed on at least one isolate of each *spa* type. All isolates were of the ST398 lineage, indicating the clonal structure of these isolates.

The PVL prevalence of all MRSA isolates found in 2007 was 12% (data not shown). However, only one of the NT-MRSA isolates gave a product in a PCR for the PVL genes. Infection with the bacteriophage carrying the genes for PVL seems to be less common in the ST398 lineage. Nevertheless, several reports about infections [12-14] and one outbreak [19] caused by the ST398 lineage have recently been published. Furthermore, methicillin-susceptible ST398 *S. aureus* (MSSA) have been isolated from three human cases with bacteraemia [20], indicating that the ST398 MRSA lineage could pose a serious threat to public health if it retained the virulence of ST398 MSSA.

SCC*mec* typing of the NT-MRSA isolates revealed two SCC*mec* types, IV and V. These two SCCmec types are considered to be community-associated. Furthermore, 32 isolates could not be typed by SCC*mec* , indicating the emergence of one or more new SCC*mec* type(s). Remarkable is the difference between the two most prevalent spa types, t011 and t108. In MRSA isolates with spa type t011, both SCC*mec* types were found, whereas in isolates with *spa* type t108, only SCC*mec* type V was detected. It is conceivable that MRSA *spa* type t108 SCC*mec* type V may have originated from MRSA *spa* type t011 SCC*mec* type V by deletion of repeat 34. Since SCC*mec* is a mobile genetic element that carries the *mec*A gene, it is also possible that MRSA isolates could acquire different SCC*mec* types.

The increasing number of articles concerning the ST398 clone is an indication of the international emergence of this clone. In some European countries, as in the Netherlands, *spa* type t011 also dominates [21,22], while other countries reported other *spa* types as the dominant type [13,23,24]. Outside Europe, ST398 MRSA has also been found in the United States [25,26], Canada [9], Singapore [27], and China [28]. Generally, ST398 isolates of human and animal origin showed similar molecular characteristics, being non-typable by *Sma*I PFGE and having closely related *spa* types, and SCC*mec* type IV, V, or variants thereof.

Today, the ST398 MRSA lineage is no longer specifically seen in pigs, but has also been found in other animals such as horses [6], poultry [7], dogs [13], and associated with cattle [5]. A Dutch study on the prevalence of MRSA in pigs identified *spa* types similar to the human *spa* types found in this study [2]. Currently, people in close contact with living pigs and cattle are screened upon admission to the hospital. Perhaps these guidelines will need to be adjusted again in the near future, since more animal species seem to serve as a reservoir for MRSA. Whether the number of NT-MRSA will increase even further remains to be seen. In the Netherlands, a country with a low prevalence of MRSA, transmission of MRSA within a clinical setting could be difficult to predict, as more than 80% of the NT-MRSA have either *spa* type t011 or t108.

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Struggling with recurrent Clostridium difficile infections: is donor faeces the solution?

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Patients with recurrent *Clostridium difficile* infections (CDI) in hospitals and the community constitute an increasing treatment problem. While most patients with a first infection respond to either metronidazole or oral vancomycin, therapy in recurrent *C. difficile* infections tends to fail repeatedly. Lack of alternative treatment options can be a tremendous burden, both to patients and their treating physicians. Most guidelines recommend prolonged oral vancomycin pulse and or tapering schedules, but evidence-based treatment strategies are lacking. The role of immunoglobulins, whey prepared from vaccinated cows, probiotics or other antibiotics is unclear. Since 1958 several case series and case reports describe a treatment strategy where faecal infusions are successfully given for the treatment of recurrent CDI. Restoring intestinal flora has been historically thought of as the mechanism responsible for cure in these patients. In the literature, more than 150 patients have received faeces from a healthy donor, either infused through an enema, or through a nasoduodenal or nasogastric tube. We summarise the literature regarding treatment with donor faeces for recurrent CDI, and introduce the FECAL trial, currently open for inclusion.

Introduction

Described as a commensal bacterium in 1935, it took until the late seventies, before *Clostridium difficile* was recognised as the most important causative agent of antibiotic-associated diarrhoea and colitis [1-3]. *C. difficile* infection (CDI) nowadays is a common nosocomial disease with substantial morbidity and mortality. The increasing incidence, partly due to the recent epidemics caused by the hypervirulent toxinotype III, ribotype 027 strain, and recent reports of community-associated infection in patients without predisposing conditions, illustrate the changing epidemiology of CDI [4-7]. Asymptomatic intestinal carriage of *C. difficile* in the normal population is estimated at 3-15%, but is much higher in hospitalised patients [8]. A prerequisite for the development of clinical *C. difficile* infection (CDI) is a disturbed homoeostasis of the normal intestinal flora, most often caused by previous antibiotic use or gastrointestinal surgery. Toxins produced by *C. difficile* disrupt the colonic epithelium, leading to an inflammatory response and clinical symptoms varying from mild diarrhoea to severe life-threatening pseudomembranous colitis [9].

Although most patients with a first episode of clinical infection respond either to withdrawal of prescribed antibiotics or to additional treatment with metronidazole or oral vancomycin, about 15–30% experience recurrent episodes [10]. Recurrent CDI can be defined as recurrence of symptoms within 8-10 weeks after cessation of specific antibiotic therapy, with exclusion of other enteropathogens and a positive diagnostic test for CDI. A subset of patients with recurrent CDI get into a spiral with several subsequent recurrences. In these cases, *C. difficile* becomes the largest hurdle for recovery, it contributes to increased mortality and morbidity and leads to prolonged isolation measures and additional costs [11,12]. Relapses or reinfections occur due to prolonged disturbance of intestinal flora, persistence of spores, incapacity to mount specific antibodies against *C. difficile* toxin, or an immunocompromised

Box 1

Treatment schedule for recurrent *C. difficile* infection

First recurrence
- Mild to moderate infection
  Metronidazole at a dose of 500 mg orally three times daily for 10 to 14 days
- Severe infection or unresponsiveness to or intolerance of metronidazole
  Vancomycin at a dose of 125 mg orally four times daily for 10 to 14 days

Second recurrence
Prolonged vancomycin orally in tapered and pulsed doses, for example:
125 mg four times daily for 14 days
125 mg twice daily for seven days
125 mg once daily for seven days
125 mg once every two days for eight days (four doses)
125 mg once every three days for 15 days (five doses)

Third recurrence
Vancomycin at a dose of 125 mg orally four times daily for 14 days, combined with any of the other options for recurrent infection (not evidence based):
- Intravenous immunoglobulin at a dose of 400 mg per kg body weight once every three weeks, for a total of two or three doses depending on effect.
- Vancomycin, followed by rifamycin at a dose of 400 mg twice daily for 14 days
- Healthy donor faeces installation*

* We feel that there is at this point not enough evidence to recommend the optimal time to introduce the procedure.

Adapted from Kelly CP, LaMont JT. Clostridium difficile--more difficult than ever. N Engl J Med. 2008;359(18):1932-40 [9]; Copyright© 2008 Massachusetts Medical Society. All rights reserved.

state [13,14]. Few studies have addressed treatment strategies for recurrent CDI. In general practice, oral vancomycin is prescribed, with limited efficacy. Restoring intestinal flora has been historically thought of as a logical mechanism to repair the host-defense against CDI. Infusion of faeces from healthy donors in patients with severe antibiotic-associated colitis was first described in 1958 [15]. We summarise the treatment options for recurrent CDI and give an overview of literature reports about the use of donor faeces as unconventional therapy in patients with recurrent CDI.

Treatment options for recurrent *C. difficile infection*

Antibiotic treatment

Vancomycin or metronidazole

Results of randomised clinical trials uniquely designed for treatment of recurrent CDI are lacking. Prospectively collected data can be derived from subgroup analysis of placebo-controlled studies comparing the combination of probiotics (or placebo) with oral vancomycin for treatment of CDI. Antibiotic treatment of a first recurrence in observational studies shows a success rate of 67%, both for metronidazole and vancomycin [16]. For additional recurrences, success rates as low as 35% are reported [10]. A subset of patients experience numerous recurrent episodes, and repeated antibiotic courses can be required for treatment of CDI, which may even persist for years [17]. Oral vancomycin is preferred for recurrent CDI because of the neurotoxic side effects of longstanding metronidazole therapy [18]. For a second recurrence, vancomycin taper and/or pulse schedules are commonly advised (Box 1) [19]. The aim of these interrupted regimens is to eradicate germinating *C. difficile* spores. In a stratified analysis including 136 patients with recurrent CDI derived from different study groups, tapered or pulsed therapy seemed with a recurrence rate of 14.3% more successful than a short course with vancomycin (recurrence rate 31%) [19].

Other antibiotic therapies

According to case reports and case series, rifamycin appeared effective for initial episodes of CDI. Rifamycin was also reported to be successful in 18 of 21 patients with recurrent CDI, in three different dosing regimens [20]. Of concern are reports about rifamycin-resistance of *C. difficile* after treatment failure [21,22] and the spreading of rifampicin–resistant *C. difficile* clones in hospitals with frequent use of rifamycins [23].

Table 1

Faecal therapy for recurrent *C. difficile* infections: overview of the literature

Year	Patients (male/female)	Mean age	No. of relapses	Entry diagnosis	Cured (%)	Follow-up	Donor related to recipient?	Prepared with whole bowel lavage	No of faecal infusions	Amount of faeces	Route of installation		Reference
											Upper GI	Lower GI	
1958	4 (3/1)	56	*	PMC	4 (100)	10 days	Md	No	1-3	Md	0	4 (e)	[15]
1981	16 (7/9)	56	*	PMC	13 (81)	5 days-3 years	If possible	No	1-24	Md	1	15	[34]
1984	1 (0/1)	65	6	CDI	1 (100)	9 months	Spouse	No	2x2	Md	0	1	[35]
1989	2 (1/1) i	60	3	CDI	1 (50)	6 months	Spouse/daughter	No	1	50 g	0	2	[36]
1991	1 (0/1)	64	7	CDI	1 (100)	3 days	Spouse	No	1	10 g	1	0	[37]
1994	7**	56	1-4	CDI	7 (100)	2 years	Spouse/relative	No	3	200 ml	0	7	[38]
1998	18**	Md	Md	CDI	15 (83)	Md	No	Md	1	Md	1	17	[39]
1999	32 (14/18)	27-89	Md	AAD	32 (100)	4-6 weeks	No	Md	1-2	5-10 g	0	32	[40]
2000	1 (0/1)	60	>5	CDI	1 (100)	1-6 months	Spouse	Yes	1	500 ml	0	1	[41]
2002	6 (1/5)	53	2-6	CDI/PMC	6 (100)	9-50 months	Yes	no	1	30 ml	0	6	[42]
2003	18 (5/13)	73	2-7	CDI	15 (83)	90 days	15 yes/3 no	No	1	30 g	18	0	[43]
2003	24 (11/13)	19-59	Md	CDI	20 (83)	Nd	Related and non-related donors	Yes	1-10	200-300 g	8	16	[44]
2006	5 (0/5)	82	>2	CDI	5 (100)	2,5-21 months	No	No	1	30 ml	0	5	[45]
2007	16 (5/11)	11-87	Md	CDI	15 (94)	4-6 weeks	Related and non-related donors	Yes	1-24	200-300 g	0	16	[46]
2008	7 (4/3)	67	3	CDI	7 (100)	30 days-1 year	6 yes/1 no	Yes	1-3	50-100 g	3	4	[47]
2008	1 (1/0)	69	1	CDI	1 (100)	2 days	Yes	No	1	45 g	0	1	[48]
	159				144/159 (91)						32	127	Total

AAD: antibiotic-associated diarrhoea; CDI: C. difficile-associated disease; GI: gastrointestinal tract; Md: missing data; Nd: not determined; PMC: pseudomembranous colitis

*unclear, since C. difficile at that time was not identified as the causative organism, so adequate antibiotics where not given.

** Sex unknown.

i = two patients treated with a faecal enema of which one failed. The failing patient and four others were treated with a new enema, consisting of a bacterial culture.

Teicoplanin (although not widely available and expensive) is another antibiotic with high reported efficacy against CDI, and limited data suggest that it may be effective in recurrent CDI [24,25]. A new and specific antibiotic against *C. difficile* is OPT-80 (PAR-101), which belongs to a new class of antibiotics, the macrocycles [26]. Data from a phase 3 study are awaited, and its role in recurrent disease is yet to be determined.

Non-antibiotic treatment modalities for recurrent CDI

Toxin targeted therapy

Binding of the pathogenic toxins (A and B) of *C. difficile* may contribute to clinical improvement and subsequent regression of CDI. However, toxin-targeted therapy (e.g. cholestyramine) has not been investigated for recurrent disease. Tolevamer, a non-antibiotic toxin-binding polymer appeared less successful for treatment of an initial episode of CDI than metronidazole or oral vancomycin [27]. Future studies should address the efficacy of combination regimens of tolevamer and antibiotics for treatment of (recurrent) CDI.

A whey product (mucomilk) isolated from cows inoculated with *C. difficile* and inactivated *C. difficile* toxin, containing high amounts of secretory IgA seems to prevent recurrence of CDI if given as adjuvant therapy in patients treated with metronidazole or vancomycin [28]. However, a randomised placebo-controlled study is lacking and the value for recurrent CDI is unknown. Vaccines containing formaldehyde-inactivated toxins A and B have been developed and some promising initial experience has been gained in a few patients with recurrent CDI [29].

Intravenous immunoglobulins

Intravenous administration of immunoglobulins (IVIG) can be considered a last resort for recurrent disease, in particular for patients with a suspected impaired immune response to *C. difficile*. Although case series suggest a beneficial effect of IVIG at a dose of 300-400 mg/kg body weight once every three weeks, a case control study did not show a reduction in recurrences [30,31].

Probiotics treatment for recurrent CDI

Several randomised trials have compared probiotics (containing *Lactobacillus* species or *Saccharomyces*) to placebo as an additional treatment to antibiotics in patients with CDI. Although the results are not uniformly negative, a recent Cochrane systematic review concludes that there is insufficient evidence to recommend the addition of probiotics to antibiotics in recurrent disease [32]. Furthermore, the occurrence of *Saccharomyces* fungaemia in patients treated with *Saccharomyces* strains merits attention [33].

Donor faeces infusion

In 1958, the surgeon Eiseman successfully treated four patients with severe antibiotic-induced colitis with an enema that consisted of donor faeces [15]. Following this initial publication, more than 150 patients with recurrent CDI have been described, the vast majority of whom was cured by the infusion of faeces. Recovery of normal intestinal flora was (and is) postulated to be the mechanism for cure.

Literature review and experiences with fecal infusions

Publications that contained original data (case reports, case series, uncontrolled studies) were selected in Pubmed and Embase. From references and through Google, additional publications were collected. A total of 16 publications (two abstracts, 14 full publications) were found (Table 1).

Success rate of faecal therapy

Taken together, 91% of all reported patients with recurrent CDI treated with donor faeces (n=159, see Table 1) were cured after one or more infusions. Clinical improvement can be noticed within a few days following donor faeces infusion. Follow-up rates vary from one week to two years. Many patients had a reported follow-up of less than one month, which implies that definite success rates are often lacking.

Necessity of donor screening

Early reports on faecal installation only mention that donors who had used antibiotics in the preceding months were excluded [15]. Although transmission of infectious diseases has not been reported after faecal infusions, most publications from the past decade report extensive screening of donors [40,43]. Our protocol for screening of (healthy) donors is summarised in Table 2. Most donors are sought in relative proximity of the patient (partners, relatives, household members). However, there is no rationale to exclude healthy volunteers. Many reports fail to mention the exact origin of the donors and an investigation of patient preferences is lacking. We do not apply any restrictions concerning the food intake of donors prior to donation. Although there can be potential important differences in the quality of the microbiota present in donor faeces from different individuals, historically their intestinal flora has not been analysed prior to use for faecal infusion. Information is lacking with regard to the specific groups and amount of bacteria necessary for optimal restoration of intestinal flora, thereby preventing *C. difficile* to become clinically significant.

Route of instillation

Of the reported patients, 80% were given a faecal installation through enema or colonoscope, and 20% received the faeces through a nasogastric or nasoduodenal/jejunal tube [43]. From our own experience, infusing faeces through colonoscopy is more difficult and strenuous, whereas (slow) infusion through a nasoduodenal tube seems safe and time-efficient [47]. To our knowledge, no other authors have discussed their experiences with different routes of administration. A disadvantage of a nasoduodenal/jejunal tube is that donor faeces may be difficult to install if patients have signs of diminished passage of fluids through their intestines. On the other hand, infusing faeces using this route has the advantage that the infused flora reaches the whole bowel. In the reported cases, no specific side effects were reported related to installation of faeces in the upper or lower tract. With the limited numbers available it is not possible to predict which route of installation is more successful in curing patients from CDI.

Virtually all publications report diluting or homogenising the faeces in saline or water, prior to infusion either in the upper gastrointestinal tract through a tube, or in the colon through enema or colonoscopy. Gustafsson et al. report homogenising faeces in pasteurised cow's milk [40]. Almost all faecal preparations are processed in a normal aerobic environment. Only Schwan et al. specifically describe preparing enemas in an anaerobic cabinet [35]. In several reports it is stated that faeces are processed and infused as quickly as possible following production by the donor, in order to preserve faecal flora. Due to lack of detailed data it is not possible to establish a relationship between a prolonged time that has passed between production and infusion, and failure of therapy.

Pre-treatment

Most early reports fail to mention antibiotic usage directly preceding the treatment. Aas et al. gave a protocolised antibiotic

regimen of 500 mg vancomycin orally four times a day during four days preceding faecal installation [43]. In addition to antibiotics, four publications describing 48 patients report pre-treatment with a laxative directly prior to donor faeces infusion [41,44,46,47]. Most publications do not report any other preparation, apart from Aas et al. who gave patients an oral proton pump inhibitor before intragastric installation of donor faeces [43].

We pretreat patients with 500 mg orally four times a day during four days and oral whole bowel lavage with a macrogol solution in an attempt to remove the pre-existent (pathological) flora and *C. difficile* spores prior to donor faeces installation. It is not known, however, whether this contributes to the efficacy of donor faeces infusion for recurrent CDI.

TABLE 2

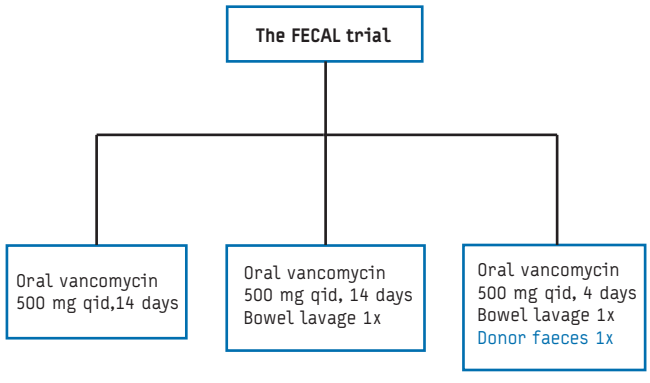
Screening of donors*

Donor	Faeces	Blood
Parasitology	Stool ova and parasites test ("Triple faeces test"[49] *Cryptosporidium* *Microsporidium*	*Strongyloides* *Entamoeba*
Microbiology	Faecal culture for common enteropathogens and *Clostridium difficile*	*Treponema pallidum*
Virology		Cytomegalovirus, Epstein-Barr virus, hepatitis A/B/C viruses Human immunodeficiency virus, human T-lymphotropic virus

*Prior to screening of faeces and blood, potential donors have to fill in an extensive questionnaire. Donors with abnormal bowel motions, abdominal complaints, symptoms indicative of irritable bowel syndrome, an extensive travel history or predisposing factors for potentially transmittable diseases are excluded. If they are considered eligible after completing the questionnaire, they are screened using the protocol above.

FIGURE

Design of the FECAL trial



qid: four times a day.

Side effects or potential adverse effects

Side effects are absent or not mentioned in all but one study which mentions (transient) side effects such as a sore throat following placement of the nasoduodenal tube, rectal discomfort following colonoscopy, flatulence, nausea and bloating [46]. We did not notice side effects in our patients treated with donor faeces infusions [47]. A possible complication could be bacterial overgrowth in the small intestine after intragastric or duodenal installation of faeces. In patients who have signs of diminished intestinal passage, infusion of faeces via the upper gastrointestinal tract should be avoided.

Faecal therapy to Eliminate *Clostridium difficile*-Associated Longstanding diarrhoea: the FECAL trial

To investigate the efficacy of faecal installations for recurrent CDI, a randomised trial comparing donor faeces infusion to conventional antibiotic treatment with oral vancomycin has been initiated in 2008 in the Netherlands. The trial follows a pilot study in which seven consecutive patients with recurrent CDI were successfully treated with one or more infusions of donor faeces [47]. Patients (over 18 years of age) are eligible if they have a proven relapse of CDI and are able to give informed consent. They are excluded if they are severely immunocompromised, have a life expectancy of less than three months, are admitted to the intensive care unit, need vasopressive therapy or if they are using antibiotics other than for the treatment of *C. difficile* for a prolonged period of time. The primary endpoint is response to treatment at 10 weeks after initiation of therapy. Secondary endpoints are response at five weeks, time nursed in isolation, and quality-adjusted life-years.

Response is defined as: absence of diarrhoea (diarrhoea is defined as ≥3 loose or watery stools per day for at least two consecutive days or ≥8 loose or watery stools in 48 hours), or persisting diarrhoea (due to other causes) with repeating (three times) negative stool tests for toxins of *C. difficile*. Treatment failure is defined as persisting diarrhoea with a positive *C. difficile* toxin stool test.

Eligible patients who have signed informed consent are randomised to one of three different treatment arms (Figure).

The conventional treatment arm (the control arm) consists of 500 mg vancomycin, given orally four times a day, for 14 days. The second treatment arm consists of 500 mg vancomycin, given orally four times a day for 14 days, combined with a whole bowel lavage by drinking four litres of a macrogol solution, taken on day four or five after initiation of the antibiotics. This arm serves as a second control arm to assess the role of whole bowel lavage in the treatment of recurrent CDI [50], since patients randomised to donor faeces infusion are also pre-treated with a bowel lavage. The

BOX 2

Amsterdam protocol used for the preparation of donor faeces

1. Faeces are collected and weighed (ca. 60-120 g, depending on production);
2. 300-400 cm3 Saline (0.9% NaCl) is added and mixed until a smooth suspension is created;
3. Faeces are poured through a double gauze and put in a glass bottle;
4. Within six hours after production by the donor, the faeces are installed through a nasojejunal tube

third (experimental) arm consists of treatment with a suspension of faeces. Patients are pre-treated with vancomycin given orally for four days and a whole bowel lavage on the fourth day. In the period before randomisation and faecal infusion, treatment is often necessary to prevent spread and deterioration of the clinical condition. Furthermore, it is logistically difficult to give a faecal infusion directly after verifying the diagnosis. We believe it may be beneficial to prepare the bowel with a short course of vancomycin for the above mentioned reasons. In the protocol, a standardised preparation period of four days prior to the faecal infusion was chosen. On the fifth day, donor faeces (Box 2 and Table 2) are infused through a nasoduodenal tube. The nasoduodenal tube is placed radiologically or endoscopically. If there is any doubt regarding the position, an abdominal X-ray will be performed. Faeces are installed within six hours after production by the donor. After this treatment, all antibiotics are stopped. Patients will be followed for 10 weeks after randomisation by a weekly telephone assessment of diarrhoea and by *C. difficile* culture and toxin stool tests (ELISA) done four times, on days 14, 21, 35 and 70.

Outpatients from the Netherlands as well as from outside the Netherlands are eligible for the trial if they are willing to travel to Amsterdam for inclusion and donor faeces installation. Patients who fail in one of the antibiotic arms (i.e. the vancomycin arm or the arm which combines vancomycin with a whole bowel lavage) are offered a treatment with a faecal infusion following their proven failure.

Conclusion

Recurrent *C. difficile* infections are a growing burden and a therapeutic challenge for patients and physicians. Current therapy consists of repeated courses of antibiotics with limited success rates and new therapeutic options are urgently needed. Faecal installations from healthy donors for the treatment of recurrent CDI seem a promising approach, restoring a normal bowel flora and preventing further outgrowth of *C. difficile* and its spores. To date, more than 150 patients treated with donor faeces have been reported in the literature. A 91% success rate is reported in case series and case reports. Due to a lack of clinical trials, faecal installations often are offered only to patients with more than two relapses, since it is still considered a last, uncommon, and rather distasteful rescue therapy. Currently, adult patients with proven recurrent CDI can be included in the first randomised controlled study comparing donor faeces installation with antibiotic therapy (FECAI trial).

Competing interest and funding

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An ongoing measles outbreak in Bulgaria, 2009

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After seven years without indigenous transmission of measles in Bulgaria, an increasing number of cases have been reported since 15 April 2009. By 19 June, the total number of notifications reached 84. To date, 64 were confirmed as measles cases and 15 cases, for whom laboratory results are pending, have been classified as probable. The present measles outbreak affects mostly the Roma population living in the north-eastern part of the country. The most affected age groups are young children below 1 year of age and children 1 to 9 years of age. An immunisation campaign was started in the affected administrative regions, targeting all persons from 13 months to 30 years of age who had not received the complete two-dose MMR vaccination.

Introduction

Measles has been a notifiable disease in Bulgaria since 1921. National case-based notification was initiated in 2004 and the European Union (EU) case definition and case classification have been adopted since 2005 [1,2]. The Bulgarian National program for elimination of measles and congenital rubella infection (2005-2010) was approved by the Council of Ministries of Republic of Bulgaria in 2005 [3].

Measles immunisation was introduced in Bulgaria in 1969 [4] and in 1972 it became universal. Until 1982 the routine vaccination included one dose measles vaccine administered at ≥10 months of age. During the period 1983-1992 a two-dose schedule using monovalent measles vaccine was applied, firstly at 12 months and 4 years of age, and later at 12 and 24 months of age. In 1993, the measles, mumps, rubella (MMR) vaccine was introduced into the national vaccination schedule. Until 2000, the routine measles immunisation consisted of the first dose with MMR vaccine given at 13 months of age and the second dose with monovalent measles vaccine at 12 years of age. Since 2001 a routine two-dose immunisation with MMR vaccine has been implemented, administered at 13 months and 12 years of age. According to the official data, collected by the National Center of Health Information, the vaccine coverage in Bulgaria with MMR is high (Table).

The last indigenous cases of measles in Bulgaria were reported in 2001 [5]. From 2002 to 2008 only six measles cases have been registered, all of them imported: three from China (2005); one from Ukraine (2006); one from Germany (2007) and one from United Kingdom (2008) [6,7].

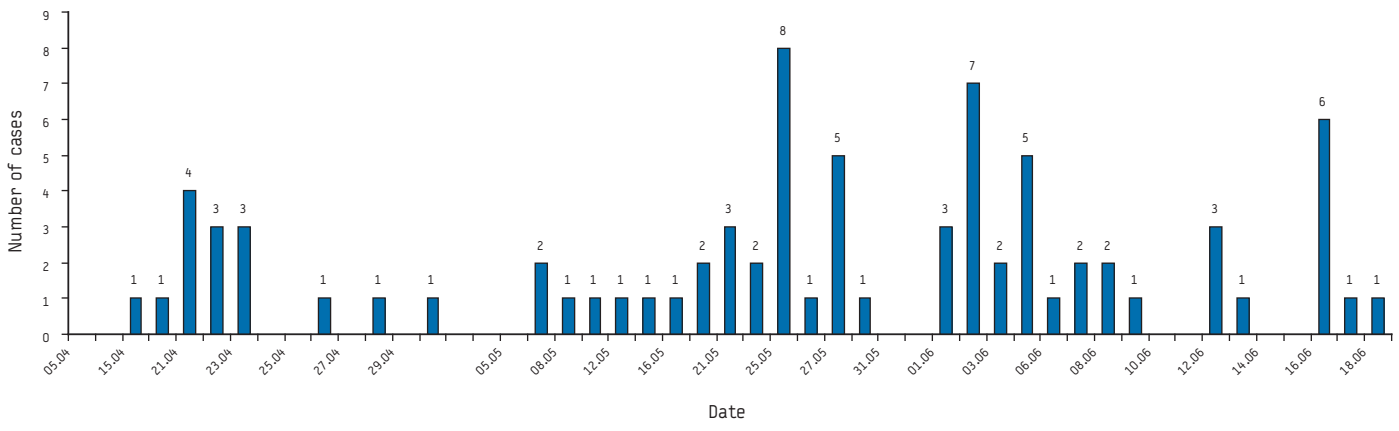
Table

National immunisation coverage with measles, mumps, rubella (MMR) vaccine, Bulgaria, 2005-2008

MMR dose	2005	2006	2007	2008
First dose (13 months)	96.2%	95.7%	96.0%	95.9%
Second dose (12 years)	92.4%	93.3%	94.0%	94.3%

Figure 1

Number of probable and confirmed measles cases reported in Bulgaria between 15 April and 19 June 2009, by date of notification (n=79)



Outbreak description

After seven years without indigenous transmission of measles in Bulgaria, an increasing number of cases have been reported since 15 April 2009 (Figure 1).

By 19 June, the total number of notifications reached 84. Of these, five were discarded (one patient who presented with a rash 10 days after MMR vaccination was considered as a case of adverse events following immunisation (AEFI), and four suspected cases tested IgM-negative). Of the remaining 79, to date, 64 were confirmed as measles cases (61 laboratory-confirmed by the National Reference Laboratory for Measles, Mumps and Rubella in Sofia, and three having clinical symptoms and an epidemiological link with laboratory-confirmed cases); the remaining 15 cases for whom laboratory results are pending, have been classified as probable.

The epidemiological investigation demonstrated that the index case was imported in March from Germany. The patient, a 24-year-old man, became ill on 12 March, four days after arrival from Hamburg, where he works. The initial symptoms included high fever, cough, runny nose, malaise and rash, developed three days later. The clinical presentation was compatible with measles and fulfilled the clinical criteria of measles. The patient was not hospitalised but consulted an infectious diseases specialist. A serum sample was tested and the case was classified as confirmed by the National Reference Laboratory and notified as an imported case (included in Figure 1).

The subsequent three measles cases occurred among his close contacts (family members). They were laboratory-confirmed by the National Reference Laboratory in Sofia. The samples were then sent to the WHO Regional Reference Laboratory (RRL) for Measles and Rubella in Berlin for reconfirmation and measles virus (MV) genotyping. The nucleotide sequences of the variable part of measles virus N gene (450 nt) derived from these three cases were identical and classified as genotype D4. Their sequence is represented by the official WHO name MVs/Shumen.BGR/15.09/1(D4). Later on samples collected from four further cases in epi-week 21 were sent to the RRL Berlin. The sequences derived from these cases (represented by MVs/Silistra.BGR/21.09/1[D4]) were identical to MVs/Shumen.BGR/15.09/1[D4] demonstrating that the seven analysed cases belonged to a single chain of MV transmission. The same genetic variant of MV was previously detected during an outbreak observed between January and June 2009 in northern Germany. This confirms the assumption that the Bulgarian index case was imported from Hamburg. Outbreaks due to the introduction of imported MV (D6, D4 and B3) into the hard-to-reach populations were recently reported also from other European countries [8]. The strain name MVs/Shumen.BUL/15.09(D4) was included in the WHO/EURO CISID database. [Information in this paragraph was kindly provided by Dr Annette Mankertz and Dr Sabine Santibanez from the Robert Koch-Institut, Berlin, Germany].*

All 78 cases following the index case occurred as a result of local transmission and were shown to be epidemiologically linked.

The present measles outbreak affects mostly the Roma population living in the north-eastern part of the country – in Razgrad, Shumen, Silistra and Dobrich regions (Figure 2). This population is characterised by large families living together and frequently moving from one place to another, looking for seasonal work in Bulgaria as well as abroad. Until now, several family clusters have been registered among this group.

The outbreak affects both genders almost equally, with male to female ratio 30/49. The most affected age groups are young children below 1 year of age (non-immunised because of the young age) and children 1 to 9 years of age, who are eligible to immunisation.

Because of the crowded households and poor living conditions of affected Roma families a large proportion of cases (51 of 79, 64.5%) were hospitalised. Complications were observed in 46.8% of cases (37/79): 33 cases developed pneumonia and four cases had abdominal disorders with diarrhea and acute abdominal pain.

The immunisation status of all reported 79 measles cases is shown in Figure 3.

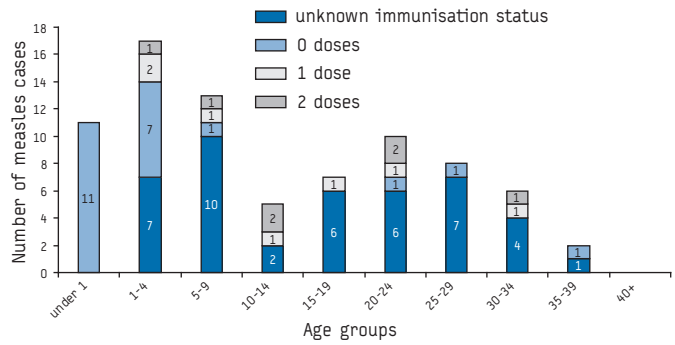
Considering the age of cases, 68 of the total of 79 measles cases should have been immunised with at least one dose of measles vaccine. However, in the majority of cases (n=43, 54.4%) the vaccination status was unknown because of the lack of documentation. Twenty-two cases were known not to have been vaccinated (including 11 below the age of one year). Only seven cases (10.3%) have received one dose and another seven (10.3%)

Figure 2

Measles cases spread by regions, Bulgaria, April-June 2009 (n=79)

Figure 3

Distribution of reported measles cases by immunisation status and age group, Bulgaria, April-June 2009 (n=79)



both doses of the measles vaccine. Of note is that among those immunised with two doses, three cases received the second dose during the catch-up campaign organised in response to the outbreak in May 2009, and it is most likely that they were harbouring a measles infection in the incubation period during that time, because soon after the immunisation, they fell ill with measles.

Control measures

The outbreak and the investigations are still ongoing, and therefore the data presented are preliminary. The public health authorities expect more cases to occur, especially among the Roma population.

The control measures are in progress: the Bulgarian Ministry of Health issued a press release regarding the situation and future immunisation and surveillance activities. General practitioners and other medical staff were requested to pay special attention to rash/fever symptoms and to strengthen routine immunisation of children aged 13 months (first dose) and 12 years (second dose) by directly reaching the parents and explaining the benefits of vaccination.

An immunisation campaign was started on 27 April in the affected administrative regions, targeting all persons from 13 months to 30 years of age who had not received the complete two-dose MMR vaccination. The MMR vaccine is supplied by the Ministry of Health and is offered free of charge through the routine immunisation services (family doctors) and special outreach teams. These supplementary immunisation activities are still ongoing.

Discussion and conclusions

Despite the high national immunisation coverage with MMR vaccine, the current measles outbreak clearly demonstrates the existence of pockets of non-immunised population, here specifically the Roma population. A quick risk assessment made by the epidemiologists investigating the outbreak concluded that the minority groups and living in closed communities as described above are at higher risk of measles infection and should be offered a supplementary measles immunisation.

In recent years, similar outbreaks, affecting unvaccinated groups, have been reported in a number of European countries, however, it seemed that the epidemic did not spread to the eastern part of Europe. During 2008, a total of 7,821 measles cases were reported to the EUVAC.NET, and most of them (90%) were from six countries: Switzerland, Italy, the United Kingdom, Germany, France and Austria [9-12].

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*Authors' correction:
On request of the authors, the paragraph on genotyping results was modified and a figure was deleted from the article after the publication. This change was made on 9 July 2009.

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Plague outbreak in the Libyan Arab Jamahiriya

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Plague is circulating regularly in localised areas worldwide, causing sporadic cases outside Africa and remains endemic or causes limited outbreaks in some African countries. Furthermore, some notable outbreaks have been reported in Asia in the last 20 years. A limited outbreak with five cases has recently been notified by the health authorities of the Libyan Arab Jamahiriya.

Introduction

Plague is a zoonosis caused by the bacillus *Yersinia pestis*. This disease may have caused over 200 million deaths in the history of humanity [1]. The disease is principally transmitted from animal to animal by fleas. Humans usually become infected through the bite of an infected flea (mainly *Xenopsylla cheopis*). The occurrence of bubonic plague cases is therefore the result of the presence of fleas, rodents and humans in a given place at a given time.

Since the first description of what may have been a plague outbreak in 430 BC in Ancient Greece [2], the plague has spread worldwide during the course of several pandemic waves. Between 1998 and 2008, more than 23,278 cases were reported including 2,116 fatalities (case fatality ratio, CFR, of approximately 9%) in 11 countries [3]. Over 95% of the 23,278 cases, however, were reported in Africa with well-identified endemic plague foci (mainly in three countries: Madagascar, the Democratic Republic of Congo [DRC] and Tanzania).

The bubonic plague is the most common form of the disease (93% of plague cases in Madagascar [4] and 81% of plague cases in the United States (US) [5]. Without adequate treatment, the case-fatality rate of bubonic plague ranges from 50 to 90%. Bubonic plague does not give rise to direct human-to-human transmission.

Pulmonary plague is not the most frequent form of the disease (3% of the plague cases in the US, 8% in DRC, sometimes more frequent in outbreaks with sustained human-to-human transmission), but is deadly in almost all cases in absence of adequate and timely treatment. This clinical presentation may give rise to human-to-human transmission through droplet transmission.

Table

Reported human plague cases/outbreaks since January 1945

Country	Year	Location	Confirmed or probable cases	Deaths
Morocco No cases reported since 1945	1945	Countrywide, mainly around Marrakech	811	ND
Algeria No cases reported from 1950 to 2003	1945-1946	Oran	12	1
	1945	Algiers	5	ND
	1946-1950	Countrywide	8	ND
	2003	Kahelia (Tafraoui, Oran)	18	1
	2008	Laghouat	4	3
Tunisia No cases reported since 1945	1944-1945	Bizerte/Ferryville	34	27
Libya No cases reported from 1984 to 2009	1972	Nofilia	18	3
	1976-1977	Tobruk	30	12
	1984	Tobruk	9	ND
	2009	Betnane (Tobruk)	12	1
Egypt No cases reported since 1947	1945	Port-Saïd, Suez, Ismailia	218	ND
	1946	Port-Saïd, Suez, Ismailia, Damietta	66	ND
	1946-1947	Alexandria	145	39

Source: Department of international and tropical diseases, Institut de Veille Sanitaire (DIT-InVS) based on numerous reports and the literature

Available evidence points to effective prevention of human-to-human transmission of pneumonic plague through isolation and treatment of cases and the observance of standard precautions completed by droplet and contact precautions during healthcare [6,7]. There is no available vaccine for large-scale use.

Outbreak report

On 14 June 2009, the health authorities of the Libyan Arab Jamahiriya reported suspected cases of bubonic plague (including one death) to the World Health Organization in compliance with the Revised International Health Regulations (IHR). The case definition proposed by the World Health Organization was used [8]. The outbreak occurred in a semi-nomadic setting. Subsequent epidemiological investigations by an international team ascertained a total of five cases. Three of these occurred in a family cluster in the Tobruk rural area (near the border with Egypt). The first identified case was a child who presented with pneumonic plague and died. Two siblings were subsequently identified as having bubonic plague. Two other epidemiologically unlinked cases occurred in young women living in the same district. Confirmatory testing is ongoing. Rodent control measures have been implemented locally.

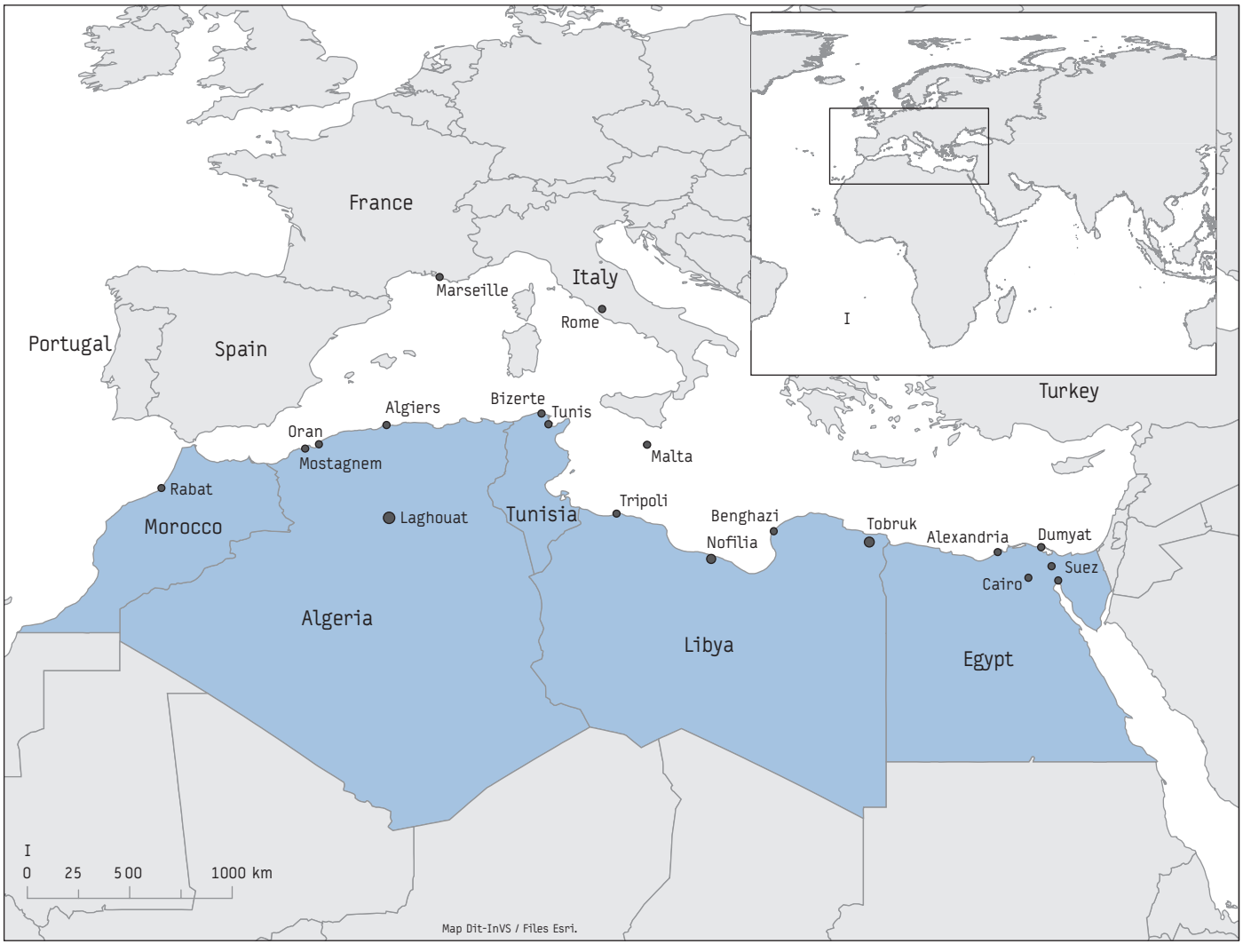
The last outbreak reported in the Maghreb to date occurred in Algeria in July 2008. At that time, the Algerian health authorities reported four cases including three fatalities in Laghouat [WHO, unpublished data]. All identified cases presented with bubonic plague. The last outbreak reported by the Libyan Ministry of Health occurred in 1984 with eight cases of bubonic plague (no deaths) [1]. The last deaths due to plague reported in that country occurred during a 1977 outbreak that affected 11 people (six deaths).

Discussion and conclusion

The implementation in 2007 of the revised IHR and improved surveillance in many countries has strengthened communication

Figure

Map of the Mediterranean region, including locations where plague cases have been reported since 1945.



Source: Department of international and tropical diseases, Institut de Veille Sanitaire (DIT-InVS)

between countries and the World Health Organization. Regional networks have also emerged which facilitate cross-border and regional exchange of public health alerts. The Libyan health authorities have been prompt in describing and reporting the outbreak described here, thereby enabling speedy confirmation and the implementation of control measures.

The Maghreb is no longer considered an endemic area for plague [9]. The recent human plague clusters, however, raise the issue of the persistence of a large focus or of several limited natural foci which have been quiescent for decades and remain capable of "re-emergence" at various dates and locations (Table, Figure). These clusters of human cases are generally sporadic and limited, but they may continue to occur despite the necessary extensive rodent control measures which will probably be insufficient to eradicate the plague reservoir in wild animals. Healthcare workers require training to better recognise signs of a disease which is no longer endemic. Informing and increasing awareness of populations living in and around plague foci, strengthening of local health systems and targeted public health measures around the cases remain the key control strategies in plague-prone areas. Improved knowledge of the natural foci is also a pre-requisite for any rational vector and rodent control

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Meningococcal disease in a backpackers' hostel in Scotland: a risk assessment for prophylaxis

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This paper outlines the risk assessment and communication strategy carried out by the Lothian Health Protection Team after notification of a probable case of meningococcal disease (later confirmed as *Neisseria meningitidis*) in a resident of a city centre backpackers' hostel. Six close contacts were identified from the hostel and given rifampicin prophylaxis. Two days after commencing rifampicin one of these contacts was admitted to hospital with a purpuric/petechial rash and thrombocytopenia. The final diagnosis for this contact was thrombocytopenia, either idiopathic or secondary to rifampicin. This example and the potential side effects of administering rifampicin prophylaxis highlight the importance of a thorough risk assessment of contacts of a case to avoid prescribing prophylaxis to anyone other than those at highest risk of becoming a subsequent case.

Introduction

The Lothian Health Protection Team (HPT) was notified of a probable case of meningococcal disease in a foreign national who was resident in a large city centre backpackers' hostel. The HPT undertook an investigation to identify close contacts requiring prophylaxis.

In the United Kingdom prophylaxis (usually rifampicin) is routinely offered to close contacts of confirmed or probable cases of meningococcal disease to eradicate carriage of the organism in those most at risk [1].

The Health Protection Agency [1] defines a close contact as:

Someone who has had an overnight stay in the household, or with whom the patient has stayed overnight, during the seven days before onset of illness in the index case.

Someone who is an intimate kissing contact.

In larger institutions defining close "household" contacts is more challenging [1,2,3]. In this case the Consultant in Public Health Medicine is responsible for deciding who constitutes the "household".

The administration of rifampicin is not without risk. Adverse effects have been reported to occur in about 4% of patients receiving "usual doses" of rifampicin (for example 10mg/kg/day) [4]. Mild adverse effects include nausea, diarrhoea, abdominal pain, headache, dizziness and skin rash [5]. More severe adverse effects include thrombocytopenia, with or without purpura, and hepatic reactions [6].

If prophylaxis is not prescribed for contacts the absolute risk to a person in the same household of developing meningococcal disease one to 30 days after an index case is about one in 300 [1].

It has been estimated that 200 household contacts need to be treated with prophylaxis in order to prevent a subsequent case of meningococcal disease in the first month [7].

Methods

For this incident a household contact was defined as:

1. Anyone who shared a room with the case in the seven days prior to symptom onset.
2. Anyone who had spent prolonged periods of time socialising with the case in the seven days prior to symptom onset.

To identify close contacts who required prophylaxis the layout of the hostel was inspected. The names of close contacts were identified through hostel records and through discussion with other residents.

Blood samples were sent from the case for confirmation and typing of *Neisseria meningitidis*.

Results

Contacts

The hostel comprised two separate buildings, a short stay facility with 170 beds and a long-stay facility with 130 beds. The index case was resident in a three bedded room of the long stay facility and had been living there for several months.

On the day of notification (day 1) six close contacts were identified who fitted the definition. All were given rifampicin prophylaxis. These contacts included: two room-mates, three friends and the case's partner.

Administration of rifampicin

Day 1: Five of the close contacts received rifampicin from the local hospital. Three of these close contacts were foreign nationals, only one of whom spoke English. Communication regarding prophylaxis and its contra-indications was done through translation by this individual. No contraindications were identified.

Day 2: One contact, travelling in Ireland, had prophylaxis arranged by public health colleagues in Ireland.

Day 3: Two days after commencing rifampicin prophylaxis one of the contacts from the hostel was admitted to hospital with a purpuric/petechial rash. This person had taken three doses of rifampicin 600mg. Differential diagnoses included idiopathic thrombocytopenic purpura, thrombocytopenia secondary to rifampicin and possible meningococcal septicaemia.

Communication to hostel residents

Day 4: Following the admission to hospital of the contact (where meningococcal disease was a possibility), information letters, written in English, were placed on each resident's bed in the hostel. These letters informed residents that there had been a confirmed case of meningococcal disease in the hostel and included information on the signs and symptoms of the disease. The HPT also visited the hostel for question and answer sessions.

Microbiology

Day 4: The samples from the index case were confirmed as *N. meningitidis* serogroup W135. The HPT advised that the previously identified close contacts of this case should be vaccinated against W135.

Day 9: The contact was discharged from hospital with a final diagnosis of thrombocytopenia which was either idiopathic or secondary to rifampicin. A blood sample sent for PCR was negative for *N. meningitidis.*

No further cases of meningococcal disease were notified from the hostel.

Discussion

Risk assessment for the administration of prophylaxis

Deciding how extensively to give prophylaxis in an institution such as a hostel is not straightforward. In this incident the HPT identified contacts requiring prophylaxis amongst those most closely linked with the case. This totalled six close contacts from the 300 bed hostel. This health protection response was similar to the response in a hall of residence in Southampton in 1997 when the first case in an outbreak was treated as a "single case" and mass prophylaxis was only advised when further cases were notified [8].

A contrasting approach was taken in a 282 bed hostel in Vancouver in 2001 when, after notification of a single case, the entire hostel was considered a "household" and ciprofloxacin prophylaxis was recommended for all staff and residents who had stayed at the hostel for up to a week before the case was admitted. It was estimated that this could have been up to 750 people [9].

The fact we have reported that a close contact who was given rifampicin was discharged from hospital with a final diagnosis of thrombocytopenia, either co-incidental or secondary to rifampicin stresses that all close contacts should be informed of the potential dangerous side effects of rifampicin prophylaxis and that a thorough risk assessment should be undertaken before administering prophylaxis to contacts.

Communications

Contact tracing proved challenging during this incident due to the limited information held about possible contacts in hostel records and by other residents.

Communication to the wider community at the hostel was also difficult due to the multiple nationalities of its residents. The letter given to individuals in the hostel was in English. Consideration was given to preparing letters in a variety of languages however this would have caused a lengthy delay in communicating the risk. Being aware of the signs and symptoms of meningococcal disease is essential to ensure that cases are given medical treatment as soon as possible. Prior preparation of information about meningococcal disease in different languages would be helpful especially in busy European tourist cities with visitors from across the world.

Acknowledgements

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The Swedish new variant of Chlamydia trachomatis (nvCT) remains undetected by many European laboratories as revealed in the recent PCR/NAT ring trial organised by INSTAND e.V., Germany

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The May 2009 round of INSTAND's ring trial "C*hlamydia trachomatis* detection PCR/NAT" included a sample with high amount of the Swedish new variant of *C. trachomatis* (nvCT). A spectrum of at least 12 different commercial diagnostic nucleic acid amplification tests (NAATs) and many different *in house* NAATs were applied by the 128 participating laboratories which reported 152 results. Approximately 80% of the results correctly reported the presence of *C. trachomatis* in the nvCT specimen. The nvCT sample was mainly missed, as expected, by participants using the Roche COBAS Amplicor CT/NG (15.5% of reported results) but also by several participants using *in house* NAATs. The trend towards using nvCT-detecting NAATs is obvious and in addition to the new dual-target NAATs from Roche and Abbott, and BD ProbeTec ET, also a number of new CE mark-certified commercial tests from smaller diagnostic companies as well as many different *in house* NAATs were used. Laboratories using commercial or *in house* NAATs that do not detect the nvCT are encouraged to carefully monitor their *C. trachomatis* incidence, participate in appropriate external quality assurance and controls schemes, and consider altering their testing system. The reliable detection of low amounts of the wildtype *C. trachomatis* strain in other samples of the ring trial set indicates a good diagnostic performance of all applied commercial NAATs while also detecting the nvCT strain.

Introduction

With the increasing acceptance of nucleic acid amplification tests (NAATs) in the field of diagnostic microbiology and the broad availability of open platforms to perform an exponentially growing spectrum of *in house* and/or commercially prefabricated NAATs, there is a growing demand for appropriate internal and external quality control (QC) activities. One comprehensive external quality assessment scheme (EQAS) for diagnostic NAATs was established in 2002 by the German Society for Promotion of Quality Assurance in Medical Laboratories, INSTAND e.V. (www.instand-ev.de). This subscheme of INSTAND's well-established quality control initiatives, named "bacterial genome detection PCR/NAT", offers certified proficiency testing panels for prominent bacterial pathogens on a biannual basis. A detailed discussion of the current and the previous EQAS schemes can be found at: http://www-nw.uni-regensburg.de/~.reu24900.mmh.klinik.uni-regensburg.de/INSTAND_e.htm.

In 2006, a new variant of *Chlamydia trachomatis* (nvCT) was identified in the Swedish county of Halland by Ripa and co-workers [1]. This mutant strain is characterised by a 377-bp deletion in ORF-1 of the multicopy cryptic plasmid, which includes the target region of both the Roche and Abbott *C. trachomatis* NAATs available at that time [2]. The currently available new redesigned dual-target assays, namely the Abbott RealTime CT/NG (CE mark-certified in January 2008) that targets another cryptic plasmid sequence in addition to the sequence affected by the nvCT deletion, and the Roche COBAS TaqMan CT v2.0 (CE mark-certified in June 2008) that detects the chromosomal *ompA* gene in addition to the sequence affected by the nvCT deletion, have replaced the former assays [3].

Immediately after the first report on the nvCT, international studies were conducted to determine whether the nvCT was present in different settings across Europe, the United States, Australia [3-5]. Only sporadic cases have so far been reported outside the Nordic countries [3-5], however, current knowledge regarding the presence and prevalence of nvCT in other countries is highly limited due to few recent studies and the fact that many European laboratories can still not detect the nvCT [4], and those that can are not aware of it because no nvCT-specific or other distinguishing NAATs are used. Ideally all laboratories should use NAATs that detect nvCT, because a wider geographic spread of this variant can not be excluded.

To supplement a recent Eurosurveillance publication on a United Kingdom National EQAS (UK NEQAS) distribution [4], the present report provides a concise reflection on diagnostic performance and NAATs used by European laboratories participating in the May 2009 INSTAND e.V. ring trial regarding detection of the nvCT. Assuming that most diagnostic laboratories are participating in one external QC scheme only, the intersection between UK NEQAS and INSTAND ring trials should be very limited and the present study represents an additional exploratory piece in the jigsaw puzzle of European *C. trachomatis* NAAT testing regimens.

Materials and methods

The May 2009 round of INSTAND's EQAS "bacterial genome detection PCR/NAT" included two panels for *C. trachomatis* detection. One set of four lyophilised blinded samples was offered for participants using combined detection of *C. trachomatis* and *Neisseria gonorrhoeae* (RV 530), and a separate set for those detecting *C. trachomatis* only (RV 531).

The latter set (*C. trachomatis*; RV 531) contained a sample with ~105 inclusion forming units (IFUs) of the nvCT strain per ml of reconstituted lyophilised specimen. This set was completed by two samples containing ~103 IFUs/ml of a wildtype *C. trachomatis* strain and one sample without *C. trachomatis* in a natural background of human and bacterial cells. The laboratories were requested to reconstitute the specimen in 300 µl of molecular grade water and analyse a 100 µl portion of the specimen, according to their routine protocols for detecting *C. trachomatis* from an endocervical swab.

Results

Response rate

NAAT results for distribution RV 531, which included the nvCT sample, were returned by 128 laboratories (100% of participants), including 115 laboratories from Germany, 12 from nine other European countries and one from United Arab Emirates (Table). For unknown reasons, some laboratories applied more than one *C. trachomatis* NAAT, which probably does not reflect their routine diagnostic workup for *C. trachomatis.* Due to this reporting of results from multiple assays and/or lack of assay specifications in the reports, the effective number of results (n=152) is higher than the number of participants (n=128).

Nucleic acid amplification tests (NAATs) used for C. trachomatis diagnostics

The change in the spectrum of NAATs applied by the participants in the German INSTAND schemes from 2006 to 2009 is depicted in Figure 1. Especially in the current round (2009), the spectrum of NAATs used in the German INSTAND ring trial substantially differed from the recent UK NEQAS ring trial [4]. In 2009, Roche COBAS Amplicor CT/NG (15.5% of participants) and BD ProbeTec ET (Becton Dickinson; 15.5%) were the most commonly used main NAATs, followed by Roche Cobas TaqMan (14.8%) and Abbott RealTime CT (11.0%). Nevertheless, from 2006 to 2009, the use of Roche COBAS Amplicor CT/NG and Roche Amplicor CT/NG rapidly decreased from 37.4% to 15.5%, and 6.8% to 0%, respectively. In contrast, the numbers of laboratories who have shifted to the new dual-target assays Abbott RealTime CT/NG and Roche COBAS TaqMan CT v2.0 significantly increased. Furthermore, especially in the recent years several new or at least less popular commercial *C. trachomatis* NAATs as well as many *in house* NAATs were in use (Figure 1).

Detection of the Swedish new variant of C. trachomatis (nvCT)

Twelve different commercial assays were used for reporting results (n=106) on the nvCT sample. Furthermore, use of "other commercial assays" was indicated in 19 results, *in house* real-time PCR assays in 23, and in four results the NAAT was not specified (Figure 2). In 80% (n=122) of the results the presence of *C. trachomatis* was reported correctly. As expected, the nvCT sample was missed by those using the Roche COBAS Amplicor CT/NG (n=15). One laboratory that used the Abbott system reported a negative result, which suggests that the older single-target RealTime CT/NG test (not detecting the nvCT) was used. Furthermore, participants using "other commercial kits" (n=5), *in house* PCRs (n=7), and completely unspecified assays (n=2) reported negative results (Figure 2).

Aside from the nvCT sample, a very good performance was observed for the detection of small amounts of the wildtype *C. trachomatis* strain in the other two positive specimens (~103 IFUs/ml) and the negative specimen of the QC panel RV 531. Ninety-two percent of all laboratories reported correct results for these three samples. A mean accuracy rate of 97% was observed among participants using commercial assays, whereas the mean accuracy rate was 86% when *in house* or "other" assay formats were used.

Discussion and conclusions

Pathogen- and method-specific ring trials (EQAS) organised by independent institutions have repeatedly proven to be valuable external quality control measures. In addition to assessing the diagnostic performance (analytical sensitivity and specificity) of different assays at individual laboratories, the statistical analysis of the results provides an actual snapshot on the technology and use of commercial or in house NAATs for detection of a given pathogen among the participants.

The results of the latest UK NEQAS [4] and German INSTAND (present study) quality assessment distributions for molecular detection of *C. trachomatis* clearly show that a substantial number of laboratories can still not detect the nvCT. A broader spectrum of NAATs, including many different internationally less popular and recognised commercial NAATs and *in house* NAATs, was applied in the INSTAND ring trial. This may reflect a trend, at least in Germany (90% of participants), towards the use of individual PCR assay formats and amplicon detection platforms mainly observed in smaller laboratories. These laboratories are typically facing a smaller number of samples per day but still try to keep the test frequency high enough to end up with short turn-around-times for their PCR results. Under these circumstances, diagnostic tests (or assay platforms) designed for really large sample numbers can usually not be operated economically and the use of customised

Table

Number and geographic location of the laboratories participating in the INSTAND scheme "bacterial genome detection PCR/NAT", distribution RV 531, May 2009

Country	Number of participants
Germany	115
Czech Republic	3
Slovakia	2
Austria	1
Bulgaria	1
Cyprus	1
France	1
Hungary	1
Russian Federation	1
Switzerland	1
United Arab Emirates	1
Total	128

FIGURE 1

Main nucleic acid amplification tests (NAATs) used by participating laboratories in the German INSTAND schemes RV 530 and 531 for molecular detection of *Chlamydia trachomatis* from 2006 to 2009

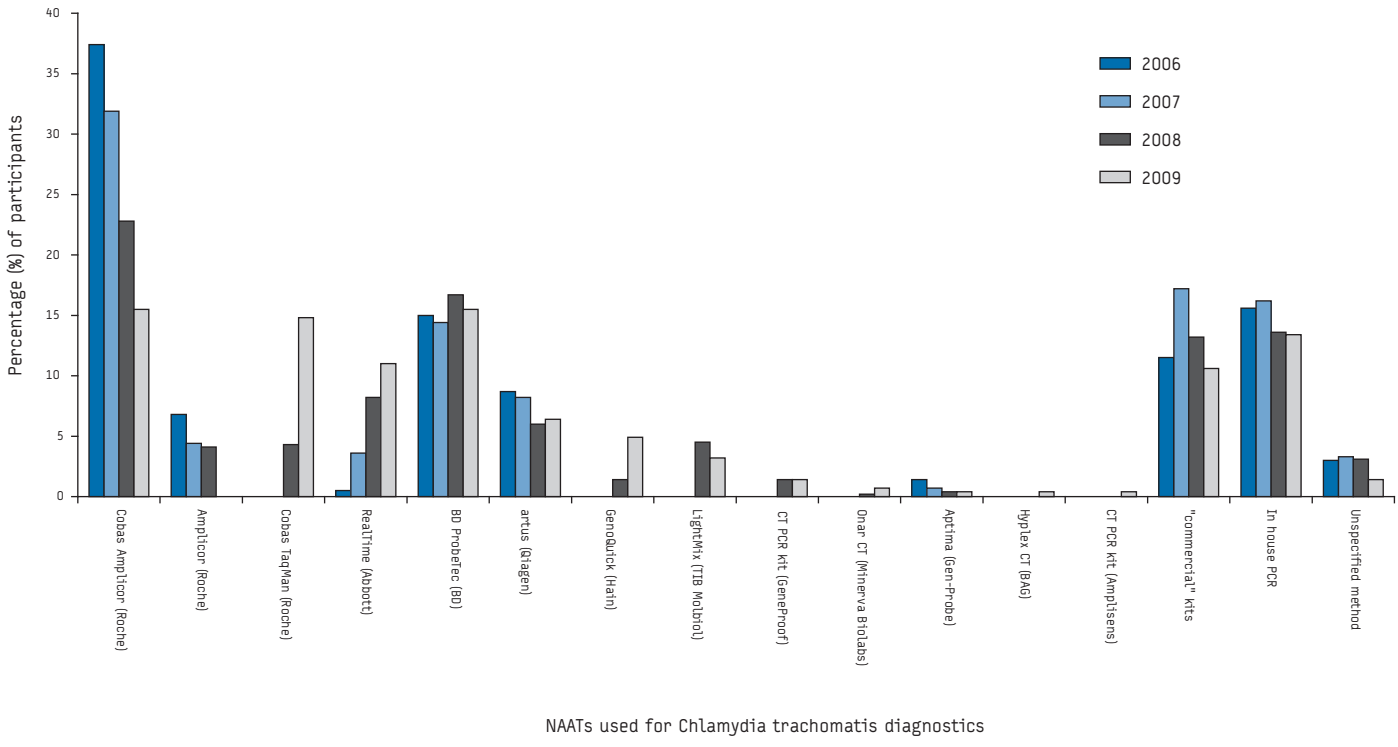
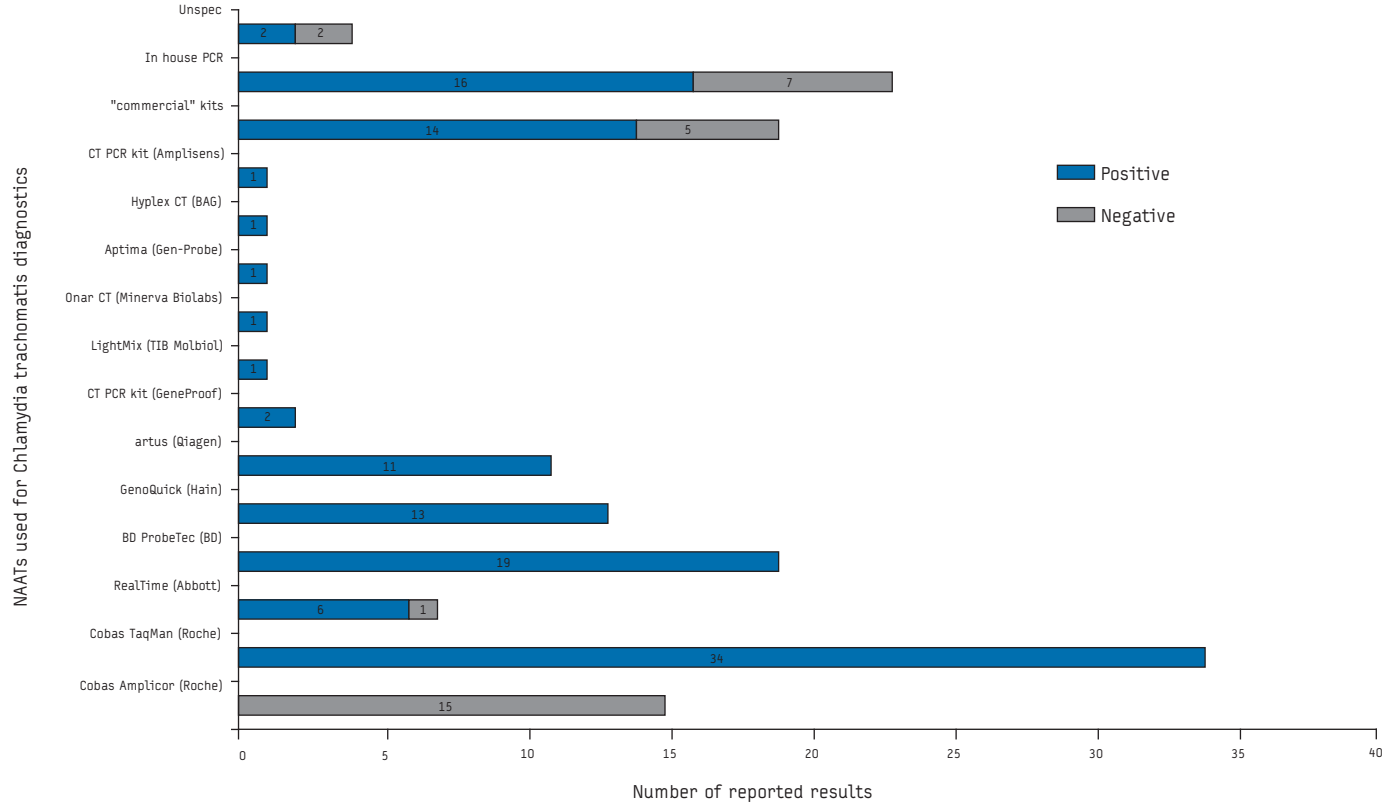


FIGURE 2

Methods and corresponding results regarding detection of the new variant of *Chlamydia trachomatis* (nvCT). Data from the INSTAND's RV 531 distribution were analysed (152 results from 128 laboratories in 11 countries)



kits and/or assay formats indeed makes sense, i.e. as long as they are thoroughly validated and reliable. As an aid to orientation, the inclusion of as many assays as possible from "smaller companies" in challenging EQAS schemes is appreciated in this respect.

As also reported from the previous UK NEQAS study [4], the use of the former versions of Roche Cobas Amplicor CT/NG and Amplicor CT/NG, which do not identify the nvCT, has rapidly declined. However, a substantial number of laboratories are still using Roche Cobas Amplicor CT/NG [4, present study] and these laboratories should consider changing their testing system. Another worrying aspect revealed by the present study is the continued use of some *in house* NAATs, which were not specified in detail by the participants, that also miss the nvCT. In order to detect the nvCT, laboratories using these *in house* PCR assays are recommended to consider changing their testing system, altering the probe and/or primer set in their *in house* NAAT, introducing an additional target in their *in house* NAAT, or introducing an additional assay not affected by the mutation, i.e. for dual testing. Dual testing is however often restricted by a more complicated workup procedure, including specimen splitting, different methodological protocols, and additional costs. Considering the currently still presumed low prevalence of the nvCT strain outside northern Europe, routine diagnostic application of nvCT-specific NAATs is not necessary. Nevertheless, as already mentioned above, at present the true prevalence of the nvCT outside the Nordic countries is mainly unknown.

In conclusion, laboratories using commercial or *in house* NAATs that do not detect the nvCT are encouraged to (a) carefully monitor their *C. trachomatis* incidence for unexplained declines, (b) frequently participate in effective internal and external quality assurance and control schemes, and (c) ideally to consider changing their testing system. This is crucial for an early detection as well as reliable surveillance of the nvCT, but also of other possibly undetected mutants, and, accordingly, the first two points are advisable for all diagnostic laboratories.

The nvCT strain will certainly be included again in one of the future rounds of INSTAND's PCR/NAT *C. trachomatis*-specific ring trials. It will be interesting to see whether the "affected" laboratories have learnt their lessons and switched to NAATs that also detect the nvCT.

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An outbreak of Shigella dysenteriae in Sweden, May–June 2009, with sugar snaps as the suspected source

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We report an outbreak of *Shigella dysenteriae* type 2 infections during May-June 2009 in Sweden, involving 47 suspected cases of whom 35 were laboratory-confirmed. The epidemiological investigation based on interviews with the patients pointed at sugar snaps from Kenya as the source. *Shigella* was not detected in samples of sugar snaps. However, *Escherichia coli* was confirmed in three of four samples indicating contamination by faecal material. During April to May 2009 outbreaks with *Shigella* connected to sugar snaps from Kenya were reported from Norway and Denmark. In the three countries trace back of the indicated sugar snaps revealed a complex system with several involved import companies and distributers. In Sweden one wholesale company was identified and connections were seen to the Danish trace back. These three outbreaks question whether the existing international certification and quality standards that are in place to prevent products from contamination by faecal pathogens are strict enough.

Introduction

Shigellosis is a notifiable disease in Sweden. Annually approximately 500 cases are notified to the Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet, SMI) and about 20% are domestic cases. The majority of the *Shigella* strains are sent to SMI for verification and further typing. Most of the cases are caused by *Shigella sonnei*. Cases with *Shigella dysenteriae* are rare in Sweden. In average five cases are reported each year, including domestic cases and cases infected abroad.

On 10 June 2009 the laboratory at SMI detected six domestic cases of *Shigella dysenteriae* from four different counties and informed the department of epidemiology. Minutes later the county medical officer of another Swedish county (not one of the four mentioned above) telephoned SMI and reported that 25 persons who had visited a restaurant on 31 May were ill with gastrointestinal symptoms. This first information also revealed that a number of them (at the time it was unclear how many) were diagnosed with *S. dysenteriae*. The restaurant was visited by 320 guests that day as it was a holiday (Mother's Day in Sweden).

Six cases of *S. dysenteriae* in a short period of time, although geographically spread, clearly indicated an outbreak. The coinciding report from another county pointed to the possibility of a large outbreak. An outbreak team including investigators from the involved county medical offices, the SMI and the National Food Administration (Livsmedelsverket, SLV) was formed. As this was a national outbreak, the outbreak investigation was coordinated from the SMI.

Routine typing of the isolates from the six domestic cases revealed *S. dysenteriae* type 2. This is a rare type of *S. dysenteriae* with only four cases reported last year and all of them acquired abroad.

All five counties with cases were contacted. In one of the counties a birthday party with 60 guests took place on 30 May. Five persons were ill and one of them was diagnosed with *S. dysenteriae.*

In cooperation with the National Food Administration an investigation was started to try to identify any common food product consumed by known cases.

Methods

Epidemiological investigation

In the county where 25 persons got ill after visit to a restaurant, a list of food items that had been delivered to the restaurant was produced. The persons affected were asked about food items they had consumed at the dinner according to the delivery list. Due to summer vacations and shortage of staff it was unfortunately not possible to perform a cohort study for the restaurant.

In the county where a case of *S. dysenteriae* was linked to a birthday party, the person responsible for purchasing food for the party was asked to list the products served and where they were bought. People who became ill after the party were asked what they had consumed.

In the remaining three counties the infected persons were either interviewed according to a general questionnaire for gastrointestinal diseases or asked by phone what they had consumed. The interviews were performed at the county medical offices and the results were gathered at the SMI for analysis and discussion with the National Food Administration.

Microbiological investigation

PFGE was performed on 12 clinical isolates with S. dysenteriae type 2 at SMI using the enzyme XbaI.

After sugar snaps had been suspected as the possible source of infection, four samples of sugar snaps were sent to the section for water and environmental microbiology at the SMI for analysis of *Shigella*. Three samples had been collected from supermarkets in two counties and one from a private person in a third county. The sample from this private person was of the same batch as a sample

from one of the supermarkets. Coliforms and *Escherichia coli* were also analysed as indicators for possible faecal contamination.

Sugar snaps were treated as environmental samples where extraction was performed by washing an appropriate amount of sugar snaps in PBS+Tween80. Extracts were then used for analysis of coliforms and *E. coli* by using Colilert-18 as well as an enrichment procedure for the analysis of *S. dysenteriae* where enriched broth was used both for plating on DC-agar and PCR.

Results

A case was defined as having a domestic laboratory-confirmed *S. dysenteriae*. The case definition was not more specific than that since the infection is so rare and there were no cases to exclude at the time. Of the 47 persons reported to have been affected by the outbreak, 35 were laboratory-confirmed, including three secondary cases (Figure, excluding the three secondary cases). One of the cases with *S dysenteriae* type 2 was identified in a sixth county more than two weeks after the earliest reported date of onset (Figure). This case was included in the outbreak as it fit the above case definition and had also consumed sugar snaps.

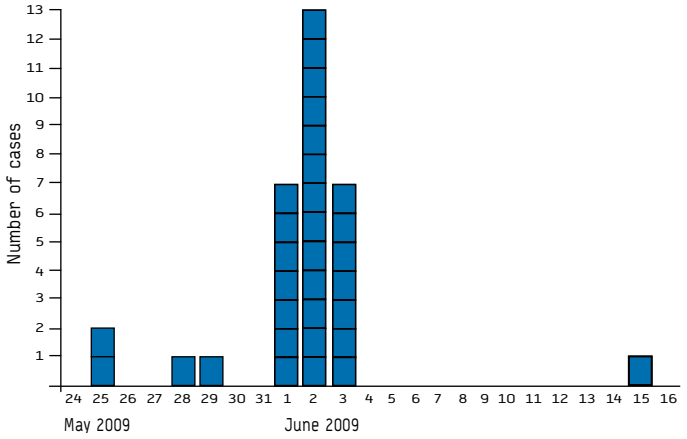
The cases were reported from six counties, all but one situated in the southern or middle part of Sweden. The cases were between 1 and 82 years old and 50% were women. 20 confirmed cases were reported from the restaurant, seven from the birthday party and eight from the remaining four counties. The cases from the birthday party were single cases in five different families. In all, seven persons were infected after the party since the parents of a child who was ill became secondary cases.

Date of onset for all cases in the outbreak was between May 24 and June 15 with the majority of cases reporting onset of symptoms on June 1 to 3 (Figure). One single case with date of onset on June 15 had kept sugar snaps in the refrigerator and consumed them continuously. This person still had sugar snaps left during the time of investigation and they were sent to SMI for analysis.

Shigella was not detected in any of the four samples of sugar snaps sent to SMI. However, *E. coli* was confirmed in three samples.

Figure

Confirmed cases of *Shigella dysenteriae* type 2 in an outbreak in Sweden in May-June 2009, by date of onset of symptoms (n=32)



11 of the 12 isolates analysed by PFGE were identical. These isolates were collected from five counties.

The investigation pointed at sugar snaps from Kenya as the source of infection since the majority of the cases from the restaurant, the birthday party and the other counties had consumed sugar snaps. As a result, the local health authority in one municipality decided to impose sales restrictions on sugar snaps, something that was not done in any other municipality. In Sweden this can be decided on local level and does not require decision by the National Food Administration.

At the time of the outbreak sugar snaps from Kenya as well as from other African countries were sold all over Sweden. At least four large wholesale companies and an unknown number of smaller companies import sugar snaps to Sweden. Information from the restaurant and the cases indicated however that the implicated sugar snaps had been distributed by the same wholesaler. Interestingly enough this company is in liaison with the wholesaler that distributed the sugar snaps suspected to have caused the outbreak of *S. sonnei* earlier this year in Denmark.

No more domestic cases with *S. dysenteriae* were reported after the case with the latest date of onset, 15 June.

Discussion

It was difficult to find samples consumed by cases representative of the suspected food batch. One package from the time of the outbreak was found in one of the case's home. *Shigella* was not isolated from this sample or from samples of sugar snaps from the other two counties. However, it is known that isolating *Shigella* from food specimens can be difficult. *E. coli*, on the other hand, was confirmed in three of the samples and since both bacterial species represent intestinal microorganisms the finding of *E. coli* could still be a good indication that the analysed sugar snaps were contaminated by faecal material.

No cohort or case control study was performed in this outbreak as these studies are time consuming and the outbreak coincided with vacations. Personnel at the county medical offices in the involved counties interviewed the persons who were ill and sugar snaps were the only common denominator. Our conclusion is therefore that the most probable source of infection in this outbreak was sugar snaps.

During April to May 2009 outbreaks with *Shigella* connected to sugar snaps from Kenya were reported from two other northern countries; Norway and Denmark [1,2]. Strains of *S. sonnei* were isolated from patients and in Norway a sample of sugar peas was tested positive for *S. sonnei* by PCR. It was probably not a coincidence that *Shigella* outbreaks were connected to sugar snaps from Kenya in three Scandinavian countries within such a short time period.

The investigation performed by the Swedish National Food Administration showed that the trade routes from Kenya are many and diversified. The wholesale companies in Sweden usually have more than one local supplier in Kenya and each supplier in turn packs products from up to 200 local farmers. Trace-back to the farm of origin thus becomes very difficult. The wholesale companies require that each local producer is certified according to GlobalGap which is the golden international quality standard for produce. The question then arises whether this programme is strict enough to

prevent products from being contaminated by faecal pathogens or whether these regulations have not been followed adequately. According to available information, the period of growth this year in Kenya was dry and that normal production volumes could not be reached. Maybe the dry conditions led local producers to use contaminated water for irrigation.

The number of cases included in this outbreak is probably an underestimation of the actual number of persons affected as is the case in food-borne outbreaks in general. The county medical officer in the county with the restaurant outbreak was convinced that a number of people who had visited the restaurant and fallen ill afterwards did not seek healthcare and were not sampled. We may suppose that this was probably the case also in other counties.

Outbreaks with *Shigella* sp. are uncommon in Sweden but in 2008 there was a large outbreak in Stockholm with 140 cases infected with a very rare type of *S. sonnei* (mannitol negative). This was the largest outbreak of shigellosis in Sweden during the last 30 years. The cases had visited the same lunch restaurant. A cohort study pointed at grated carrots of Swedish origin as the suspected vehicle in the outbreak but this was not laboratory-confirmed [3].

The recent *Shigella* outbreaks in Denmark, Norway and Sweden, most likely associated with imported sugar peas from Africa, revealed a complex import system for sugar peas involving various wholesalers and distributors and numerous growers. The dimension of the system raises concern whether the existing international certification and quality standards that prevent products from being contaminated by faecal pathogens are strict enough.

As sugar peas are sold as a ready-to-eat product, consumers should be aware of the risk of possible contamination by faecal bacteria that can cause gastroenteritis. It is advisable to wash the vegetables or even better heat them up quickly. During the outbreak information on correct handling of vegetables to avoid infection was published on SMI and SLV websites. However, it will be discussed whether this kind of information should be disseminated more widely to prevent similar outbreaks in the future.

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A case of verocytotoxin-producing Escherichia coli O157 from a private barbecue in South East England

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The following case report describes a cluster of *Escherichia coli* O157 cases in the United Kingdom related to undercooked beef at a barbecue, resulting in an intensive care admission in France with haemolytic uraemic syndrome and highlighting the need to cook beef properly.

Introduction

A 32-year-old British woman became ill with diarrhoea on 1 June 2009 and travelled to France on 2 June. She was subsequently hospitalised in France on 7 June and was transferred to an intensive care unit with haemolytic uraemic syndrome (HUS). Her sister-in-law notified the Health Protection Agency about the case on 12 June. From the information she provided it was suspected that the infection occurred at a barbecue held by the case and her husband on 30 May at their home in Oxfordshire in which two other couples participated.

Methods

Case finding and epidemiological investigation

Information was gathered primarily from the sister-in-law who did not participate in the barbecue. On 15 June the other diners at the barbecue were contacted and food history was obtained from all participants. Faecal samples were sought first from another symptomatic guest, on 15 June, and subsequently, on 18 June, from others who ate at the barbecue but did not have any symptoms.

Environmental investigation

The local Environmental Health Officers were informed and went to the house of the case. They sampled a packet of unopened frozen minced beef bought at the same time as that used at the barbecue and, from the bin, the empty mince packet used for the barbecue with some residual meat and blotting paper in which the meat was wrapped. These samples were sent to the Food, Water and Environmental Microbiology Laboratory in Southampton for testing.

Laboratory confirmation and typing

For testing the empty beef mince packet the entire interior was swabbed and the swab, together with the small piece of raw meat and the blotting paper from the bottom, were placed in enrichment medium. Faecal and environmental isolates were confirmed, phage typed and tested for the presence of verocytotoxin (VT) – encoding genes by the Laboratory of Gastrointestinal Pathogens at the Centre for Infections, Colindale. The isolates were compared by pulsed-field gel electrophoresis (PFGE).

Results

Of the six people who ate at the barbecue only two were symptomatic: the index case hospitalised in France with HUS and an adult male with diarrhoea. He reported having eaten part of an undercooked beef burger at the barbecue. Other guests were well and reported eating similar foods to the two cases at the barbecue, which also included sausages, chicken kebabs and fish but none of them reported having undercooked beef burgers.

Stool specimens from the two cases were positive for *E. coli* O157. Three specimens from guests without illness were negative. The index case was tested in France and the isolate was not available for comparison. The other case in the UK was confirmed as *E. coli* O157 phage type 2, VT2 gene positive. The frozen beef did not grow any presumptive *E. coli* O157 but *E. coli* O157 was identified from the empty beef mince packet (which had contained the meat used to make the beef burgers at the barbecue). The empty meat packet was noticed to be very smelly and contained a bloody sheet of blotting paper at the bottom. The isolate from the meat packet was also phage type 2, VT2 gene positive. PFGE was performed on the PT2 isolates and their profiles were indistinguishable from each other.

Of 290 cases of *E. coli* O157 tested in the first half of 2009 by the Laboratory of Gastrointestinal Pathogens at the HPA 18 were PT2. These 18 PT2 cases were from six regions in England but none from the region in which this cluster occurred. None of the 18 PT2 isolates had the same VNTR type as the case in this cluster. PFGE is not routinely performed on all cases, only on those from suspected clusters.

Conclusions

There was a cluster of verocytotoxin-producing *E. coli* O157 cases related to homemade beef burgers at a private barbecue. Phenotypic and genotypic typing showed that the strain isolated from one case was indistinguishable from that from the investigated food source.

VTEC O157 is a potentially life threatening infection and it has not yet been eliminated from meat products. The public health message of the importance of cooking meat properly, particularly beefmeat products, therefore continues to be an important one. HUS is a rare sequela of VTEC O157 infections, particularly unusual in adults. The only risk factor identified in the case described here was that the patient was epileptic and was taking anti-epileptic medication.

Diagnosis of a British traveller in another European Union member state led to the identification of a cluster in the UK, thanks to the information provided to the Health Protection Agency by the family of the patient. Although identified late, when the second case was discovered, laboratory testing and typing of samples taken from this person and from residual food wrapping allowed identification of the source of infection. No other *E. coli* O157 cases were identified in the Thames Valley region during this time.

An outbreak of viral gastroenteritis linked to municipal water supply, Lombardy, Italy, June 2009

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We report an outbreak of viral gastroenteritis linked to municipal drinking water in a town in northern Italy in June 2009. Over one month we identified 299 probable cases of whom 30 were confirmed for at least one of the following viruses: norovirus, rotavirus, enterovirus or astrovirus. Water samples and filters from the water system also tested positive for norovirus and enterovirus. Control measures included treating the water system with chlorine dioxide and filters with peracetic acid, while providing temporary alternative sources of drinking water to the population.

Introduction

On 9 June 2009, a general practitioner from the municipality of San Felice del Benaco notified to the local health authority of Brescia (Lombardy region, north Italy) 21 cases of gastroenteritis among guests of a hotel. Patients presented with vomiting, diarrhoea and fever. In the following days, there were also reports of cases among local residents. Located near the lake of Garda, San Felice del Benaco has 3,360 residents but is very touristic during the summer months. We investigated the outbreak in order to identify the source of infection and implement appropriate control measures.

Methods

We defined a probable outbreak case as a person who fell sick with vomiting or diarrhoea after 7 June 2009 and who stayed prior to disease onset in San Felice del Benaco. A confirmed outbreak case was defined as a person who fulfilled the criteria of a probable case and whose stool sample was laboratory-confirmed for at least one of the following viruses: norovirus, rotavirus, enterovirus or astrovirus. Probable cases who tested negative for the presence of virus in the stools were still considered as probable cases.

Active case finding was performed as follows: a public hotline was set up where people could call the health authority for information regarding the disease and report symptoms, date of onset and basic demographic data. In parallel, the outbreak investigation team collected daily information on case-patients presenting at the emergency unit of the local hospital and collected stool samples when possible.

The local and regional health authorities initiated an environmental investigation at the hotel on 9 June 2009, taking food samples from the kitchen, interviewing and collecting stool samples for microbiological testing from 20 probable cases (both guests and hotel staff). When it was clear that the outbreak was spreading to the larger community (apart from three campsites with their own private water supply, where no cases were reported), the environmental investigation was extended and included collection of water samples from the municipal water supply. Municipal water comes from the nearby lake. Before being distributed to the town as drinking water, it is treated with chlorine dioxide and hypochlorite and passes through sand filters. The investigators collected a total of 94 water samples from the lake at the location where the water is pumped, from filters and from public fountains. Samples were sent to the Lombardy and Emilia Romagna Experimental Zooprophylactic Institute (IZSLER) to test for the presence of bacterial pathogens (*Salmonella* sp., *Shigella* sp., *Campylobacter* sp., *E. coli* O157, *Yersinia enterocolitica, Aeromonas* sp., *Clostridium perfringens* toxins), parasites (*Cryptosporidium* sp.) and viral pathogens (norovirus, rotavirus, enterovirus, astrovirus). Virological methods included negative staining electron microscopy, type A rotavirus ELISA and PCR methods for norovirus, rotavirus, enterovirus and astrovirus.

Results

A total of 299 persons fulfilled the outbreak case definition, including 269 probable and 30 confirmed cases. The epidemic curve in Figure 1 shows the probable and confirmed outbreak cases by date of onset. The outbreak occurred between 8 June and 4 July 2009 and peaked on the 15 and 16 June with 47 outbreak cases per day.

The attack rate for the town of San Felice del Benaco was 8.9% (299/3,360). Age group-specific attack rates ranged from 7% (50/713) in persons aged 65 years and older to 14% (34/242) in

the age group 15-24 years (Figure 2). Four cases were hospitalised, all of them children.

There was no fatality. Stool samples obtained from 36 probable cases were examined at the laboratory. Of these, 17 (47.2%) tested positive for norovirus, 19 (52.8%) for rotavirus, 12 (33.3%) for enterovirus and 4 (11.1%) for astrovirus. Eight cases had both norovirus and rotavirus in the stools and two cases tested positive for norovirus, rotavirus and enterovirus. The laboratory did not find any virus in six cases (but we still included them among probable outbreak cases because of compatible symptoms). *Salmonella* sp., *Clostridium perfringens* and *Campylobacter* sp. were found in samples from two, one and one cases, respectively.

The mean age of confirmed cases of rotavirus was 29 years (range: 0-71) compared to the mean age of 39 years (range: 0-88) for cases of norovirus and 39 years (9-88) for cases of enterovirus. The age distribution of confirmed cases is shown in Figure 3.

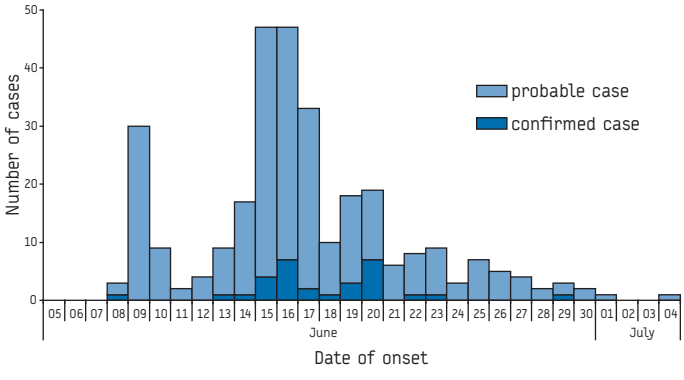
Food samples from the hotel tested negative for the presence of pathogens. On 16 June 2009, preliminary environmental investigation results showed abnormally high levels of *Clostridium perfringens* (4 UFC/100 ml) and *Aeromonas hydrophyla* (16 UFC/100 ml) in water samples from two public fountains. Tests on 44 water samples from from the municipal water system (water from fountains and filers) showed the presence of norovirus and enterovirus. Examination of the municipal water network revealed that: 1) the water company had undertaken work on the collection reservoir which might have limited the effect of chlorination; 2) two filters were 10 years old (cleaned weekly but not disinfected); 3) the chlorine concentration in the water before it passed through the filters was 0.4 mg/l; in filtered water it was only 0.08 mg/l.

Control measures

On 17 June 2009, a special ordinance from the municipality restricted the use of municipal water (inhabitants were told not to use municipal water for drinking and cooking purposes) and provided alternative water supplies to the population via water tankers. Local authorities organised a door-to-door information campaign and distributed leaflets in order to reach as many people as possible. On 19 June 2009, the municipality started disinfecting the water system with chlorine dioxide (0.2 mg/l) and sand filters with peracetic acid. When the presence of norovirus in water and stools of cases was confirmed, the residual concentration of chlorine dioxide in terminal points of the network was increased to 3.4 mg/l for three consecutive days from 23 June 2009. Regular water sampling and testing was performed to monitor the efficiency of control measures. The ordinance on drinking water was maintained

Figure 1

Probable (n=269) and confirmed (n=30) cases of viral gastroenteritis, by date of onset of symptoms, San Felice del Benaco, Italy, 8 June 2009 - 4 July 2009



Table

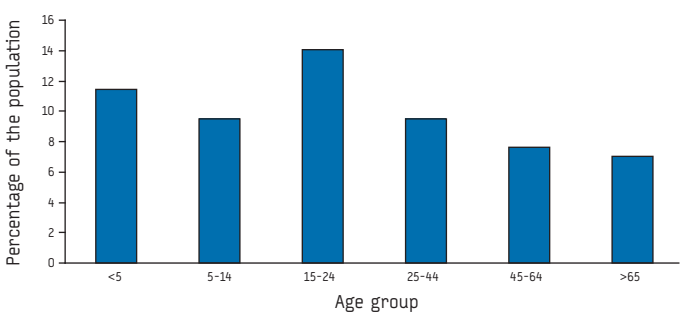
Pathogens found in stools samples of 36 cases of gastroenteritis, San Felice del Benaco, Italy, 8 June 2009 – 4 July 2009

Pathogen	Number of patients with positive results (multiple infection possible)	Percentage (%)
Rotavirus	19	52.8
Norovirus	17	47.2
Enterovirus	12	33.3
Astrovirus	4	11.1
Salmonella sp.	2	5.6
Clostridium perfringens	1	2.8
Campylobacter sp.	1	2.8

Source: Lombardy and Emilia Romagna Experimental Zooprophylactic Institute (IZSLER), Brescia, Italy

Figure 2

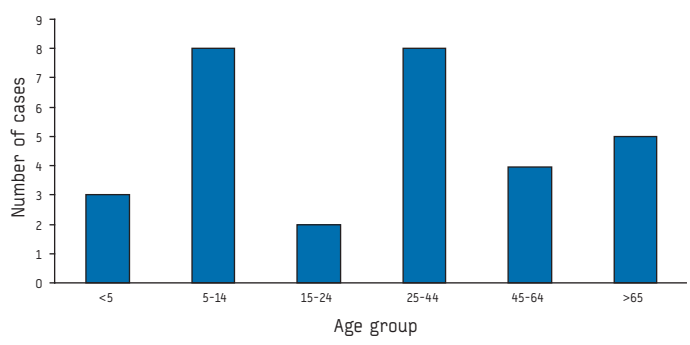
Attack rate of gastroenteritis per age group, outbreak in San Felice del Benaco, Italy, 8 June 2009 - 4 July 2009 ((n=299 cases)



Source: Azienda Sanitaria Locale (ASL) Brescia, Italy

Figure 3

Age distribution of confimed cases of viral gastroenteritis, San Felice del Benaco, Italy, 8 June 2009 - 4 July 2009 (n=30)



Source: Azienda Sanitaria Locale (ASL) Brescia, Italy

until all water quality tests complied with safety norms. Water samples collected after the first treatment with chlorine dioxide and peracetic acid all tested negative for the presence of norovirus.

Conclusion and discussion

An outbreak of viral gastroenteritis has been microbiologically linked to a contaminated municipal water supply in a small town of Lombardy. Timely control measures and good compliance of the population following the information campaign prevented a much higher attack rate.

The alert came from a cluster of gastroenteritis in a hotel. The direction of the hotel promptly informed a general practitioner who notified the cluster to the public health authorities. An increase of gastroenteritis in the general population was noticed one day after the initial alert. The hotel is located along the lake, near the water reservoir, which could explain why the guests and its staff were among the first to be affected (see the first peak on the epidemic curve on 9 July 2009).

Although the number of residents in San Felice Del Benaco is 3,360, it is important to note that in the summer season many tourists stay in the town and the total population is multiplied by three. Therefore, the attack rates reported above (based on the resident population) are probably overestimates even though the surveillance system did not capture all cases. All age groups were affected. This is consistent with an exposure that is equally distributed across all ages. The relatively high mean age of confirmed rotavirus cases (29 years) is also consistent with an exposure that is not limited to young children.

The municipal water is taken from the lake at a place where the water is stagnant. So far, water samples from the lake tested negative for the presence of norovirus, rotavirus, enterovirus or astrovirus. However, we cannot exclude contamination of the lake due to over-capacity of the sewage system and/or illegal wastage.* In Italy, municipal water systems have been identified as the source of water-borne infections in several norovirus outbreaks (1, 2). It reminds us of the public health importance of well-maintained and monitored water supplies in our towns and cities (3).

Acknowledgements

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* Authors' correction

Upon the request of authors, one sentence was deleted from the discussion after the publication of the article. The correction was made on 3 August 2009.

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Verocytotoxin-producing Escherichia coli O157 outbreak in Wrexham, North Wales, July 2009

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An outbreak of *Escherichia coli* O157 involving four people in North Wales is currently being investigated. Laboratory typing shows all the isolates belong to phage type 2. All four cases reported eating different products from a fast food outlet in the area. The possibility of other common exposures is being explored.

The National Public Health Service for Wales (NPHS) and Environmental Health Officers from Wrexham County Borough Council (WCBC) are currently investigating four cases of verocytotoxin-producing *Escherichia coli* O157 (VTEC O157) in the Wrexham area.

The cases are all females, aged 3, 23, 32 and 32 years. Case 1 had an onset date of 20 July and was reported to the NPHS on 22 July after a positive stool sample result. She later developed haemolytic uraemic syndrome and thrombocytopaenic purpura and was admitted to hospital on 28 July. She is currently receiving renal dialysis and ongoing plasmapheresis. Case 2 had an onset date of 21 July and was reported to the NPHS on 24 July. She is recovering at home. Case 3 and 4 are a mother and daughter, both with onset of symptoms on 21 July. The child was admitted to hospital on 27 July with haemolytic uraemic syndrome and required dialysis for five days. She has now been discharged. Samples were taken from mother and child at the hospital, and the results were reported to the NPHS on 30 July. All four cases reported eating different products (chicken, beef and vegetarian burgers) from a fast food outlet in the area in the week before becoming unwell. The possibility that the cases have links involving other common exposures is still being explored.

Faecal samples from all the cases were confirmed as positive for *E. coli* O157. Confirmation and typing at the Laboratory of Gastrointestinal Pathogens (LGP) at the Health Protection Agency in London have shown them all to belong to phage type (PT) 2 and to possess genes encoding verocytotoxin VT2. The isolates were indistinguishable from each other by pulsed field gel electrophoresis (PFGE) of XbaI fragments. Variable number tandem repeat typing showed that they had the same profile that was not found in other isolates of PT2 from 2009 tested so far.

The food outlet was visited by Environmental Health Officers from WCBC on 30 July. Several problems were identified, such as poor food handling techniques, lack of hand washing equipment, no evidence of food hygiene training for staff and no food safety management system in place. As a precaution the outlet is currently the subject of a Hygiene Emergency Prohibition Order, and is closed until further notice. This means that the owners have to demonstrate that systems are in place to correct the deficiencies identified and satisfy the Environmental Health Officers that food handling practices will change before reopening. Food and environmental samples were taken from the food outlet for laboratory investigations. Results are pending.

Active case finding has been pursued using local general practitioners, but there have been no further cases reported to date.

VTEC O157 PT2 strains may be associated with the development of serious illness. They have represented around 10% of isolates in England and Wales since 2005, compared with the most prevalent type, PT21/28, that accounted for up to 40% of reports [1,2].

Twenty four isolates of VTEC O157 were confirmed from Welsh laboratories in 2009 up until 3 August. Prior to the cases reported here, there were only two sporadic infections with PT2 (in mid-March) and neither was from North Wales. Food or animal sources were not investigated for these unlinked cases.

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Vibrio cholerae non-O1 non-O139 infection in an immunocompromised patient returning from Spain, July 2009

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We describe a severe gastroenteritis with non-O1, non-O139 *Vibrio cholerae* in an immunocompromised patient returning from a holiday in Spain in July 2009. Predisposing factors and possible cholera enterotoxin production could explain the unusually grave symptomatology. The patient recovered after doxycyclin treatment.

In July 2009, a Dutch man in his fifties presented to an emergency department in Amsterdam with profuse diarrhoea. He had recently been diagnosed with systemic sclerosis complicated by a renal crisis, myositis and reduced motility of the stomach and small bowel (especially the duodenum). His medication included prednisone and esomeprazole.

The patient became ill the day before presentation with severe diarrhoea (more than 30 evacuations per day), vomiting and abdominal cramps. One day before onset of symptoms he had returned from Canary Islands, Spain. He had not swum in natural water nor eaten seafood during his stay. None of his family members who had accompanied him on his holiday had symptoms of gastroenteritis. On examination he was afebrile with normal pulse and blood pressure. He was severely dehydrated having lost more than 10% of his bodyweight. Laboratory tests showed an acidosis, hypokalaemia and elevated creatinine and C-reactive protein. He was hospitalised and treated with intravenous fluids and potassium.

A faecal culture was sent to the microbiology department. Its rice water appearance guided the technician to include testing for *Vibrio cholerae*. A lactase-negative, oxidase-positive, Gram-negative rod was identified by the Vitek system (Biomerieux, France) as *V. cholerae*. Serotyping classified it as non-O1, non-O139 serotype. Disk diffusion results showed susceptibility to cefotaxime, ciprofloxacin, trimethoprim-sulfamethoxazole and tetracycline.

When culture results became available six days later, the patient was still having diarrhoea (diminished to 10 evacuations a day) and was feeling unwell. Antibiotic treatment was started with oral doxycycline 100mg for three days and this led to quick recovery from his gastroenteritis. Further worsening of the systemic sclerosis prevented the patient from being discharged in the following days.

In contrast to *V. cholerae* serotype O1 and O139, the non-O1, non-O139 *V. cholerae* (NCV) are not associated with cholera epidemics but with sporadic cases or small outbreaks of gastrointestinal disease [1,2]. Occasionally these can cause extraintestinal disease including wound infections and septicaemia [1,2]. Few NCV strains produce cholera enterotoxin, the toxin responsible for massive dehydrating diarrhoea. Some strains can have other virulence genes leading to less severe intestinal symptoms [1,2]. The presence of typical choleric rice water stools and the extent of dehydration in our patient are uncommon in NCV infection and suggest cholera enterotoxin production [3]. The predisposition of this patient may have contributed to the severity of disease. He was on immunosuppressive medication, his gastric acid production was blocked by esomeprazole and the intestinal mobility was impaired [4,5].

NCVs are part of the normal bacterial ecosystem of estuaries and coastal areas and these strains seem to persist in the environment, similar to *V. cholerae* O1 and O139 strains [5]. NCVs are found in salt and fresh water in both the Mediterranean and temperate parts of Europe. Warm summer months favour *Vibrio* growth and it is in late summer and early fall that most cases occur, either through eating contaminated seafood or by direct contact with contaminated water [6-8]. A recent study in Italy, in a population with high dietary seafood intake, showed that 3.4% of the acute diarrhoea cases admitted to hospital were caused by NCV infection [7]. Most European countries do not routinely check for the presence of NCV in clinical samples, foodstuff or the environment. Our case underlines the importance of testing for V. cholerae in potentially exposed patients with acute diarrhoea, especially when predisposing factors like immunosuppression and acid-blocking medication are present.

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Finland introduces rotavirus vaccine into the national vaccination programme in September 2009

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Supported by an economic evaluation, rotavirus vaccine is introduced into the national immunisation schedule in Finland. The vaccination programme has been estimated to be reasonably cost-effective. Given at the age of two, three and five months, the vaccine is expected to prevent annually in Finland among children under the age of five years approximately 2,000 rotavirus diarrhoea episodes needing hospitalisation, and over 10,000 outpatient visits. The impact of the programme will be evaluated in 2011 by repeating the economic analysis and carefully monitoring adverse events.

Rotavirus causes epidemics every year during the months of winter and spring in northern Europe. Especially in young children, the infection manifests as acute gastroenteritis with high fever, vomiting and watery diarrhoea, with 10-20 stools per day, lasting for a total of three to eight days. The first rotavirus infection in a person is usually symptomatic, and can easily lead to severe dehydration. The typical clinical picture is usually observed in children between the ages of six months and two years. Almost all children are infected with rotavirus, either with symptoms or asymptomatic, before they are five years old. Rotavirus infection is easily transmitted, since a lot of virus is excreted in stools during diarrhoeal bouts.

As in Europe in general, serotypes G1 and G4 have been the dominant serotypes causing annual rotavirus diarrhoea epidemics during 1980s and 1990s in Finland [1,2]. In recent years, serotype G9 has gained importance and was the most common serotype found in 2005. Among the total 125 isolates serotyped from the Helsinki metropolitan area during the epidemic season in 2006-7, the G1P[8] was dominant (57%) followed by G9P[8] (29%). The G4P[8] serotype was found in only four isolates [3].

Presently there are two live rotavirus vaccines on the market, which differ in their antigenic composition and protective principle [3]. The RotaTeq vaccine is a live reassortant vaccine derived from human and bovine rotaviruses. For sufficient protection, three doses are needed. Rotarix is a live attenuated vaccine based on human rotaviruses (RIX4414). For sufficient protection, two doses are needed. The vaccine preparations contain different rotavirus serotypes: RotaTeq is composed of serotypes G1, G2, G3, G4 and P[8] and Rotarix of G1P[8].

In clinical trials, vaccine efficacy of either vaccine against severe rotavirus diarrhoea that requires rehydration therapy was over 90 %, and against any rotavirus diarrhoea 60–70 % (3). Although no formal comparative efficacy analysis was performed, there is no scientific reason to believe that the protective efficacy of these two vaccines would be significantly different that could guide the choice of one vaccine over the other. Based on the trial outcome, the risk profiles of the two vaccines are also fairly similar.

In Finland, a new vaccine can be introduced to the national immunisation programme if it fulfils four key criteria. There needs to be a public health disease burden that is to be prevented, the vaccine needs to be safe and able to reduce the disease burden, it should not have any significant adverse events on the population level, and finally, the intervention should be reasonably cost-effective to justify the expense from the state budget [4].

Evaluation of the cost-effectiveness of rotavirus vaccination

In order to understand the burden of disease caused by rotavirus, we estimated the proportion of healthcare resource use attributable to rotavirus. We regressed [5,6] the weekly laboratory reports of gastrointestinal pathogens on the weekly infectious and non-infectious intestinal disease episodes (constructed from the hospital outpatient visits and inpatient hospitalisations) and weekly primary healthcare visits according to a model. According to this estimation of the burden of disease, approximately 11,100 children under five years of age annually need health care services due to rotavirus in Finland [7]. We estimated that rotavirus gastroenteritis annually leads to 2,400 episodes needing hospitalisation, 3,700 hospital outpatient visits and 9,000 visits to healthcare centres.

To investigate the potential cost-effectiveness of the vaccination programme, a cohort model was constructed to compare the costs and outcomes of the two rotavirus vaccines to a scenario without intervention [8]. A hypothetical birth cohort was followed over the first five years of life. The analysis was conducted from the perspectives of the health care provider and of society.

It was estimated that a rotavirus vaccination programme in Finland could prevent annually approximately 2,000 rotavirus diarrhoea episodes requiring hospitalisation and over 10,000 outpatient visits among children under the age of five years. The estimated annual costs to the healthcare provider of rotavirus infection among children under five years were EUR 4.2 million without vaccination. The cost per quality-adjusted life year (QALY) gained from the perspective of the healthcare provider was EUR 25,218 for Rotarix (assuming EUR 39.5 per dose) and

EUR 45,199 for Rotateq (assuming EUR 29.5 per dose). In the probabilistic sensitivity analysis (healthcare payer perspective), the 95% confidence intervals for cost per QALY gained ranged from EUR 20,370 to EUR 30,498 for Rotarix and from EUR 38,177 to EUR 48,506 for Rotateq.

The Finnish National Institute of Health and Welfare (THL) and National Advisory Boards of Vaccination and Infectious Diseases who reviewed the analysis in 2007 agreed that the parameter values were based on good quality national data and that the assumptions chosen were conservative enough to give relevant guidance for national decision making [4]. Based on this analysis, the rotavirus vaccination programme appeared to be not cost-saving but reasonably cost-effective, especially if nosocomial infections and home-treated rotavirus cases were included. Thus, rotavirus vaccine was recommended to be included into the national programme – a recommendation which the Ministry of Social Affairs and Health as well as the Ministry of Finances agreed to in 2008.

Choosing the vaccine to be used

In Finland, the procurement of vaccines for the national programme is centralised. In the competitive bidding done in 2008, the only decisive factor was the price. The offer of RotaTeq manufactured by Sanofi Pasteur MSD was cheaper; at this price the programme was cost-saving. Finland has now agreed to include RotaTeq into the national programme for two years after which a new tender will be launched. Today, given the present price of Rotateq, the rotavirus vaccine programme is estimated to be cost saving both from the societal and health care provider perspective. Also, it is to be expected that the vaccine provides indirect protection to the society as a whole when transmission of rotavirus is reduced [9].

In Finland, the three doses of the vaccine will be given at the ages of two, three, and five months thus increasing by one the visits to a well-baby clinic for vaccination (the one at two months of age). As a precaution, the first dose is recommended to be given before the age of 12 weeks, but not earlier than six weeks. Also, the child should not be older than 26 weeks (i.e. 6.5 months) when the third dose is given. These age limits approved by the European Medicines Agency (EMEA) are somewhat stricter than those recommended by the United States Food and Drug Administration (FDA), which has recently raised the upper limit of the third dose to the age of eight months. In addition, the Strategic Advisory Group of Experts (SAGE) and the Global Advisory Committee on Vaccine Safety (GACVS) of the World Health Organization (WHO) have suggested that these limits be raised even more in resource-poor countries where the rotavirus disease burden is very high, and where it is important for rotavirus vaccine coverage to be as high as possible. In such countries the recommended upper limit is 15 weeks for the first dose and 36 weeks for the third dose [10].

Safety of the RotaTeq vaccine

The clinical safety of RotaTeq was proven in trials involving approximately 70,000 children in 12 countries. One third of these were Finnish children (11). By spring 2009, the manufacturer had sold approximately 22 million doses of RotaTeq. In those countries where the vaccine was introduced into the national programme (i.e. Australia, Austria, Luxemburg, and the United States), it has proved to be safe. In the US, the reported incidence of intussusception (1/25–50,000 first doses) did not differ from that expected, i.e. from the observed incidence before starting the vaccinations [12]. Cases of intussusception were reported somewhat more often after the first than after the second or third doses. Another cause for concern has been Kawasaki disease. During the clinical trials the suspicion of an increased incidence of Kawasaki disease arose, although the difference between the vaccines and controls was not significant. After the introduction of the vaccine and careful monitoring, there has been no evidence that would point to an increased risk of Kawasaki disease among those vaccinated [12]. The most common expected adverse events are mild and transient gastrointestinal and respiratory symptoms [11]. After the first dose, less than 9 % of the vaccinees excrete the vaccine virus into stools. This is even more rare after receiving the second or third dose.

Monitoring the impact of rotavirus vaccination

Rotavirus vaccinations will be started in September 2009 in all the well-baby clinics in Finland, which cover approximately 99% of the Finnish cohort of newborns. For the time being, Finland does not have an operational vaccine registry. Thus, vaccine coverage, which traditionally has been high in the country, i.e. above 90% for most vaccines used in the national programme [13], will be monitored using the administrative method combined with periodic surveys of the vaccination status of a randomly chosen sample of 1,000 children. Adverse events associated with rotavirus vaccination will be monitored through the existing passive surveillance system, i.e. health care personnel will notify of any suspect case of adverse events following immunisation (AEFI) to THL. In addition, certain clinical manifestations like intussusception and Kawasaki disease will be actively monitored as part of the VAESCO project, a project for harmonising vaccine safety in Europe (www.vaesco.net). A systematic monitoring of the effectiveness of the rotavirus vaccination programme is planned for the year 2011 repeating the collection of morbidity and mortality data as done for the economic evaluation [7,8]. In addition, isolated rotavirus vaccine strains will be sero- and genotyped to understand the possible impact of vaccination on new reassortments and shifts in the proportions of the prevailing serotypes [2].

Details on the rotavirus vaccines used, vaccinating, adverse event monitoring and frequently asked questions can be found at the THL website both in Finnish and Swedish language (www.thl.fi).

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West Nile virus infection in Veneto region, Italy, 2008-2009

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We report here an update on human cases of West Nile virus (WNV) infection in Veneto region, northeastern Italy. In addition to two cases of WNV neuroinvasive disease notified through a surveillance programme started in September 2008, further four cases were retrospectively identified (in May 2009) by investigating patients with aseptic meningoencephalitis of unknown aetiology occurring in Veneto region in June-September 2008. All six patients had symptom onset in August-September 2008 and were resident in a wetland area close to the Po river delta in Rovigo province. Further five cases of asymptomatic WNV infection, including four residents of the same area in Rovigo, were identified in a seroprevalence study in farm workers from Veneto region. To date, no human cases have been notified in 2009.

Introduction

In Italy, the first outbreak of West Nile virus (WNV) infection was reported in the late summer 1998 among horses residing in a wetland area in Tuscany. At that time, 14 horses had neurological illness and six of them died, but no human cases of WNV disease were reported [1]. Subsequently, a national veterinary surveillance plan for WNV was activated in 2002 in Italy, aiming to identify risk areas and to monitor WNV circulation based on observation of wild bird mortality, and on entomological and sentinel chicken surveillance, as well as to check for WNV seroconversion in horses residing in risk areas. Thereafter, sporadic seroconversions have been identified in sentinel chickens and horses [2,3], but no equine or human cases of symptomatic WNV infection had been notified until September 2008, when an outbreak of WNV infection was identified in the northeastern part of Italy [4,5].

The first possible case of WNV neuroinvasive infection in a horse was notified on 8 September 2008 in Emilia-Romagna region, Italy. A special plan for WNV surveillance was subsequently activated in Emilia-Romagna on 16 September, which led to the identification of other horses with WNV neuroinvasive illness [4] and, on 20 September 2008, to the identification of the first human case of meningoencephalitis caused by WNV infection in a female patient who lived in a rural area between Ferrara and Bologna in Emilia-Romagna region, and had symptom onset on 15 September 2008 [5].

In Veneto region, the first WNV-seropositive horse was identified on 24 September 2008 in a stable in Rovigo province, where a horse presented neurological symptoms after being brought back from Emilia-Romagna region. Thereafter, on 29 September 2008, Veneto region activated a special veterinary surveillance plan in horse stables of Rovigo, Venezia and Padova provinces, and started a seroepidemiological investigation of all workers on farms where infected horses were identified, as well as a surveillance programme for possible human cases of WNV infection in Veneto region. To identify cases that might have occurred before the implementation of these surveillance activities, in May 2009, we performed a retrospective investigation of cases of aseptic meningoencephalitis of unknown aetiology occurring in Veneto region in June-September 2008. Here we describe the results of this retrospective study as well as provide an update on cases reported through the surveillance programme and on those identified in the seroepidemiological study of stable workers, and present the results of screening of blood and organ donations from the affected area.

Retrospective study of cases of aseptic meningoencephalitis

Methods

To identify cases of WNV neuroinvasive disease occurring before the activation of the surveillance programme in Veneto region, we retrospectively analysed cerebrospinal fluid (CSF) samples referred to our Regional Reference Centre from hospitals of Veneto region in the period June-September 2008 for the presence of specific immunoglobuline M (IgM) antibodies against WNV. This study was performed in May 2009.

CSF samples from patients aged ≥15 years with suspected viral encephalitis, but with negative viral test results (routine PCR and serology tests for herpes simplex virus (HSV), varicella zoster virus (VZV), enteroviruses, tick-borne encephalitis virus (TBEV), Toscana virus (TOSV) and other neurotropic viruses), were selected for the study, according to definition criteria for possible cases of WNV neuroinvasive disease (Table 1).

WNV IgM testing was done by using WNV IgM capture DxSelect™ ELISA (Focus Diagnostics, Cypress, California) according to the manufacturer instruction, with the exception that CSF was diluted 1:2, as recommended by Prince *et al.* [6]. CSF samples which were positive at WNV IgM capture ELISA were tested for neutralising antibodies by plaque-reduction neutralisation test (PRNT) for WNV and for tick-borne encephalitis virus (TBEV), a flavivirus commonly found in northeastern Italy, to rule-out cross-reactivity. PRNT was

conducted in a biosafety level 3 lab, according to the protocol described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2008 of the World Organisation for Animal Health (OIE). Briefly, heat-inactivated CSF or serum samples were tested at 1:100 final dilution. Equal volume of serum and medium containing 100 plaque-forming units of WNV were incubated for 75 min at 37 °C before inoculation onto confluent monolayers of Vero E6 cells grown in 25 cm2 flasks. After the inoculum was adsorbed for 1 h at 37 °C, cells were overlayed with agarose-containing medium, and then incubated for 72 h at 37 °C. Then, a second agarose overlay containing 0.003% neutral red dye was applied to each flask for plaque visualisation. Following a further overnight incubation at 37 °C, the number of virus plaques per flask was assessed. Endpoint titres were assigned as the greatest dilution in which >90% neutralisation of the challenge virus was achieved. Samples with reciprocal 90% neutralisation titres of >10 were considered positive. WNV IgM-positive CSF samples were also tested by real-time RT-PCR for WNV-RNA detection using the oligonucleotide primers and TaqMan probe targeting the WNV E gene designed by Lanciotti et al. [7]. For real-time RT-PCR, nucleic acids were purified from 200 µl CSF or plasma samples by using an NucliSENS® easyMAG® system (bioMérieux, Inc., Durham, NC) and eluted in a final volume of 50 µl. Then, 5 µl of RNA was combined with Superscript® One Step RT-PCR System reagents (Invitrogen Ltd, Paisley, UK), primers and probe in a 20-µl total reaction volume and amplified in a LightCycler® 2.0 Real-Time PCR System (Roche Diagnostics S.p.A., Monza, Italy).

Results

Of the 74 investigated patients (40 males and 34 females; median age 51.5 years, range 21-94 years) with aseptic meningoencephalitis of unknown aetiology, four (a 69-year-old woman and three men aged 69, 70, and 86 years) had IgM antibodies against WNV in CSF, as demonstrated by IgM capture ELISA (Table 2). The presence of WNV-specific neutralising antibodies in CSF was confirmed in all four cases by PRNT, which showed neutralisation titres >1:40, while WNV-RNA testing gave negative results. The presence of WNV-reactive neutralising antibodies was also demonstrated in a convalescent serum specimen, subsequently provided. For two patients, two consecutive serum samples were available, which showed an increase of WNV-specific antibody titre. All four WNV-positive patients were resident in Rovigo province and were hospitalised in the period from 25 August to 9 September. One of these patients (male, 70 years old), who had encephalitis in early September 2009, was described as a probable case in a previous report, based on the detection of high titre WNV IgG in February 2009 [8].

Table 1

Case definition of West Nile virus (WNV) neuroinvasive disease, surveillance programme in Veneto and Emilia Romagna regions, Italy, 2008-2009

Subjects ≥ 15 yr with fever ≥ 38.5°C and neurological symptoms (e.g., encephalitis, meningitis, Guillain-Barré syndrome or acute flaccid paralysis).
Cases were classified as:
Possible: clinical symptoms and aseptic CSF.
Probable: clinical symptoms and at least one of the following laboratory criteria: - presence of IgM antibodies against WNV by ELISA; - seroconversion by ELISA; - fourfold increase of IgG antibodies against WNV in two consecutive samplings (>5 days, preferably 15-20 days between the two samples) by ELISA.
Confirmed: clinical symptoms and at least one of the following laboratory criteria: - isolation of WNV in blood or CSF; - presence of IgM antibodies in CSF (by ELISA); - detection of WNV-RNA by RT-PCR in blood or CSF; - detection of increased levels of WNV IgM and IgG by ELISA and confirmed by PRNT.

WNV: West Nile virus; CSF: cerebrospinal fluid; PRNT: plaque-reduction neutralisation test.

Table 2

Summary of data on cases of West Nile virus (WNV) infection in Veneto region, Italy, 2008-2009

Province	Retrospective analysis of cases of meningoencephalitis of unknown aetiology (June-September 2008) Number of confirmed/total investigated (%)	WNV disease surveillance (October 2008 - July 2009) Number of confirmed/total suspected (%)	Seroepidemiological survey of farm workers (October-December 2008) Number of confirmed/total investigated (%)
Rovigo	4/15 (26.7%)	2/24 (8.3%)	4/212 (1.9%)
Padova	0/21 (0%)	0/17 (0%)	0/92 (0%)
Venezia	0/11 (0%)	0/2 (0%)	1/17 (5.9%)
Vicenza	0/1 (0%)	0/4 (0%)	-
Verona	0/1 (0%)	0/4 (0%)	-
Treviso	0/13 (0%)	0/10 (0%)	-
Belluno	0/12 (0%)	-	-
TOTAL	4/74 (5.4%)	2/61 (3.3%)	5/321 (1.6%)

WNV infection surveillance in Veneto region, 2008-2009

Methods

A surveillance programme for possible human cases of WNV infection was activated in Veneto region on 29 September 2008, after the notification of the first equine case on 24 September 2008. All infectious disease units of hospitals in Veneto region were asked to report suspected cases of aseptic encephalitis and/or meningitis of unknown aetiology from all provinces of the region and cases of fever and rash from areas where WNV infection had been documented in horses (initially only Rovigo, eventually also Venice and Padua provinces), and to collect blood and CSF samples from these patients. Specimens of blood and CSF were sent to our Regional Reference Centre and investigated for IgM and IgG antibodies against WNV by ELISA testing (Focus Diagnostics), PRNT to confirm ELISA-positive samples, and WNV real-time RT-PCR, as above described.

Results

Within this ongoing surveillance programme, to date, 61 patients from Veneto region (33 males and 28 females; median age 47 years, range 19-85 years) were reported with suspected WNV infection and referred for further investigation. Of these, 37 were referred in October-November 2008, and 24 were reported in June-July 2009. Of these, only two cases in 2008 were confirmed for WNV infection, as described in a previous report [8]. The first was an 81-year-old woman from Rovigo hospitalised in the end of August 2008 for suspected viral meningoencephalitis with fever, headache, and altered mental status. On October 16, serology testing demonstrated the presence of IgM and IgG antibodies against WNV, confirmed by PRNT; retrospective analysis of a CSF sample collected on 6 September demonstrated the presence of IgM antibodies against WNV, while WNV RNA testing was negative. The second case was a 48-year-old female patient resident in Rovigo province, who had an episode of fever, severe headache, maculopapular rush, pharyngitis, adenopathy, and arthralgia starting in early August 2008. WNV serology testing, performed in the end of November for persistence of symptoms, was positive for IgM and IgG antibodies, confirmed by PRNT.

To date, no human cases of WNV infection have been identified in 2009.

Seroepidemiological survey

Methods

A seroepidemiological survey was started in Veneto region on 29 September 2008 involving all workers employed in farms where WNV-positive horses were identified by the veterinary surveillance. The aim of the study was to investigate the prevalence of WNV infection and to promote the awareness of the disease in this at-risk population. In the survey, local Public Health Services conducted interviews with farm workers to ascertain their risk for WNV infection and collected serum samples, which were sent for analysis to our Regional Reference Centre. We tested the samples for IgM and IgG antibodies against WNV by ELISA and used PRNT for confirmation, as above described.

Results

Of 321 investigated subjects (178 males and 143 females, median age 45 years; range 4-84 years), two men (71 and 76 years old) and three women (51, 60, and 67 years old), all asymptomatic, were IgM and IgG WNV-reactive (two cases) or only IgG WNV-reactive (three cases) and confirmed by PRNT. Four of these persons were resident in Rovigo province and one in Venice province (Table 2). Four have been previously reported [8].

Screening of blood and organ donations

Methods

Following the notification of the first human case of WNV infection in Veneto region, in accordance with the European Union blood safety directive [9], a nucleic acid test (NAT) for WNV RNA screening was started on 28 October 2008 in all blood, stem cells, tissue, and organ donations collected in the period from 1 September to 5 December 2008 from donors who were resident in Rovigo province or who stayed for at least one night in Rovigo province during the last 28 days before donation. In 2009, based on estimates of WNV circulation in Italy, WNV-RNA NAT screening will be done on all donations collected from 1 August to 31 October in Rovigo province, as well as in the provinces of Ferrara (Emilia-Romagna region) and Mantova (Lombardia region).

Results

During 2008, our Regional Reference Centre individually screened a total of 5,500 donations by using the Procleix WNV Assay (Chiron, Novartis). All donations resulted WNV RNA-negative.

Discussion

Surveillance of suspected cases of WNV infection and retrospective investigation of cases of meningoencephalitis of unknown aetiology occurring in Veneto region led to the identification of six patients with WNV neuroinvasive disease. All cases were resident in a wetland area of about 40 km in diameter in Rovigo province and had symptom onset in the period ranging from early August to mid-September 2008. The incidence of WNV disease in this area could be estimated at 12 cases per 100,000 population, but this is probably an underestimation because based in part on retrospective data.

In the neighbouring provinces of Ferrara and Bologna in Emilia-Romagna region, three human cases of WNV neuroinvasive disease were reported, with symptom onset in early, mid-, and late September 2008 [5,8].

The seroprevalence study in farm workers from Veneto region demonstrated a low prevalence (<2%) of WNV infection, but, notably, four of the five cases with asymptomatic infection were resident in the above mentioned wetland area in Rovigo province. Moreover, the veterinary survey in horse stables reported the highest seroprevalence in Rovigo province, where 58% horses had WNV-neutralising antibodies [10]. WNV infection appears to be widespread among horses in northeastern Italy. In fact, in 2008, several equine outbreaks of WNV infection were identified in Veneto, Emilia-Romagna, and Lombardia regions, with a total of 794 seropositive horses out of 2,030 investigated (39.1%), including 32 horses with WNV neuroinvasive disease [10,11]. On 28 July 2009, a case of equine WNV disease was notified in Reggio Emilia province (Emilia-Romagna region), which is located outside the area where WNV circulation was identified [12].

We could not recover and characterise the virus responsible for the human cases described here. It was isolated from birds, a horse, and a donkey by the National Reference Veterinary Laboratory [11,13]. Genome sequencing and phylogenetic analysis showed that the virus isolated in 2008 was closely related to the WNV strain isolated during the equine outbreak, which occurred in Tuscany

region in 1998, and to other European strains [11,13,14]. So, both 1998 and 2008 Italian outbreaks could be related to a continuous endemic circulation of WNV, although a recent new introduction of WNV by migratory birds cannot be excluded, since the location of the current outbreak is very close to a migratory bird resting area.

To date, no human cases have been notified in 2009, but it is conceivable that new cases will present this year. In fact, the virus has been frequently isolated from local birds and mosquitoes [10] thus indicating it has established an endemic infection cycle.

In conclusion, a relatively high incidence of WNV infection was observed in August-September 2008 in Veneto region, in an area close to the Po river delta. The burden of WNV infection in this area is probably still underestimated. To clarify this issue, Veneto region has recently started a seroepidemiological study in blood donors form Rovigo province. This will be done on samples obtained from 2,550 blood donors (about 1/3 of all donations) from Rovigo province, for 17 weeks, starting on 15 July 2009.

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An analysis of a short-lived outbreak of dengue fever in Mauritius

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During the month of June 2009, Mauritius experienced a short-lived outbreak of dengue fever localised in its capital city Port Louis. *Aedes albopictus*, a secondary vector of dengue viruses, was the probable vector. We introduce a method which combines Google Earth images, stochastic cellular automata and scale free network ideas to map this outbreak. The method could complement other techniques to forecast the evolution of potential localised mosquito-borne viral outbreaks in Mauritius and in at-risk locations elsewhere for public health planning purposes.

Introduction

Dengue fever is a mosquito-borne viral disease which affects 50-100 million people every year in tropical and sub-tropical regions of the world. Dengue viruses (DENV) appear in four serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) and can cause dengue fever, dengue haemorrhagic fever and dengue shock syndrome among other illnesses [1]. Sporadic cases of dengue fever occurred in Mauritius [2] at the time of a major dengue virus (DEN-2) epidemic in Réunion Island in 1977-1978 [3]. *Aedes aegypti* mosquitoes are the major vectors of DENV but were eradicated in Mauritius and nearly eradicated in Réunion Island during the anti-malaria campaigns in the early 1950s. *A. albopictus*, a secondary vector of DENV, was the probable mosquito vector during the 1977-1978 Réunion Island epidemic [3].

Dengue fever re-emerged in Mauritius in June 2009 as a mild fever localised in the capital city Port Louis (population of 144,000 and size of 45.6 km2) on the north-west coast of the island, with *A. albopictus* as the probable vector. A first suspected case was detected on 3 June 2009. There were 192 serologically confirmed cases from 3 to 18 June 2009. The number of cases decreased over the next five days with 16, 4, 4, 3 and 0 cases, respectively. Most of these 219 cases were from the Port Louis region. Mosquito fogging and larviciding started on 3 June 2009, covered the whole of Port Louis and were repeated every seven days. Mosquito fogging was carried out outdoors early in the morning, early evenings and sometimes late in the evenings, when wind speeds were less than 15 km/h. The insecticide used was Aqua K-Othrine® and thermal foggers were used for the spraying. Public awareness campaigns on the necessity to search and eliminate mosquito breeding sites at home and in the neighbourhood and to protect oneself against mosquito bites were carried out through radio, television and the press through a public private partnership. Detailed information leaflets were also distributed. Target groups included the public, community groups and school children.

We introduce a method which uses Google Earth images, stochastic cellular automata [4] and scale free network [5] ideas to map the evolution of dengue fever in Port Louis in June 2009, and compare a scenario without mosquito control or behavioural change (Scenario 1) with a scenario with mosquito control and human behavioural change (Scenario 2).

Methods

The outbreak was assumed to have been started by the introduction of a human index case into a completely susceptible human and mosquito population. An area of interest of Port Louis where most of the serologically confirmed dengue fever cases occurred was selected from a Google Earth digital image of Port Louis. The area of interest, an area of 2.9 km x 3.6 km, was divided into cells each 0.1 km x 0.1 km in size. The number of houses in each cell was estimated using colour image analysis, and the human population in a cell was estimated by assuming an average number of five inhabitants per house. The mosquito population in a cell depended on the human population as shown in the Table.

Table

Parameters for the evolution of dengue fever in Port Louis for Scenarios 1 and 2

	Scenario 1					Scenario 2			
Intervention	1	2	3	4	5	1	2	3	4
Day of intervention	1	7	14	21	30	1	7	14	21
Human viraemic period [days]	5	5	5	5	5	5	5	5	5
Human infectious period [days]	5	5	5	5	5	5	3	2	2
DENV latent period in humans [days]	5	5	5	5	5	5	5	5	5
DENV latent period in mosquitoes [days]	6	10	12	15	22	6	10	12	15
Mosquito lifetime [days]	30	25	20	20	20	30	20	15	10
Mosquito infectious period [days]	30	25	20	20	20	30	20	15	10
Ratio vector/humans	3	2	2	2	2	3	2	1.5	1
DENV transmission probability	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mosquito bite rate [per week]	2	2	2	2	2	2	1	1	1

DENV: Dengue virus

The index case was assumed to reside in an index cell. Individuals in a cell were assumed to interact with mosquitoes in the cell following a SEIR (susceptible-exposed-infected-removed) model for human-mosquito interaction [6]. Individuals in a cell were assumed to be able to move locally with equal probability to each of the eight neighbouring cells and to interact with mosquitoes. They were also assumed to move globally on a scale-free network [5]. Only 40% of the human population of a cell was allowed to move globally (and 50% locally) at any time step (one day) and they returned to their original cell at the end of the time step. Mosquitoes were restricted to their cells.

The scale free network was set up as follows:

1. Four most frequently visited places (hubs) in the area of interest were chosen.
2. Each hub was represented by one cell.
3. The index cell was randomly linked to two of the hubs.
4. Another cell was chosen that was allowed to link itself with the hubs or with the index cell using the Barabási–Albert algorithm [5].
5. Steps 3-4 were repeated for the remaining cells to generate a scale-free network.

The evolution of the outbreak was computed for the two scenarios for the parameter values given in the Table. It was assumed that the mosquito latent period increased with falling temperatures as the month of June passed, accompanied by a decrease in the mosquito lifetime. The decrease in mosquito lifetime was assumed to be greater for Scenario 2 with vector control measures. The human infectious period decreased in Scenario 2 because confinement of affected humans and protection against mosquito bites led to a decrease in the bite rate.

Results

The human population size for the area of interest was computed as 82,580. Figure 1 shows the evolution of the number of infected cases over time for the two scenarios averaged over 100 runs. The average final epidemic size 3,662 cases for Scenario 1 was and 549 cases for Scenario 2.

A histogram of the final epidemic size for 1,000 runs for Scenario 2 is shown in Figure 2.

Figure 3 shows an example of the spread of infected humans over the region of interest in Port Louis 21 days after the first intervention. The outbreak is well-developed and spread over Port Louis with maximum incidence at and around the index cell.

Discussion

We have introduced a method which combines Google Earth images, stochastic cellular automata and scale-free network ideas to yield quantitative estimates for the outcome of a localised dengue fever outbreak. An average of about 550 infected people was computed in Scenario 2 for the period in June 2009 when cases were reported. This number compares well with the actual number about 220 serologically confirmed cases. However, the histogram indicates that larger epidemics can occur, although

Figure 1

Temporal evolution of the number of infected humans (averaged over 100 runs) for Scenarios 1 and 2

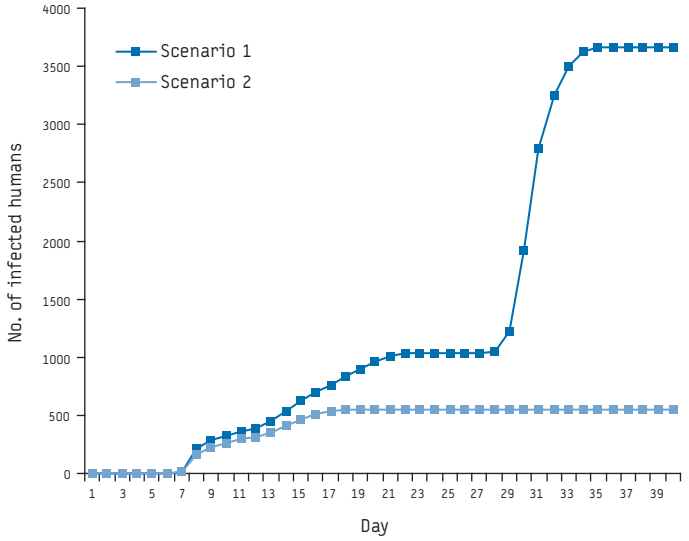


Figure 2

Histogram for the final epidemic size for 1,000 runs for Scenario 2

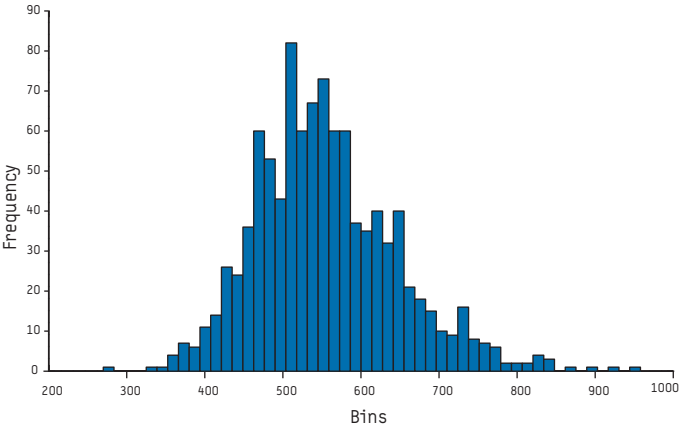
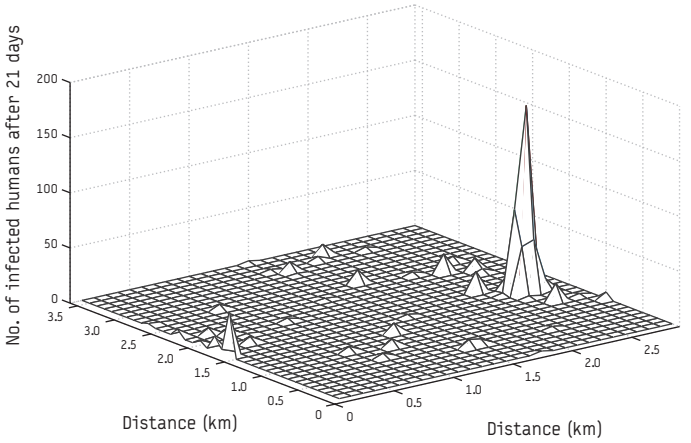


Figure 3

Example of the spread of infected humans over the region of interest in Port Louis 21 days after the first intervention for Scenario 2



with lower probability. Computations for Scenario 1 indicate that, without the intense mosquito fogging campaign and – to a lesser extent – the public awareness campaign carried out by Mauritius authorities in June 2009, the number of cases could have been in the thousands. Larviciding is unlikely to have played a major role in controlling the outbreak, given the very short duration of the outbreak.

The localised nature of the dengue virus outbreak in Mauritius in June 2009 suggests an isolated event limited by by falling temperatures, by the fact that only one secondary vector (*A. albopictus*) for DENV was present, and by the fact that infected mosquitoes outside of the outbreak area did not generate additional cases. The occurrence of the outbreak is not surprising considering the recent resurgence of dengue fever in many countries [7] and global air travel. However, the timing of the outbreak at the beginning of winter in Mauritius is surprising and highlights the risk of an emergence of dengue fever in those countries in the north temperate zone which have established populations of *A. albopictus* and where climatic conditions favourable for the propagation of dengue viruses may prevail in the summer [7]. The modelling technique described here could complement other techniques to forecast the evolution of potential localised mosquito-borne viral outbreaks in Mauritius and in at-risk locations elsewhere for public health planning purposes.

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Sporadic cases of chikungunya, Réunion Island, August 2009

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On 28 August 2009, French authorities reported five cases of chikungunya fever on Réunion Island: three confirmed, one probable, and one suspected case under investigation. All three confirmed patients presented with an acute febrile syndrome, arthralgia, myalgia and cutaneaous rash. All live in the same area on the western side of the island.

Introduction

In 2005-2006 a major epidemic of chikungunya virus (CHIKV) infections occurred on Réunion Island [1] and in the southwestern Indian Ocean region [2]. In Réunion, the cumulative attack rate was 36% [1] and corresponded to the seroprevalence rate of 38% that was measured at the end of the epidemic [3]. After December 2006, no new autochthonous confirmed case of CHIKV was detected on Réunion Island [4].

On the neighbouring island of Madagascar, chikungunya virus was responsible for a large outbreak in 2006 in Toamasina (Tamatave), a coastal town located 350 km northeast from the capital Antananarivo. Within 4,242 randomly selected patients over near 200 000 dengue like syndromes reported among representative residents, 67,5% were found positive for a recent Chikungunya infection [5]. Further outbreaks of chikungunya fever occurred in May 2006 in Mahajanga (northwest coast), in February 2007 in the Sava region (northeast coast), in March to June 2007 in Antsiranana (northern coast), with 20 (29 sampled), 10 (15 sampled) and 14 (28 sampled) confirmed cases, respectively [6]. Since March 2009, the sentinel surveillance network of the Malagasy Ministry of Health has reported sporadic confirmed cases in Toamasina [6]. In June 2009 viraemic cases imported from Madagascar (Toamasina) to continental France were documented [6].

Cases of chikungunya on Réunion Island in 2009

On 5 April 2009 a Malagasy patient travelled for a medical visit from Madagascar to Réunion where he developed symptoms typical of chikungunya fever. A blood sample on 10 April 2009 was positive for CHIKV by specific anti-CHIKV IgM and by real time RT-PCR [6,7].

An autochthonous confirmed case of chikungunya fever was reported to the regional office of the French Institute for Public Health Surveillance in Réunion (Cire Réunion-Mayotte, Institut de Veille Sanitaire) in August 2009 by the Pasteur Cerba laboratory. The Pasteur Cerba laboratory is a central laboratory that receives specimens (on average 3,500 specimens per month) from all over the country including the French overseas territories (West Indies, Guyana, Polynesia). This autochthonous confirmed case lives in Saint-Gilles-Les-Bains on the western side of the island. On 18 July 2009, she had presented with an acute febrile syndrome associated with arthralgia, myalgia, and a cutaneous rash. A blood sample, drawn on 24 July 2009 was found positive for specific anti-CHIKV IgM but negative for IgG and in the RT-PCR. The case was confirmed at the National Reference Centre for Arboviruses at the Institut Pasteur in Paris by detection of anti-CHIKV IgG in a second blood sample taken on 11 August 2009.

Two further autochthonous cases of CHIKV infection in people from the same town were diagnosed at two local hospitals. Both patients reported an acute febrile syndrome associated with arthralgia, myalgia, and cutaneous rash on 23 July and 3 August 2009, respectively. The first one was found positive for CHIKV by RT-PCR and the second by seroconversion demonstrated on paired sera. All results were confirmed by the National Reference Centre for Arboviruses at the Institut Pasteur in Paris.

None of these three confirmed cases of chikungunya virus infection reported a recent travel history off the island or a contact with persons with a travel history or having received a package from abroad.

Currently two other probable cases are being investigated: a tourist who reported an acute febrile syndrome associated with arthralgia and cutaneous rash on 4 August 2009 and had stayed in the same area as the confirmed cases and a permanent resident

of Saint-Paul, a neighbouring town of Saint-Gilles-Les-Bains who had reported a stay in Saint-Gilles-Les-Bains.

Sequence analysis is in progress at the National Reference Centre for Arboviruses at the Institut Pasteur in Paris to tentatively determine the origin of the chikungunya virus in order to establish whether the 2005-2006 Réunion strain has re-emerged or whether a new isolate has been introduced.

Conclusion

Epidemiological and biological investigation of theses cases provides evidence for active transmission of chikungunya virus in Saint-Gilles-Les-Bains, a tourist location on Réunion Island. In reponse to this outbreak, control measures are being organised by the Cire Réunion-Mayotte and the Vector Control Team of Drass Réunion. Active mosquito control measures and information to the population on how to prevent mosquito bites have rapidly been implemented.

Entomologic investigation found low vector activity correlated to winter in the southern hemisphere. Nevertheless, mosquito density seems to be sufficient to support CHIKV transmission. The current austral winter may contribute to moderate the transmission, but special attention in the next weeks is needed. Reinforcement of epidemiological and entomological surveillance has been organised to prevent the risk of potential spread of the virus on the island. Medical staff on the island has been informed about the situation and recommendations on how to react to suspected cases have been issued to them.

Currently, health services in Réunion are under intense strain because of the current H1N1 influenza pandemic. However, despite the small number of cases of CHIKV infection, special attention should be focused on arbovirus activity to prevent, or at least minimise, the spread of the virus during next summer in the southern hemisphere starting in November. Physicians should be aware to sample patients for chikungunya infection when facing a patient presenting an influenza-like syndrome without respiratory symptoms. The Réunion-Continental France laboratory network, built up in 2005 to support local laboratories confronted with the emergence of Chikungunya virus, has been reactivated to reinforce diagnostic capabilities. Specific information of persons living in the area or visiting this island, focusing on individual mosquito bite prevention, should be intensified both locally and in northern hemisphere countries.

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First identification of tick-borne encephalitis in Denmark outside of Bornholm, August 2009

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The incidence of tick-borne encephalitis (TBE) in Scandinavia is increasing and spreading geographically. Following two clinical cases of TBE hospitalised after tick bites in northern Zealand, Denmark, specific IgM and IgG antibodies against tick-borne encephalitis virus (TBEV) were demonstrated in acute serum samples of these patients. TBEV was identified by RT-PCR in ticks collected from the same location. This is the first report of TBEV in Ixodes ricinus leading to clinical cases in Denmark outside of Bornholm island.

Background

Tick-borne encephalitis (TBE) is caused by TBE virus (TBEV), a member of genus *flavivirus*, family *Flaviviridae*. The incidence of TBE has been increasing in neighbouring countries of Denmark (Sweden and Germany) over the past years and mirrors the geographic spread and increased number of ticks [1,2]. The vector for the European subtype, TBEV-Eu, is *Ixodes ricinus* (the Common Tick) that is seen in most of Europe and is the dominant tick species in Denmark (>90%). In Denmark, TBE is endemic only on the island Bornholm in the Baltic sea, with a stable annual incidence of 4 per 100,000 inhabitants [3]. Three serum samples from roe deer from Zealand examined during the 2002-2003 hunting season were found to be antibody-positive for the TBE-complex of viruses [4]. However, a cross-reaction with Louping ill virus could not be excluded. Importantly, no clinical cases of TBE have been reported or TBEV detected outside Bornholm.

Clinical case and virological analysis

In July 2009, a man in his 40s developed fever and other influenza-like symptoms as well as arthritis about one week after receiving four tick bites in his own garden. The patient is a forest worker living in a house in the forest. After about four days of recovery he was hospitalised two weeks after the bites with symptoms of meningoencephalitis and mononuclear cells in the spinal fluid. Serum samples from the time of admission to hospital at the beginning of the encephalitis were negative for *Borrelia* but had positive IgM (optical density (OD450nm) 1,190, cut-off 224) and IgG (OD450nm 695, cut-off 224) titres to TBEV as measured by a validated ELISA (Enzygnost, Siemens) [5]. Spinal fluid was negative in the PCR for herpes simplex virus, enterovirus, varicella zoster virus and TBEV. At the time of publication of this report, the patient was recovering but continued to feel dizzy.

The patient reported about a man in his 30s who was working in a kindergarten in the same forest about 500 meters away, who had a similar unidentified viral meningoencephalitis after tick bites the year before (October 2008). When re-examining this second patients' acute serum from 2008, the antibody test was positive for anti-TBEV IgM (OD 609, cut-off 224) and IgG (OD 1,109, cut-off 243), and a recent follow-up convalescent serum (taken approximately one year later) was still IgG-positive (OD 942, cut-off 243) but IgM-negative for TBEV [5]. He was therefore rediagnosed as a TBE patient.

A TBEV antibody plaque neutralisation test (kindly performed by Dr. Matthias Niedrig, Robert Koch-Institute, Berlin) using TBEV K23 according to Reinhardt *et al*. [6] was positive on the convalescent serum but not on the acute serum drawn during the encephalitis from both patients.

Environmental analysis

Ticks were collected by "flagging"(dragging of a 1x1 m cloth through the grass) at the edge of the forest surrounding the forest worker's garden, identified by species and sorted into three pools of approximately 50 nymphs, 30 adult females and 25 adult males, respectively. Ticks were also collected (nine pools containing a total of 219 larvae and 62 nymphs and adults) at three different sites in an adjacent forest with the highest density of deer in Denmark. RNA was extracted from the ticks using MagNA Pure total NA kit (ABI), and a real-time RT-PCR was run in a quality-controlled routine PCR diagnostic laboratory using specific primers and probes as described in [7]. The PCR is specific for viruses of the TBEV complex as validated by the European Network for Diagnostic of Imported Viral Diseases, ENIVD (www.enivd.de). Only the pool of nymphs from the patient's garden was strongly positive (RT-PCR cycle threshold value of 22).

Discussion

These are the first two cases of TBE in Denmark outside Bornholm that are confirmed by identification of viruses of the TBEV complex in *I. ricinus* nymphs collected at the same location and same time of transmission to a patient. Both cases had a typical biphasic disease starting with influenza-like symptoms, easily misdiagnosed during the present influenza A(H1N1)v pandemic, and with some neurological sequelae (dizziness, fatigue) after the meningoencephalitis. Both patients were TBE IgM- and IgG-positive in the acute serum. For the patient from 2008, we had the

opportunity to obtain convalescent serum approximately one year later, which, as expected, was TBE IgG-positive and IgM-negative, and positive in the neutralisation test confirming the qualitative ELISA test. It takes time for neutralising antibodies to develop and they are normally not present during the acute illness [5].

It was expected that the distribution of TBE would expand in Europe [1,2,8], and spread in Denmark has been suggested based on serology in roe deer [4]. However, the investigation of roe deer serum antibodies has in itself limited relevance to human medicine, partly because of the uncertainty of the serology method used. So far the distribution on Zealand and the rest of Denmark is not known and could be either very local or very wide. The finding of two confirmed human cases in 2008 and 2009, respectively, suggests that TBEV has been present but unnoticed for a longer time.

According to the Danish legislation, TBE is not a notifiable disease. However, diagnostic tests for TBEV are only performed at the Department of Virology at Statens Serum Institut, and we have not seen any cases of TBE in Denmark outside Bornholm before these cases. We have begun a systematic collection of ticks in Denmark and have so far identified TBEV only in one of two likely locations in north Zealand. The PCR is specific for the TBEV complex, but in addition, we are in the process of culturing or otherwise amplifying the viruses isolated from the collected ticks in collaboration with ENIVD in order to obtain sequences for confirmation and molecular epidemiology. Further sampling, molecular characterisation of the Zealand TBEV, increased clinical awareness and continued monitoring should confirm and clarify the spread of TBE in Denmark.

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Imported human African trypanosomiasis in Europe, 2005-2009

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Physicians in Europe are likely to see more African trypanosomiasis cases because of the increasing popularity of travel to Africa. In this paper the literature on imported cases in Europe, since 2005 is reviewed. Because of the high mortality risk associated with acute Rhodesian trypanosomiasis, travellers should be informed about preventive measures and the early disease manifestations.

Introduction

Human African trypanosomiasis (HAT) is endemic in sub-Saharan Africa. *Trypanosoma brucei rhodesiense* (East Africa) and *T. b. gambiense* (West Africa) are transmitted to humans by tsetse flies of the *Glossina morsitans* group (*T. b. rhodesiense*) and of the *G. palpalis* group (*T. b. gambiense*) which are found only in Africa. West African sleeping sickness has almost exclusively a human reservoir, while East African trypanosomiasis is a zoonosis involving antelopes, cattle and humans. Infections by both *T. b. gambiense* and *T. b. rhodesiense* are generally under-reported in humans due to acuteness and lack of specific symptoms at the onset of disease as well as its rural distribution. *T. b. rhodesiense* is focally endemic in many eastern and southern African countries. It tends to occur in form of epidemic outbursts. Human infections have been reported mainly from Malawi, south-east and central Uganda and Tanzania, and sporadically from Kenya, Mozambique, Rwanda, Zambia and Zimbabwe. *T. b. gambiense,* the parasite causing West African sleeping sickness is focally endemic in Angola, Democratic Republic of the Congo, Central African Republic, Chad, Republic of the Congo, Côte d'Ivoire, Guinea, southern Sudan and north-west Uganda. Cases have been sporadically reported from Burkina Faso, Cameroon, Equatorial Guinea, Gabon, Nigeria, Benin, Ghana and Mali [1]. All countries listed so far have a surveillance system for HAT, however, there is no dedicated structure for surveillance in Burundi, Ethiopia, Gambia, Guinea-Bissau, Liberia, Niger, Senegal and Sierra Leone, where under-reporting may be likely [2]. A new HAT atlas initiative for sub-Saharan Africa has led to the creation of a geographic database to store and regularly update HAT epidemiological data. The resulting detailed, high quality regional level maps allow the geo-location of autochtonous cases that have been detected through active and passive surveillance [3].

HAT has always been an exceptional travel-associated disease. It is a rare cause of fever [4] cutaneous lesions and/or neurological signs in travellers returning from endemic areas. Although it has been estimated that about 50 cases are reported yearly outside Africa [5], no recent estimate is available. In Europe, the largest published data on imported HAT included 109 cases registered between 1904 and 1963 [6]. Over the last decades, 26 cases (including 24 West African HAT) seen in France between 1980 and 2004, were reviewed [7]. In addition, imported cases were reported in Italy [8,9], Spain [10], the United Kingdom [11-13], Germany [14], the Netherlands [15-19], Belgium [20], Norway and Sweden [21,22], Switzerland [23], Poland [24] and France [25-26].

We present the clinical and epidemiological characteristics of published HAT cases imported in Europe since 2005 (Table).

Diagnosis

T. b. gambiense represents more than 90% of all reported cases of HAT worldwide (autochthonous and imported cases) but *T. b. rhodesiense* accounts for 60% of imported cases. *T. b. rhodesiense* infection in humans is characterised by high grade fever, an inoculation chancre and substantial parasitaemia in its acute stage. Incubation period is about 6 to 10 days, but may be as short as three days. Gambian HAT may follow an indolent course with a very low or absent parasitaemia. It may remain

unrecognised for years [5]. *T. b. gambiense* is better adapted to its human host, allowing humans to be infective for extended periods thereby sustaining its endemicity. In active infection, *T. b. gambiense* and *T. b. rhodesiense* specific IgG and IgM antibodies are present in high concentration and can be detected by ELISA or immunofluorescence from about three to four weeks after infection. Parasite detection using blood concentration techniques should be done to confirm the infection. Furthermore, in 60% of infections with *T. b. gambiense*, parasites can be detected in lymph aspirate from enlarged cervical nodes. Cerebrospinal fluid examination is always required to evaluate neurological involvement which determines the choice of therapy [5].

Implications for travellers

Whereas imported HAT due to *T. b. gambiense* is more often seen in migrants and expatriates residing in rural endemic areas, HAT due to *T. b. rhodesiense* is more likely to be seen in travellers to East African game parks where the ungulate wildlife serves as a reservoir for the pathogen. In recent years almost all reported cases have been infected in northern Tanzania (Serengeti, Tarangire) or in Uganda (Queen Elizabeth National Park) [17,18,22,24,28]. Some emerging tourist destinations (Malawi: Kasungu National Park, Waza Game Reserve; Rwanda: Akagera National Park; Zambia: South Luangwa National Reserve; Tanzania: Moyowosi Game Reserve) are known foci of *T. b. rhodesiense*, and may pose a risk for travellers.

In travellers infected with *T. b. rhodesiense*, an evolving chancre on the bite site precedes the onset of high grade fever, and usually persists for a few days thereafter. This is an important clinical sign not to be missed by the attending physician. Fulminant disease progression has been reported in a German tourist in her forties with a history of tsetse bites during a visit to the Serengeti National Park. She died only six days after fever onset (13 days following tsetse bites), in Nairobi Hospital, after air ambulance evacuation from a private clinic in Dar es Salaam where the HAT diagnosis was made. She had two typical chancres that were missed when she first presented with fever in another clinic seven days after the tsetse bites, and a malaria diagnosis was alleged [29]. A history of tsetse fly bites in patients with clinical symptoms has to be considered a medical emergency. Early treatment with suramin (Germanin®, Bayer 205) or in case of non-availability, with pentamidine is essential to prevent severe complications and death. All available drugs for HAT treatment, including suramin can be obtained through the World Health Organization (WHO) trypanosomiasis control and surveillance unit, by contacting Dr. Simarro (simaropp@who.int) and Dr. Franco (francoj@who.int). A small stock of HAT drugs should be made available at one tropical medicine/travel medicine centre per country, in order to enable early treatment when required.

Table

Imported cases of African trypanosomiasis in Europe, since 2005, by date of publication

Sex	Age	Nationality	Clinical features (time before first symptoms and diagnosis)	Sub-species	Country of exposure (reason for travel)	Treatment	Reference (year)
M	44	Italian	Fever, headache, fatigue, weight loss, paresthesia, day-time somnolence, insomnia, hepatosplenomegaly, lymph nodes, ataxia (6 months)	*gambiense*	Gabon (expatriate)	Eflornithine	9 (2005)
F	54	Italian	Fever, headache, fatigue, splenomegaly, insomnia, hyperesthesia (3 months)	*gambiense*	Central African Republic (expatriate)	Eflornithine	9 (2005)
F	52	Dutch	Fever, headache, vomiting, diarrhea, confusion, depression, hallucinations, sleepiness. One relapse episode. Death. (4 days)	*rhodesiense*	Serengeti national park of Tanzania (tourist)	Suramin, Melarsoprol	17 (2006)
M	37	French	Fever, fatigue, anorexia, headache, arthralgia, insomnia, rash, pruritus, paresthesia, lymph nodes, weight loss (8 months)	*gambiense*	Gabon, Camerron, Guinea (expatriate)	Pentamidine	26 (2007)
M	72	French	Fever, fatigue, pruritus, lymph nodes, weight loss (5 months)	*gambiense*	Gabon (expatriate)	Pentamidine	26 (2007)
M	26	British	Fever, insomnia, lethargia, vomiting chancre, erythema, jaundice (5 days)	*rodhesiense*	Malawi (military)	Suramin	12 (2007)
M	38	British	Fatigue, somnolence, headache, fever, lymph nodes, hepatomegaly, myalgia. One relapse episode. (4 months)	*rhodesiense*	Namibia, Mozambique, Malawi (unknown reason, travel for 2.5 years)	Suramin, Melarsoprol	13 (2007)
F	25	Dutch	Fever, headache, cellulitis, red papule, lymphangitis (4 days)	*rhodesiense*	Serengeti National park of Tanzania (tourist)	Suramin	18 (2009)
M	61	Polish	Fever, jaundice, respiratory distress, bleeding (disseminated intravascular coagulation - DIC), oliguria, skin rash, hepatosplenomegaly (8 days)	*rhodesiense*	Queen Elizabeth national park of Uganda (tourist)	Pentamidine	24 (2009)
M	50	French	Fatigue, fever, double skin ulceration, lymph nodes (7 days)	*gambiense*	Gabon (expatriate)	Pentamidine	27 (2009)
F	27	Dutch (immigrant from Angola)	Fatigue, apathy, sleepiness, loss of apetite, depression, coma (32 months)	*gambiense*	Angola (immigrant)	Eflornithine	19 (2009)

Conclusions

Physicians in Europe are likely to see more HAT cases because of the increasing popularity of travel to Africa, the only region that has recorded a growing number (3%) of tourist arrivals in 2009 according to the United Nations World Tourism Organization (UNWTO, www.unwto.org). The average annual growth of tourism in some sub-Saharan countries such as Tanzania and Uganda has ranged between 10 and 20% with a focus on safari travel. Because of the high mortality risk associated with acute Rhodesian trypanosomiasis, European travellers to destinations where the disease is endemic, particularly game parks and safari areas in eastern and southern Africa, should be informed about the early disease manifestations and advised to report tsetse bites to their physician upon return, when presenting symptoms. Although thousands of travellers are bitten by tsetse flies each year, the majority will not develop HAT. Nevertheless, caution is recommended. Preventive measures against tsetse fly bites are helpful. The tsetse fly is active during the daytime and is particularly attracted by motion and dark colours, with a marked preference for blue. Bites are painful and can be prevented by wearing wrist- and ankle-length clothing of thick material and avoiding bright or contrasting coloured clothing. Because the tsetse fly is able to bite through thinly woven fabric, the impregnation of clothing with permethrin is recommended together with the application of a skin repellent [30].

At present, imported HAT cases are not systematically reported through the existing channels to signal emerging infections (ProMED) or in the accessible medical literature. To harmonise reporting, we would recommend the creation of an electronic reporting form. This would allow for the evaluation of long term trends in imported HAT, and contribute to identifying risk factors and risk areas.

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Trichinellosis outbreak in Lithuania, Ukmerge region, June 2009

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An outbreak of trichinellosis due to wild boar meat was detected in Lithuania in June 2009. The outbreak affected 107 people all of whom had consumed sausages made of wild boar meat. Inspection of food samples confirmed the presence of *Trichinella* larvae in the meat.

Background

Several human cases of trichinellosis are reported in Lithuania every year. Between 1999 and 2008, a total of 359 cases were registered, including 66 sporadic cases and 42 outbreaks. During these ten years the incidence of trichinellosis decreased from 1.7 to 1.2 cases per 100,000 population [1].

The epidemiological investigations show that human trichinellosis in Lithuania is mostly spread by consumption of meat from infected pigs and wild boars. Of all outbreaks reported from 1999 to 2008, 58% occurred due to consumption of meat from home-raised pigs, 10% due to infected wild boar meat and about 8% due to illegal sale of meat. Some 24% of outbreaks were unexplained.

Outbreak investigation

On 11 June 2009 the Lithuanian Centre for Communicable Disease Prevention and Control received an urgent report about five suspected cases of human trichinellosis in Ukmerge municipality. An epidemiological investigation was started on the same day in order to determine the extent of the outbreak, identify its source and propose control measures. The investigations involved specialists from the Ukmerge department at Vilnius public health centre and the Ukmerge district State Food and Veterinary service.

Case finding

A standardised questionnaire was used to collect information on the clinical features, date of onset of symptoms, consumption of meat products, and dates and places of meat purchase. Investigation of the first cases quickly revealed that they had consumed homemade sausages from wild boar.

A **confirmed case** was defined as a person with the following clinical symptoms: fever (> 38 °C), with myalgia, or facial or orbital oedema, who had consumed homemade sausages from wild boar, produced on 16 May 2009, and had positive serology for *Trichinella*. A **probable case** was defined as a person with the following clinical symptoms: fever, myalgia, facial or orbital oedema, or hypereosinophilia, who had consumed homemade sausages from wild boar, produced on 16 May 2009. A **suspected case** was defined as a person with hypereosinophilia alone or associated with fever, myalgia or orbital oedema, who had consumed homemade sausages from wild boar, produced on 16 May 2009.

Patients and their family members were interviewed and active finding of persons who had consumed suspected meat was implemented after receiving an urgent report from a personal healthcare institution (general practice and hospital) about a suspected case of trichinellosis. Active case finding was started every time a healthcare institution reported a suspected trichinella case. Persons who had consumed suspected meat were referred to their local healthcare institutions for laboratory examination and medical observation. Blood samples were tested for eosinophilia and serological investigations for antibodies against trichinellosis were performed by ELISA.

Food investigation

On 11 June the State Food and Veterinary service collected the remainder of wild boar sausages (13.4 kg produced from several animals) from hunters and their family members and tested them for trichinellosis. The food samples were tested using the artificial digestion method.

Results

Human cases

As a result of the investigations, it was established that 128 persons had consumed sausage made from wild boar meat suspected as the source of infection. Of these, 107 people were considered to have been affected by the outbreak. Fourteen cases (13.1%) were laboratory-confirmed, the remaining 93 (86.9%) were regarded as probable cases fulfilling clinical and epidemiological criteria. Blood serological reactions for the detection of antibodies against *Trichinella* were performed three to four weeks after meat consumption. It is presumed that this time was too short for finding antibodies against *Trichinella*, which can explain the relatively small proportion of confirmed cases.

The first patient fell ill on 20 May; the last case was detected on 26 June (Figure 1). The outbreak lasted 37 days. The shortest incubation period was five days and the longest was 25 days.

Most cases were reported from Ukmerge municipality but the infection spread beyond this region, affecting six municipalities in total (Table).

The majority of cases were adults (88.8 %), only 12 cases in children were registered (Figure 2).

The main clinical symptoms were: fatigue (100%), nausea (94.6%), fever (91.6%), muscular pain (88.2%), facial oedema (52.3%), orbital oedema (946%), and haemorrhagic rash of the skin (14.6%). Eosinophilia was found in all patients. The clinical symptoms of disease were serious in five cases (4.7%), medium in 50 cases (46.7%) and mild in 52 cases (48.6%). A severe course of disease was defined by fever higher than 39 °C, face swelling, pain of neck, shoulders and trunk, myalgia, and neurological complications (lethargy, apathy and excitement). A medium course of disease was defined by fever of up to 39 °C, orbital oedema and lesser myalgia. A mild course of disease was defined by subfebril temperature and insignificant orbital oedema.

Fifty-five patients (51.4%) were hospitalised. The patients were treated by mebendazole. Corticosteroides were administered for patients with a severe and medium course of disease. All patients were followed up after treatment and all recovered. Persons with a severe or medium course of disease will be followed for six months by their local healthcare institutions. For these patients, an assessment of eosinophilia and myalgia will be done regularly.

Food source

On 12 June the laboratory department of the State Food and Veterinary Risk Assessment Institute found *Trichinella* pathogens in the collected sausage samples. In 1g of meat about 20 larvae were found. The samples will be sent to Italy for the determination of the *Trichinella* type.

Conclusion

The source of infection was identified to be wild boar meat. Several wild boars were hunted on 10 May 2009 in Ukmerge region. The meat was not inspected for the presence of *Trichinella*. On 16 May 2009, 50 kg of cold-smoked sausages were produced from the wild boar meat in a joint stock company 'Alekniskis'. The sausages were not produced for trade but only for private consumption. The sausages were distributed to huntsmen who ate this meat themselves and distributed it further among their family members, neighbours, relatives and acquaintances.

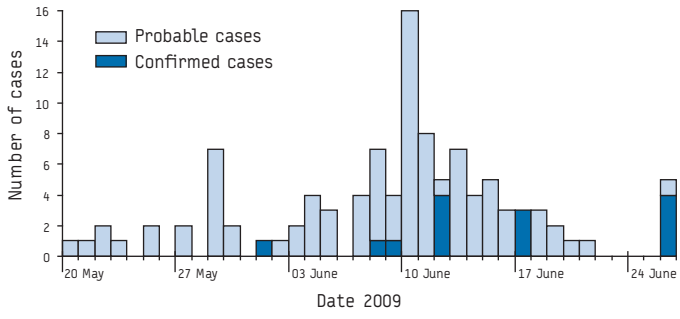
It is believed that a large number of cases were due to the significant invasion of *Trichinella* larvae in the meat.

Wild boar meat is the second most common cause of trichinellosis in Lithuania. Another larger outbreak had been registered in 2001, in which 65 persons fell ill with trichinellosis (69 cases or 65% of all cases in 2001). Investigations performed in 2000-2002 showed that about 0.5% of wild boars in Lithuania are infected with *Trichinella* [2]. The infected animals are evenly distributed in the whole country. Therefore the meat of wild boar remains an important source of infection in Lithuania.

According to legislation in Lithuania all slaughtered pigs and hunted wild boars must be examined for *Trichinella*. Presently there are two methods used to detect *Trichinella* in meat: trichinoscopy (compressorium) and artificial digestion method. Epidemiological data suggests that in spite of these regulations, consumption of uninspected meat still occurs. Therefore, intensive public education, especially for small pig breeders and hunters, is needed in order to prevent human trichinellosis in Lithuania.

FIGURE 1

Cases of trichinellosis reported in an outbreak in Lithuania, May-June 2009, by date of disease onset (n=107)



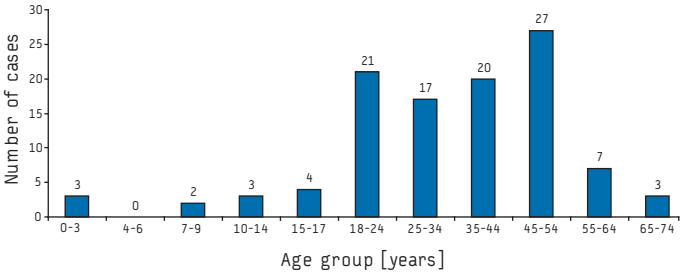
TABLE

Geographical distribution of cases of trichinellosis, Lithuania, May-June 2009, by municipality (n=107)

Municipality	Number of cases (%)
Ukmerge	56 (52.3%)
Vilnius	9 (8.4%)
Kaunas	16 (15.0%)
Kedainiai	21 (19.6%)
Jonava	4 (3.7%)
Zarasai	1 (0.9%)
Total	**107 (100%)**

FIGURE 2

Age distribution of cases of trichinellosis, Lithuania, May-June 2009 (n=107)



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http://www.infectieziektebulletin.be

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Monthly, online. In Slovene.
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Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid.
Fortnightly, print and online. In Spanish.
http://www.isciii.es/jsps/centros/epidemiologia/boletinesSemanal.jsp

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Communicable Disease Surveillance Centre, Northern Ireland, Belfast.
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http://www.cdscni.org.uk/publications

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Health Protection Scotland Weekly Report
Health Protection Scotland, Glasgow.
Weekly, print and online. In English.
http://www.hps.scot.nhs.uk/ewr/index.aspx

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Norwegian Institute of Public Health, Folkehelseinstituttet, Oslo, Norway
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