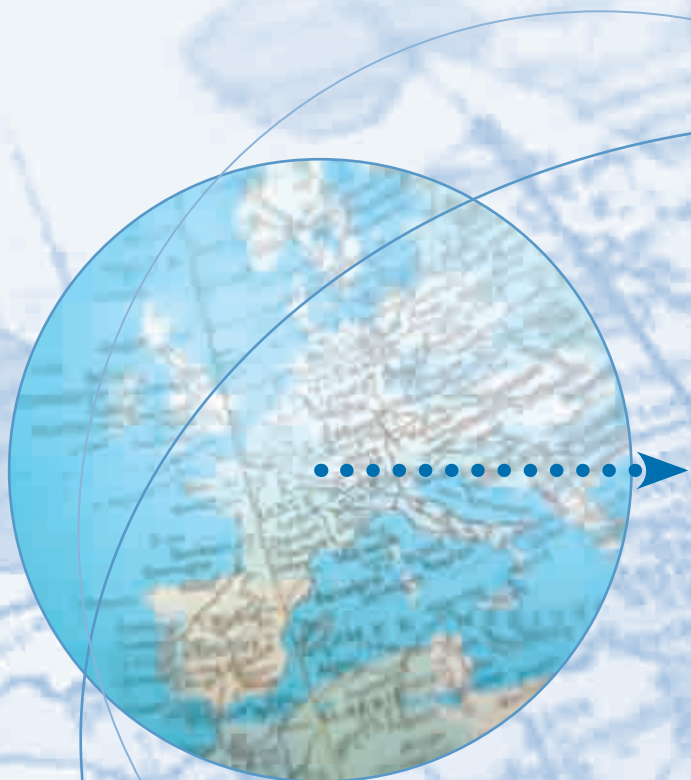




Eurosurveillance



In this edition

- **Special issue: Pregnancy and infectious diseases**

Also

- **Focus on tuberculosis**
- **Human case of swine influenza A (H1N1), Aragon, Spain, November 2008**
- **Use of oseltamivir in 12 European countries between 2002 and 2007 – lack of association with the appearance of oseltamivir-resistant influenza A(H1N1) viruses**
- **Six short reports and an editorial on outbreaks and imported cases of hepatitis A in Europe**



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Editorials

PREVENTION OF CONGENITAL AND PERINATAL INFECTIONS

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Perinatal and congenital infections cause morbidity and mortality throughout the world. While there are a large number of pathogens that can occasionally be harmful for the unborn child, some are of considerable public health impact, for example rubella, varicella, syphilis, hepatitis B, toxoplasmosis, or infections with cytomegalovirus (CMV) or human immunodeficiency virus (HIV). The advances in the field of clinical microbiology have increased our options in terms of preventive strategies, early diagnosis, clinical interventions and therapeutic alternatives to combat these infections.

When a considerable public health impact of a given infection is evident, preventive measures can be discussed in relation to the epidemiology, the available resources and the acceptance in the population. Information on hygienic measures and other means to avoid infection is another cornerstone in the preventive work. Immunisation before pregnancy is an option for rubella, hepatitis B and varicella. In cases where intervention to prevent damage to the unborn child is possible, large screening programmes can be organised in order to identify maternal infections that may otherwise not be recognised due to uncharacteristic or subclinical symptoms. Congenital syphilis can be prevented by antibiotic treatment in early pregnancy, transmission of HIV by antiviral treatment of mother and newborn, and transmission of hepatitis B by vaccination and immunoglobulin treatment of the newborn. Identification of a neonate with congenital CMV infection or toxoplasmosis allows treatment to reduce harm.

Lack of resources and appropriate maternal care stand in the way of efficient programmes, but there is also a need of increased knowledge. Many of these interventions could be improved not only by a better understanding of the epidemiology and the impact of the diseases as well as further research into improved diagnostics and treatments and development of vaccines, but also by distribution of information to pregnant women and health care professionals. This issue of Eurosurveillance is dedicated to infectious diseases and pregnancy and contains a number of articles highlighting different aspects of the epidemiology and control and prevention efforts for diseases such as toxoplasmosis, rubella, varicella, sexually transmitted diseases e.g. HIV and hepatitis B, and cytomegalovirus infections.

Safe vaccines against, for example, rubella, exist and are very efficient in preventing infection in the mother [1]. It is clear that congenital rubella can be eradicated by vaccination programmes, a goal that has already been reached in the Scandinavian countries and the United States (US). The combination of high uptake two-dose childhood vaccination programmes in combination with postpartum vaccination of susceptible women has eliminated rubella within a decade [1-3]. Whatever the strategy –adherence and efficacy of the programmes will be decisive for the end result. This is clearly illustrated in an article in this issue by Pandolfi and co-workers that gives an overview of data from surveillance systems of the World Health Organization (WHO) and the European surveillance community network for vaccine-preventable infectious diseases, EUVAC-NET [4]: rubella still circulates in many parts of the world, including Europe. And not only in countries in which programmes

for rubella elimination have only recently been implemented, but also in countries with a long history of vaccination with low uptake of the vaccine such as Italy. The authors review different strategies to improve the situation with the goal to achieve not only herd immunity but also protection of all women of childbearing age as long as the global circulation cannot be

stopped. They further propose to include varicella vaccine in such programmes in order to prevent varicella in pregnancy, which bears a risk for foetal embryopathy and neonatal disease [5]. A vaccine against varicella has been available for a long time, and has been employed in universal childhood vaccination for example in the US since 1995 [6]. However, only few countries in Europe, e.g. Germany, have so far followed the US example. It is, however, likely that more countries will introduce this vaccine as combination vaccines become more available, which implies a considerable reduction in the costs. As the authors point out, the use of the vaccine for the prevention of adult varicella is today suboptimal. Wherever possible, the vaccine should be offered to non-pregnant women of childbearing age who do not have acceptable evidence of previous infection (susceptibility should preferentially be confirmed by immunity testing), especially women working as carers for children and adolescents, and parents of young children.

Vaccines may also be used in newborns in order to protect against maternal transmission during birth, as is the case for hepatitis B [7]. If vaccination is not possible such as for syphilis and infections with human immunodeficiency virus (HIV), transmission of the

Many of these interventions could be improved not only by a better understanding of the epidemiology and the impact of the diseases as well as further research into improved diagnostics

infection to the child may be prevented by antimicrobial therapy of the mother [8,9]. The cornerstone for such programmes is testing pregnant women when they have their first contact with maternity care. Surveillance of the efficiency of the preventive programmes is crucial for their success. As described by Giraudon et al., an efficient surveillance programme has been in place in London for nearly ten years, which monitors the extent of antenatal testing and the prevalence of susceptibility to HIV infection, hepatitis B, syphilis and rubella [10]. If the acceptance rate for testing is low, this will be investigated rapidly and the information used promptly to improve the uptake. It is noteworthy that this study also investigated how children of women infected with hepatitis B are followed up by vaccination, a programme that frequently suffers from a high rate of non-adherence.

A more cumbersome strategy has to be adopted if treatment needs to be given to the mother within a short interval after contracting the infection, in order to prevent permanent damage of the child. This is the case with *Toxoplasma gondii* infections, and seronegative women, who may be exposed any time, have to be screened at monthly intervals starting at the latest in the 12th week of pregnancy [11]. C. Cornu et al. studied factors influencing adherence to such a programme in the French Rhone-Alps region [12]. The authors conclude that there are gaps in the adherence to the screening schedule, regarding the adequate time of first visit, intervals between visits and numbers of subsequent visits for sampling. They suggest that simplification of the logistics of the procedure (prescription, reimbursement) might improve adherence, but also point out that special attention needs to be paid to the background of the women who are not able to follow the schedule.

A study from Greece by I. Elefsiniotis et al. highlights the, often significant, differences in the epidemiology of hepatitis B that exist between immigrants and indigenous inhabitants [13]. The very high rate of hepatitis B in immigrants from Albania illustrates the well-known fact that special attention has to be taken to include immigrants and refugees in ongoing public health programmes.

Other populations who need special attention and care are drug users who are at high risk of contracting blood borne and sexually transmitted disease. These aspects and the need for social and psychiatric support is well summarised in the review from Lisbon by V.A. Gyarmathy and co-workers [14].

The present status and knowledge of congenital CMV is the topic of three more papers in this issue of Eurosurveillance. The fact that CMV infections are without symptoms in pregnant women and in most of the congenitally infected neonates, has made the exploration of the topic cumbersome, but much information has been gained from large prospective studies (see the review by Ludwig and Hengel [15]). Previously, retrospective diagnosis of congenital CMV at the time of the appearance of late sequelae had not been possible as differentiation between congenital and the very frequent early postnatal infection could not be done. However, advances in molecular diagnostics now allow a retrospective diagnosis, provided that dried blood spots from metabolic testing are preserved, which at the same time provide an easier approach to epidemiological investigations, as exemplified by P. Paixão et al. [16].

The epidemiology of CMV varies widely in different populations but, wherever tested to date, congenital CMV is a major cause

(20-25 %) of severe neurologic deafness, often with delayed onset. Severe neurologic disability and ocular problems may also occur. The present knowledge on congenital CMV infection is summarised in the review by A. Ludwig and H. Hengel [15] and the report by A. Vossen et al. [17] from the International Conference on Congenital CMV, held at the Centers for Disease Control and Prevention (CDC) in Atlanta in November 2008.

A first-grade conference on this topic being held at the US CDC underlines the great efforts directed to the prevention of congenital CMV in the US. The development of a CMV vaccine is considered a first priority, followed by seroepidemiology in different populations and mapping of the extent of congenital CMV among deaf children, pathophysiology and many other aspects.

In Europe, a screening programme to identify primary CMV infection in pregnancy has been in place for some years in Italy, now withdrawn but considerable voluntary testing is still ongoing, and valuable experience has been gained [18-19]. Several other projects are ongoing or starting (e.g. in Belgium, France, Germany, Sweden, the United Kingdom and several other European countries). Controlled studies are being initiated on the effectiveness of immunotherapy in preventing or alleviating foetal damage and on antiviral therapy for the treatment of children with symptoms affecting the central nervous system [20].

There is also a role for hygienic measures in avoiding transmission of CMV, a ubiquitous infection among young children. The European Congenital CMV Initiative (ECCI), a collaborative organisation of European CMV researchers from many disciplines, initiated by G.M. Revello, T. Lazarotto and M. Barbi is now distributing information to the public and to health professionals through the London-based website (www.ecci.ac.uk). The website also contains a case register. Further information is available at the website of the US CDC (www.cdc.gov/cmV) as well as on a national basis on a Swedish website on congenital-perinatal infection (www.infpreg.se). As it is apparent that the public health impact of congenital CMV damage is considerable, more resources are now needed in Europe as in the US in order to make further progress in prevention. The strength of a European collaboration has previously been well illustrated in the European Union collaborative study of mother-to-child transmission of HIV, hepatitis C and toxoplasmosis [21-23]

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Surveillance and outbreak reports

ANTENATAL SCREENING AND PREVALENCE OF INFECTION: SURVEILLANCE IN LONDON, 2000-2007

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In the United Kingdom (UK), it is recommended to universally offer antenatal infection screening for human immunodeficiency virus (HIV), hepatitis B and syphilis infections, and susceptibility to rubella for the benefit of the mother and to reduce vertical transmission of infection. This paper describes the surveillance of antenatal infection including uptake of screening, and the results of testing in pregnant women in London between 2000 and 2007. Antenatal screening coordinators in liaison with midwifery heads and microbiologists at all thirty London National Health Service (NHS) Trust maternity units supplied quarterly data on the number of pregnant women booked for antenatal care, tests done, and tests results. The overall estimated uptake of screening increased since 2000 and reached 95.6% for HIV, 96.5% for syphilis, 96.2% for hepatitis B and 97% for rubella susceptibility by the second half of 2007. There is considerable variation in the performance between NHS Trusts. The overall estimated prevalence of HIV infection was 3.4/1,000 women (ranging from <1/1,000 to 10/1,000 across Trusts), of hepatitis B (HBsAg-positive) was 11.3/1,000 (2.6/1,000- 23.9/1,000), of syphilis was 4.4/1,000 (<1/1,000-16.3/1,000) and of rubella susceptibility was 39.3/1,000 (19-103/1,000). Antenatal infection screening has improved and there has been some success in implementation of national policy. However, screening uptake and prevalence of infection vary considerably across London NHS Trusts and some women are evidently disadvantaged. Improvements in information systems should help local partners to focus their interventions in those Trusts where work is still needed to increase testing as well as the capacity to monitor the uptake of screening.

Introduction

Universal antenatal infection screening aims to identify infection early so that mothers can be offered advice and interventions in pregnancy and afterwards for their own health benefit as well as to reduce the chance of vertical transmission. In 1998 in the United Kingdom (UK), the Department of Health recommended that all pregnant women should be offered antenatal screening for hepatitis B infection [1]. In 1999, UK national policy stated that all pregnant women should be offered and recommended testing for human immunodeficiency virus (HIV) infection, along with other antenatal screening tests, as an integral part of their antenatal care, and that this offer as well as the patient's decision to undergo the testing should be recorded [2]. National guidelines also require robust systems to monitor the uptake of testing. In 2003, the

Department of Health reinforced the policy by publishing a set of antenatal screening standards including those for syphilis infection and susceptibility to rubella virus infection [3].

In 2007, there were an estimated 77,400 people living with HIV in the UK, of whom over a quarter (28%) were unaware of their infection. Almost half (48%) of those individuals who had been diagnosed were resident in London [4]. Antenatal Infection Screening Surveillance (AISS) was implemented in London in 2000 in collaboration with the National Health Service (NHS) in London, by the then Regional Epidemiology Services of the Public Health Laboratory Service now the Health Protection Agency (HPA) [5]. It monitors the implementation of the national screening policy in London NHS Trusts (i.e. public hospitals), as well as the antenatal prevalence of HIV, hepatitis B and syphilis infections, and susceptibility to rubella. The results are reported quarterly to NHS Trusts and health authorities, to assist them in understanding the infection burden among pregnant women and to facilitate targeting of interventions where needed in London.

This article describes the AISS system as well as the gradual increase in antenatal infection screening during 2000 to 2007 and prevalence of infection in pregnant women reported at NHS Trusts across London.

Methods

The surveillance system was developed throughout London in collaboration with 30 maternity units in 28 NHS Trusts (two of the Trusts comprising of two maternity units). In each Trust, the head midwife of the maternity unit and the antenatal screening coordinator liaise with the microbiologists to obtain the information and provide it to the HPA. Currently 96% of births take place in obstetric units in hospital, and these Trusts are estimated to cover the large majority of the birth cohort (around 115,000 births per year in London) [6]. Staff at each Trust return a six-monthly (since 2005 quarterly) form to the HPA London regional office. Forms include source of information, aggregated data for the total number of pregnant women registered for antenatal care (hereafter called "booked" for antenatal care), tests carried out for HIV, hepatitis B, syphilis and rubella antibody, and the total number of positive tests. Positive tests are defined as HIV antibody positive, hepatitis B surface antigen (HBsAg) positive, syphilis positive with enzyme immunoassay test and rubella antibody <10 iu/ml.

The uptake of screening for each infection was estimated by calculating the proportion of tests done per total number of women booking for antenatal care. Prevalence of infection was calculated as the total number of positive tests per 1,000 tests done.

Results

By the end of June 2008, all 30 maternity units at the 28 London NHS Trusts had returned completed forms for the years 2000 to 2007. In 2006 and 2007, reports were received for all four quarters from all Trusts. There were some gaps in information provided by individual units, but all Trusts participated in the scheme.

Uptake of screening

Estimated uptake of antenatal screening in 2007 was 96.4% for hepatitis B, 96.6% for syphilis and 96.8% for rubella susceptibility. HIV screening uptake, which had been less than 70% in 2000, rose to an estimate of 95.1% in 2007 (Figure 1). Nevertheless, in 2007, valid quarterly reports where information on booking for antenatal care and test was given (108/112 reports) indicated that for at least 6,744 out of 138,618 booked women no HIV testing was reported. Based on average prevalence of antenatal

HIV infection in London as obtained through the AISS in 2007 (3.6/1,000), we estimated that around 24 babies were potentially at risk of vertical transmission of HIV and remained unrecognised. Three Trusts reported that in at least one quarter in 2007 less than 4/5 women had been screened for HIV.

Prevalence of infection

In 2007, the estimated overall prevalence of HIV infection, slightly decreasing, was 3.6/1,000 varying across Trusts (<1/1,000 to 10/1,000), of hepatitis B (HBsAg-positive) was 11.7/1,000 (3/1,000 to 24/1,000) and of syphilis was 4.7/1,000 (<1/1,000 to 16/1,000) (Figure 2). Prevalence of rubella susceptibility was 41/1000 (16/1,000 to 78/1,000) in 2007, compared to previous estimates of 37/1000 in 2001 and 34/1000 in 2004.

The prevalence of antenatal infection varies considerably across London's NHS Trusts (Table) and sectors ("pre 2006 NHS reorganization" London Strategic Health Authorities). HIV prevalence in 2007, ranged from 1.6/1,000 pregnant women in the North West London sector to 4.9/1,000 in the South East London sector, and was 50-fold higher at the NHS Trust with the highest prevalence compared to the one with the lowest prevalence (range from 0.2/1,000 to 10.1/1,000). For hepatitis B, the disparity was 11-fold (2.6/1,000 compared to 23.9/1,000), and for syphilis prevalence was 81-fold higher at the Trust most affected (range 0.2/1,000 to 16.3/1,000).

Data source, participation and data provided

In 2007, the source of information was missing from only two reports. Sources of data were derived from maternity and laboratory records such as manual records, delivery figures (birth register), electronic patient records, range of laboratory and maternity computer systems including Euroking K2, Winpath and Telepath. Only one Trust was unable to provide information about the number of bookings made. All Trusts were able to supply information on the number of screening tests performed apart from one Trust that did not provide syphilis data. All Trusts provided their positive results apart one that did not provide syphilis data and one unable to provide rubella data for two quarters.

Discussion

Overall in London, antenatal infection screening has improved and the implementation of the national policy can be regarded as a success to some degree. However, screening uptake and prevalence of infection does vary considerably across London NHS Trusts, and it is likely that in some pregnant women HIV infection remains undiagnosed thus putting unborn babies at risk of vertical transmission. Many NHS Trusts in London serve a population with high levels of HIV infections. This reflects the demography of the capital, with areas where a high proportion of women come from high prevalence countries. In 2006, overall 53% of women who gave birth in London had been born outside of the UK. In the same year, the prevalence of HIV among women born in sub-Saharan Africa who gave birth in the UK was 25/1,000 [4]. Though certain groups are at higher risk, it is essential that all women in London can benefit from early diagnosis and interventions to prevent their infants from becoming infected.

There were some problems with data completeness in some Trusts and thus there are limitations to the system. However, we believe that its overall results and conclusions are sound.

FIGURE 1

Uptake of antenatal screening for HIV, hepatitis B and syphilis; Health Protection Agency Antenatal Infection Screening Surveillance, London, half-years 2000-2007

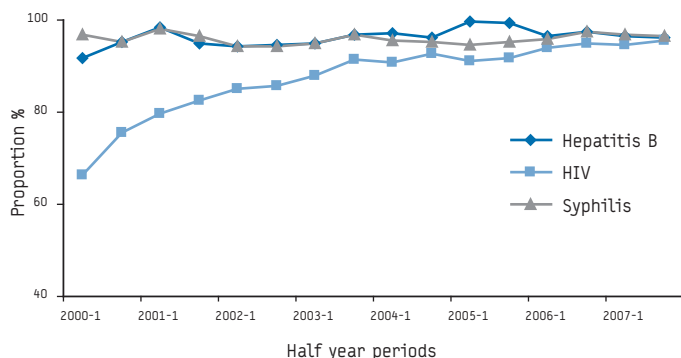
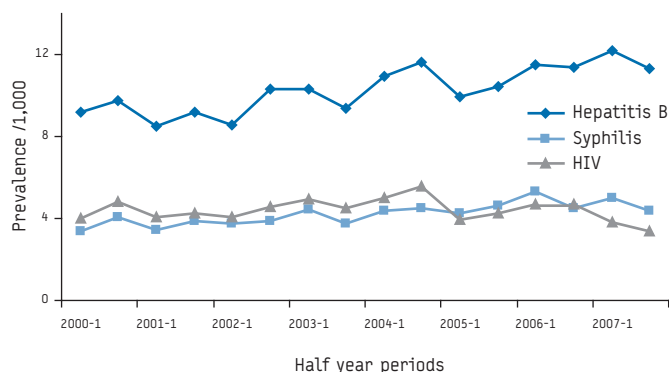


FIGURE 2

Prevalence of HIV, hepatitis B and syphilis; Health Protection Agency Antenatal Infection Screening Surveillance, London, half-years 2000-2007



It is possible that some women reportedly not tested in the current pregnancy may have been tested prior to pregnancy [7]. Irrespective of this, they should be screened in the current pregnancy. An underestimation of screening uptake could also result if women booked for testing had pregnancy loss before being tested. However, screening uptake could also be overestimated, with tests repeated during pregnancy, or reported for women who had miscarried or women who were not booked, typically because they presented very late in the pregnancy. Detailed local audit would be necessary to accurately assess to what extent low uptake reported in some Trusts may reflect limitations in the reporting system. Exploration of this and further review at Trusts level is recommended along with an assessment of characteristics of women who were not screened. Those who decline screening may constitute a particular risk group and may have higher prevalence of HIV or other infections [8]. Variability in the monitoring systems in place may make comparisons across Trusts less meaningful but observations and trends within single Trusts should be fairly reliable.

The findings mirror the trend in HIV prevalence found in the HPA HIV Unlinked Anonymous Survey of Pregnant Women through Dried Blood Spot Surveys, showing stability since 2004 (29/10,000 cards tested positive in 2000, 40/10,000 in 2002 and 42/10,000 in 2006) [4, 10]. There are evident inequalities in the prevalence of HIV across London, consistent with findings from the confidential reports of HIV-positive pregnancies to the Royal College of Obstetricians and Gynecologists in the National Study of HIV in Pregnancy and Childhood; data for 2003-2004 indicated that prevalence of maternal HIV infection was the highest in North Central London (5.8/1,000) [11].

Data sources and availability

Not all London NHS Trusts appear to be able to provide all the required information. Implementation of simple and robust methods for monitoring uptake of screening in antenatal patients has been recommended [9]. There are still some limitations though, as the surveillance is not based on individual records, but on aggregated numbers. Denominators and numerators are often obtained from

TABLE

Prevalence of HIV, hepatitis B and syphilis infection and susceptibility to rubella per 1,000 pregnant women tested, by London Strategic Health Authority and National Health Service Trusts. Health Protection Agency Antenatal Infection Screening Surveillance, London, 2007)

Strategic Health Authority	National Health Service Trusts (i.e. public hospitals)	Prevalence per 1,000 tested			
		HIV	Hepatitis B	Syphilis	Rubella susceptibility
North Central London	Barnet & Chase Farm	1.9	7.9	3.2	47.9
	North Middlesex	10.1	20.9	16.3	55.4
	Royal Free	0.5	8.0	0.2	37.4
	University College	2.9	8.3	-	42.9
	Whittington	2.3	16.1	4.7	40.5
North East London	Barking, Havering and Redbridge	2.8	13.2	5.7	42.3
	Homerton	4.7	23.9	8.8	35.3
	Newham	6.3	14.9	6.7	56.3
	Royal London	0.8	13.4	5.4	64.7
	Whipps Cross	3.5	21.6	6.8	62.5
North West London	Central Middlesex	2.4	15.7	5.2	59.4
	Chelsea & Westminster	1.5	4.3	1.0	34.7
	Ealing	0.6	10.8	4.8	59.9
	Hillingdon	1.0	7.0	1.8	34.6
	Northwick Park	2.5	7.1	2.0	78.4
	Queen Charlotte's	0.6	5.9	1.6	17.4
	St. Mary's	2.6	9.3	6.4	31.5
	West Middlesex	2.6	5.8	2.0	31.2
South East London	Farnborough	0.2	2.6	1.2	23.0
	Guy's & St. Thomas'	6.7	17.5	5.9	45.8
	King's College	4.3	19.0	7.2	36.4
	Lewisham	7.9	16.1	5.9	59.6
	Queen Elizabeth	5.6	17.2	2.2	26.7
	Queen Mary's	2.2	4.8	0.9	41.2
South West London	Kingston	1.0	3.9	0.5	24.5
	Mayday	3.9	11.3	0.8	19.4
	St. George's	4.4	10.0	2.5	52.2
	Epsom & St. Helier	1.1	4.5	1.7	16.2

different sources (i.e. laboratory and maternity units and their various computer systems). Aggregated data cannot be cross-checked and do not allow us to explore co-infection or correlations in high rates of infections (e.g. in some Trusts, hepatitis B rates are very high but not correlated to high rates of HIV or syphilis infection). The HPA-coordinated AISS does not involve the private sector. However, almost all antenatal care in London is provided through the NHS [6].

The AISS is of particular public health interest for infections other than HIV, which are not monitored through alternative dedicated scheme. The estimated prevalence of syphilis (screen test positive) in pregnant women is around one in 200 but the increasing numbers of cases of syphilis among women in the UK [12,13] suggests that high rates of antenatal testing should be maintained to prevent future cases of congenital infection.

An assessment of vaccination coverage among babies at risk of vertical transmission of hepatitis B in 2006 showed that less than half of the babies born to HBsAg-positive mothers in London had received the four recommended hepatitis B vaccinations by the first year of age. There were important variations in performance across London [14]. This study enabled a limited validation of AISS data as it provided baseline information on the expected numbers of those at risk at each Trust in a particular timeframe. Aggregated data for pregnant women who decline antenatal tests recently have been added to the AISS questionnaire, to help better understand why uptake is not complete in all maternity units. A better understanding of the characteristics of individual infected women is needed as well. For this purpose a pilot of an individual based enhanced surveillance of HBsAg-positive mothers also began in London in 2008.

In the UK, as in many other European countries [15-21] there are different policies for universal antenatal infection screening. From a health-economics point of view, there is recent evidence in Europe that universal antenatal HIV screening is justified [22]. In the UK, cost benefit analysis has concluded that syphilis antenatal screening is worth continuing [23]. A recent study in France showed that surveillance of congenital syphilis cases, as well as assessment of syphilis screening practices during pregnancy, should be performed to prevent the occurrence of congenital syphilis cases [24]. An Italian study found prevalence of positive syphilis serology among 0.49% of pregnant women and authors concluded that antenatal syphilis screening is important, facilitates treatment during pregnancy and prevents vertical transmission [25]. Syphilis screening tests need to be followed by further diagnostic tests to confirm infection and assess its stage as well as any potential infectivity and risk to the unborn child.

We believe that the Antenatal Infection Screening Surveillance system described here is an effective method of monitoring policy implementation through provision of simple, relatively cheap and timely information. This provides the local health care providers with comparative data and indicators of their relative success. Maternity unit practices have been described as the most important predictor for determining uptake of HIV testing [26]. Local studies of possible reasons for not achieving universal testing are needed. This would help to ensure that practices are appropriately monitored at local level and results of this monitoring are used to improve antenatal screening, provision of treatment for infected mothers and interventions to prevent infection in the unborn child and among contacts of the mother.

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Research articles

PREVALENCE OF HUMAN CYTOMEGALOVIRUS CONGENITAL INFECTION IN PORTUGUESE NEWBORNS

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Human cytomegalovirus (HCMV) is considered the most frequent cause of congenital infection, occurring in 0.2 to 2.2% of all live births. Since this is a wide range of prevalences observed in different studies, it would be desirable to investigate the prevalence of this infection at national level. The aim of this study was the evaluation of the national prevalence of HCMV congenital infection. We analysed a total of 3,600 Guthrie cards collected from Portuguese newborns during a period of 14 months (August 2003 to September 2004). The cards covered all regions of Portugal and were proportional to the number of births in each region. A heat DNA extraction method was used, followed by DNA amplification by nested PCR. Sensitivity and specificity of this method were evaluated as 93% and 100%, respectively, using 28 cards from HCMV-positive and 280 cards from HCMV-negative children. The national prevalence of congenital HCMV was determined as 1.05% (95% confidence interval: 0.748-1.446). This is the first study of the prevalence of HCMV congenital infection at national level in Portugal. It suggests that Portugal may have one of the highest prevalences of congenital HCMV infection in Europe.

Introduction

Human cytomegalovirus (HCMV) is considered the most frequent cause of congenital infection, occurring in 0.2 to 2.2% of all live births [1]. Since this is a wide range of prevalences observed in different studies, it would be desirable to study the prevalence of this infection at national level. To our knowledge, only one study about the prevalence of HCMV congenital infection in Portugal has been published. It used urine detection by the shell-vial method, and the estimated prevalence was 0.7% [2]. However, the above study was performed at only one hospital, making it difficult to extrapolate the results to the national prevalence. For an estimation of the national prevalence, urine samples from all the regions in the country would have to be collected, proportional to the number of births from each region, and HCMV would have to be detected by cell culture. However, this approach is not feasible to perform.

Dried blood spots (DBS), also known as Guthrie cards, have been used both for late detection of HCMV congenital infection (after the first three weeks of life) and for epidemiological studies with promising results [3,4]. Since all the DBS collected in Portugal for metabolic neonatal screening are sent to a national reference laboratory – the Jacinto de Magalhães Institute of Medical Genetics – and stored there for several years, the use of these cards

allow sampling from all Portuguese regions, which would be very difficult with viruria tests.

The aim of this work was the study of the prevalence of HCMV congenital infection in Portuguese newborns, based on the above collection of DBS. The methodology used was adapted from a technique previously described by an Italian team [5]. The first step of this study was therefore the determination of the sensitivity and specificity of this technique, when compared with the reference method, i.e. the detection of HCMV in urine by cell culture. The second step was the study of the national prevalence.

Material and methods

Sensitivity and specificity of nested PCR on DBS

Samples

Sensitivity was studied using Guthrie cards from 28 children with HCMV congenital infection as determined by detection of HCMV in the urine by shell-vial culture during the first three weeks of life. Specificity was studied using Guthrie cards from 280 neonates without HCMV infection (no detection of HCMV in the urine by shell-vial culture during the first three weeks of life). All cards were from children between one month and eight years of age at the time of our study. The DBS had been collected in the first week of their life and stored at the Jacinto de Magalhães Institute of Medical Genetics. For our study, this institute sent the cards, with the parents' consent, to the Hospital Centre Cova da Beira.

Molecular analysis

We used a heat-induced DNA extraction method, followed by amplification of HCMV DNA in a nested polymerase chain reaction (PCR), using a protocol adapted from Barbi et al. (2000) [5]. Each sample was tested in triplicate.

Blood was eluted from the Guthrie cards, and the DNA was extracted using a heat-induced protocol. A nested PCR protocol was used to amplify a region of the HCMV genome coding for the gp58 subunit of glycoprotein B [6]. The following oligonucleotide primers were used:

Outer primers:

- gB1: 5'-gAggACAACgAAATCCTgTTgggCA-3'
- gB2: 5'-gTCgACggTggAgATACT-gCTgAgg-3'

Inner primers:

- gB3: 5'ACCACCgCACTgAggAATgTCAg-3'
- gB4: 5'TCAATCATgCgTTTgAAGgTA-3'

The sensitivity of this nested-PCR technique had previously been tested by us and shown to consistently detect HCMV in a suspension of 900 copies/ml of the laboratory strain AD-169 (unpublished data). Two different concentrations of AD-169 were processed as positive controls in each set of PCRs, and water was processed as negative control in quadruplicate. Disks punched from blank Guthrie cards were processed as additional negative controls and tested along with the samples. The cards that gave a positive result in at least one of the triplicate amplifications were retested with a new series of disks.

Viral culture

Viral cultures were grown at the Hospital Centre Cova da Beira or at the Hospital de Santa Cruz, using shell-vial assays in human foetal lung fibroblast cells (MRC-5 line), using a protocol previously described by protocol Gleaves et al. (1985) [7], with minor modifications. The cells were analysed by immunofluorescence using anti-HCMV pool I.E.A.+E.A. monoclonal antibodies which give a typical nuclear signal in HCMV infected cells.

Prevalence of HCMV congenital infection in Portuguese newborns

We studied a total of 3,600 dried blood spots (DBS), that had been collected from Portuguese newborns during a period of 14 months (August 2003 to September 2004) and sent to the national screening laboratory. These newborns were from all regions of Portugal, including the Azores and Madeira. The number of Guthrie cards studied was proportional to the number of births in each region (data from the Jacinto Magalhães Institute of Medical Genetics). Within each region, the cards were randomly chosen and sent anonymously to the Hospital Centre Cova da Beira. The study was approved by the Comissão Nacional de Protecção de Dados (National Data Protection Commission).

DNA extraction and PCR were performed as described above.

Results

Sensitivity, specificity and negative and positive predictive values of the nested-PCR on DBS

Specificity

Of the 280 cards from uninfected individuals, 267 were negative in all three PCR tests. The remaining 13 cards were positive in one of the three PCR tests. On repetition, these 13 cards were negative in all three PCR tests (total: 1/6 positive tests). Therefore, no card among the 280 negative controls had more than one positive amplification out of six, and this was established as the cut-off for discrimination between positive and negative cards. The 13 single-positive PCR results were assumed to have been caused by a laboratory contamination during the amplification step, and were considered false-positive results.

Sensitivity

Of the 28 cards from HCMV-infected individuals, 26 had more than three positive amplifications in six PCR tests and were considered positive. Two cards were under the cut-off described above ($\leq 1/6$ positive amplifications) and were considered negative.

With these results, the sensitivity, specificity, negative and positive predictive values of this nested-PCR with the criteria described above were, respectively, 93%, 100%, 99% and 100%.

Prevalence of HCMV congenital infection in Portuguese newborns

The above method and cut-off were used to estimate the prevalence of HCMV congenital infection in Portugal. Of the 3,600 Guthrie cards tested, 38 were positive, according to the criteria

described above. This corresponds to a prevalence of 1.05% (95% confidence interval; exact binomial method: 0.748-1.446).

Discussion

The importance of studying the prevalence of congenital HCMV infection, the most frequent congenital infection [1], should not be underestimated. Updated evaluations of the impact of this infection are needed in order to raise awareness of the true burden of congenital HCMV infection and disease, allocate public health resources, and determine the cost-effectiveness or cost-benefit of potential interventions [8]. Determination of the congenital HCMV prevalence in each country would certainly benefit this purpose.

This is the first study on the prevalence of HCMV congenital infection at national level in Portugal. The 3,600 cards tested covered all regions of Portugal and were proportional to the number of births in each region, so that the samples represented all the Portuguese territory. To our knowledge, this is also the first study using Guthrie cards from all regions in one country to estimate a national prevalence, although one multicentric study used this technology for the determination of the prevalence of congenital HCMV in Italy [4].

The methodology used in the present study was adapted from a method described by an Italian team [5], which was reported to have 100% sensitivity and 99% specificity compared to the reference method, virus isolation in cell culture. Therefore, the first step of our study was to determine the sensitivity and the specificity of the adapted protocol used by us.

For the sensitivity analysis, we included DBS from all children diagnosed with CMV congenital infection between 1995 and 2001 who had been tested with the reference method at the Hospital Centre Cova da Beira and the Hospital de Santa Cruz and who fulfilled the inclusion criteria, i.e. signed informed consent from the parents and availability of a DBS collected in the first week of life before receiving any blood transfusion. A total of 28 cards were tested, obtained from symptomatic and asymptomatic infections, but also from children for whom clinical information was not available, which was the case for the two negative results. These two had had a positive urine culture result and we therefore consider them false-negatives.

Because the viral load was not determined in the above mentioned laboratories in 2004 and urine specimens were not preserved until 2007 (when they introduced routine determination of CMV viral load), the relationship between false-negative results, clinical information and low viral loads could not be ascertained in this study.

Interestingly, our recent experience with an external proficiency panel of samples (CMV DBS, organised by Quality Control for Molecular Diagnostics from the European Society of Clinical Virology) suggests that low viral loads could be the main factor responsible for false-negative results (unpublished data). Other possible explanations for the two false-negative results could be ineffective DNA extraction or the presence of inhibitory substances in the specimen; this was not checked in this evaluation because the technique described by Barbi et al. (2000) does not include an internal control [5]. Nevertheless, the 100% sensitivity obtained by the Italian team suggests that PCR inhibition is not a significant problem inherent to this technique.

Our results of 93% sensitivity and 100% specificity were encouraging and allowed us to proceed with the aim of this study, the determination of the prevalence of HCMV congenital infection in Portuguese newborns. The observed prevalence of 1.05% was within the expected range [1], albeit a little higher than in some of the latest European reports [4,9,10]. According to a recent meta-analysis of selected studies which had used the reference method and analysed at least 800 urine or saliva samples, the prevalence in European countries ranged from 0.3 to 0.5%, but only studies from Belgium, Denmark, Italy, Sweden, and the United Kingdom were included in this review [11].

One possible explanation for our results could be the high seroprevalence of around 80% in pregnant women in Portugal [12], because a positive correlation between maternal seroprevalence and birth prevalence has been described: one meta-analysis suggested maternal seroprevalence as a significant predictor of birth prevalence, with every 10% increase in maternal seroprevalence corresponding to a 0.26% increase in birth prevalence [8]. However, this cannot be the only explanation, since Barbi et al. (2006) also described a seroprevalence of 80% in women of childbearing age, whereas it identified only 0.18% of newborns with congenital infection in. Interestingly, the same team reported 0.47% congenital infection in a previous study [13]. Whether this discrepancy was the result of different methodologies or sample size (9,032 pooled DBS in the study from 2006 versus 1,268 urine cultures in 1998) or due to other factors, is unclear.

In the present study, a higher sampling could have narrowed the confidence interval, but for practical reasons, the sample size had to be limited to 3,600 cards. Since the methodology implied that each card must be tested in triplicate, followed by a further three amplifications for those with a positive result, more than 10,000 individual nested-PCR reactions had to be performed in the current setup of the study.

Considering that the sensitivity of the method was 93%, the maximum proportion of expected false-negative results would not have had a significant influence on the final prevalence (1.05% could have been 1.14%). On the other hand, the specificity in the first phase of the study was 100%, assuring a very low probability of false-positive tests in the second phase. However, specificity was studied in only 280 cards and it cannot be ruled out that a false-positive result may have occurred if more cards had been analysed.

Since the cards for prevalence determination were sent anonymously, we could not obtain clinical information, including maternal serological evolution during pregnancy. Another study coordinated by the Portuguese Society of Paediatrics, currently addresses this point in order to figure out the relative importance of maternal primary and recurrent infections in the Portuguese children with congenital CMV infection.

With around 105,000 births per year, a prevalence of 1.05% translates to about 1,103 children (between 785 and 1,518, with a 95% confidence interval of 0.748-1.446) born in Portugal each year with this congenital infection. Assuming that around 11% of these infections will be symptomatic at birth [8], about 121 (between 86 and 167) of the infected newborns will have signs and/or symptoms, and at least half of them will present late sequelae. To these numbers must be added an estimated 13.5%, i.e. about 149 (between 106 and 205) children with asymptomatic infection at birth, who will suffer from late sequelae, particularly sensorineural hearing loss and cognitive impairment [11].

In conclusion, for the first time in Portugal a nationwide study using DBS allows us to estimate the number of children congenitally infected with CMV. Our data suggest that Portugal may have one of the highest prevalences of congenital CMV infection in Europe, although nationwide studies in other European countries are needed before any conclusions can be drawn.

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Research articles

PREVENTION OF CONGENITAL RUBELLA AND CONGENITAL VARICELLA IN EUROPE

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Rubella and varicella zoster virus (VZV) infections during pregnancy can cause severe adverse outcomes in the embryo or foetus. Despite the availability of safe and efficacious vaccines, cases of congenital rubella and varicella syndrome still occur in Europe. As of 2004, several countries had high proportions of women of childbearing age that were susceptible to rubella and varicella virus infection. Effective immunisation strategies to enhance prevention should include an active role of different medical specialists in order to include all medical consultations a person may have at different points in their lives as an opportunity to immunise susceptibles. Linkage of data on infectious diseases with those from congenital defects registries may be helpful to monitor the epidemiology of congenital rubella and varicella.

Introduction

Women have an increased risk of acquiring certain transmissible diseases during pregnancy due to transient immunosuppression [1]. Although many infectious diseases can be prevented by vaccination during childhood, appropriate immunisation of women of childbearing age is crucial in preventing diseases in their offspring that may occur during embryonal/foetal life or early after birth. Because many immunisations, if performed during pregnancy, may theoretically pose a risk for the unborn child, immunisation strategies should be integrated where possible with preconceptional care.

Prevention of congenital rubella syndrome is one of the priorities set by the World Health Organization (WHO) Regional Office for Europe. In 1998, the target of one case of CRS per 100,000 live births by 2010 was approved as a goal of immunisation programmes in the Region [2,3].

This paper tries to draw a picture of the epidemiology of rubella and varicella infections in Europe and the potential for their transmission to pregnant women and presents with possible strategies to enhance prevention of these infections.

Rubella

Epidemiology

Reliable data on the incidence of CRS are difficult to obtain for various reasons: because of weakness of the surveillance systems, because rubella in pregnancy can be asymptomatic, because CRS can present with incomplete clinical signs, and because specific symptoms may appear late in the infection.

From 2001 to 2003, a total of 47 cases of CRS were reported from member states of the WHO European Region, decreasing from 21 cases in 2001 to 12 cases in 2003 [4-6]. Moreover, 36% of these cases were reported from Romania and 32% from the Russian Federation, whereas the last CRS cases in Finland and Denmark, where coverage for MMR vaccine has been high for many years, was recorded in 1986 [7]. In 2004, 15 member states did not report information on CRS to the WHO, but 14 member states reported 17 cases of CRS [2]. In Italy, where a national campaign for measles and CRS elimination has been reinforced since 2003 [8], the annual incidence rate of CRS has consistently exceeded the WHO goal of one per 100,000 newborns between 1996 and 2002, with a peak in 2001 of six per 100,000 [9,10]. Recent data suggest that rubella outbreaks still occur in women of childbearing age in Italy. In the period between 2005 and 2008, 30 confirmed cases of rubella have been reported in pregnant women, and four confirmed CRS cases have been diagnosed [11].

The trend of rubella infections in European countries can be obtained from data reported to the WHO by the countries of the WHO European Region, and from data reported to EUVAC.NET, a European surveillance network for vaccine preventable diseases that includes 18 European Union countries [12]. Data reported to the two systems from 2000 to 2007 are shown in figure 1 [12]. Data from both surveillance systems indicate a sharp decrease in the number of cases after 2003, and a stable number of cases since 2005.

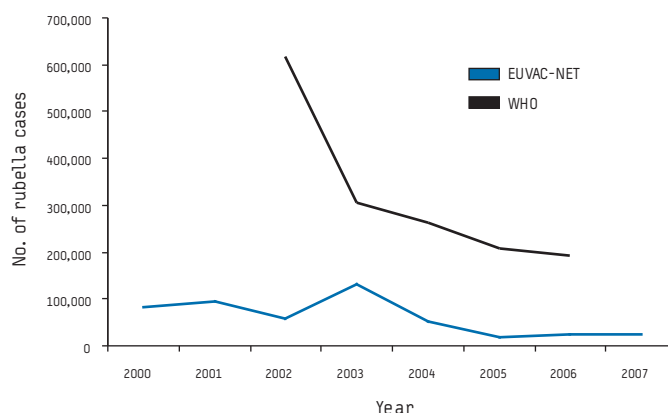
According to the European Centre for Disease Prevention and Control (ECDC), 1,498 rubella cases were reported from 22 countries in 2005. The highest incidences were reported by Lithuania (3.44 per 100,000) and the Netherlands (2.23 per 100,000). The overall incidence in the 22 countries was 0.51 per 100,000 [13]. As a result of suboptimal immunisation coverage for rubella, several outbreaks have been recorded in Europe in the last decade. In the period from 2002 to 2003, a large rubella outbreak was observed in Romania with 115,000 reported cases mainly in school-aged children with no difference in incidence by sex [14]. A large rubella and CRS outbreak was described in 1993 in Greece, with 25 serologically confirmed cases (24.6 per 100,000 live births); the incidence decreased after this, but another epidemic occurred in 1999, mainly in young adults, with four cases of CRS (4.0 cases per 100,000 live births). The CRS incidence in Greece remained low until 2003 [15,16]. Rates of CRS as high as 350

per 100,000 live births have been described during outbreaks in the Russian Federation between 2002 and 2004 [1]. In Turkey, there was no surveillance system for rubella and CRS until 2005. In 2005, with a new surveillance system, 2,245 rubella cases were reported – an incidence rate of 3.1 per 100,000 inhabitants – and only one case of CRS in the same year [17]. In the United Kingdom (UK), measles-mumps-rubella (MMR) vaccination controlled rubella in children and women of childbearing age, but an epidemic in 2005 showed that individuals born between 1982 and 1986 who had never been previously exposed to natural infection were still susceptible [18-21].

Seroprevalence data from the European Sero-Epidemiology Network (ESEN) study performed between 1996 and 2003 showed that women in several countries included in the study were not sufficiently protected against rubella infection (Figure 2) [22].

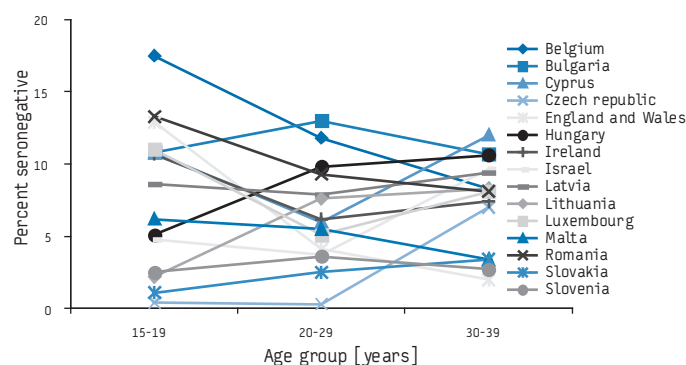
In Finland and the Netherlands on the other hand, a low rate (<5%) of susceptibles in childhood and adolescents of both sexes was observed in the period from 1996 to 2004 [7,23]. In Italy,

FIGURE 1
Reported number of rubella cases to WHO and EUVAC.NET, 2000 - 2007 (n=2,073,229)*



* source: [11,23,24]

FIGURE 2
Proportion of women susceptible to rubella, by age group, 1996 - 2003*



* source: [16]

seroprevalence data from 2004 showed 11% of susceptible women in the age group of 15-19 year-olds, and 8% in the 20-39 year-olds [11].

Prevention strategies

In order to meet the WHO target of one case of CRS per 100,000 live births by 2010 and to achieve elimination of measles, a measles and CRS elimination strategy was launched in 2002 [3]. The success of current policies in countries using the rubella vaccine has been considerable. The use of rubella combined vaccine has markedly increased since 2002 in the European Region. However, eastern European countries have only recently introduced the MMR vaccine, and some countries in western Europe, where the vaccine has been used for a longer time, have historically had inadequate coverage rates (Table) [18]. In addition, several countries have only recently moved from a one-dose strategy to a two-dose strategy for rubella-containing vaccine [3,12,24].

Use of rubella-containing vaccine in WHO/Europe member states has increased from 38 (75%) of 51 countries in 2001 to 48 (92%) of 52 countries in 2007; Currently 47 member states use at least one dose of a combined MMR vaccine in their childhood immunisation programmes [3,24]. Given that most countries in Europe have chosen to use combined measles-rubella (MR) or MMR vaccines, rubella elimination is feasible within a framework of measles elimination [12].

Rubella-susceptible women immigrating from outside Europe have been identified as an important target group for immunisation. Programmes to immunise newly arrived women and adolescent girls are necessary, because they may have contracted rubella in a high-incidence country that does not have a rubella immunisation programme and give birth to an infant with CRS. International vaccination centres should make an effort to immunise immigrant people visiting friends and relatives outside Europe. Several supplementary immunisation activities targeting measles- and/or rubella-susceptible individuals have been conducted in several countries since 2001, including Albania, Cyprus, Italy, Kazakhstan, Kyrgyzstan, Moldova, Montenegro, Serbia, Tajikistan and Turkey [24].

Overall, about 70% of member states had national immunisation plans in 2004, 60% had measles elimination plans, but less than 50% had rubella elimination plans and/or plans for CRS prevention [24].

Varicella Epidemiology

The epidemiology of congenital varicella (CV) can be derived only indirectly from ad hoc studies because no European country has a specific surveillance system in place. Moreover, in some European countries Denmark, Iceland, Ireland, Northern Ireland, Norway, Sweden, Switzerland and Turkey, varicella disease is not under surveillance. Others Belgium, England and Wales, France, Germany, the Netherlands and Portugal have data derived from sentinel surveillance systems [25,26].

More than 90% of European children contract chickenpox in the first 10-12 years of life [27-30]. In 2002-2003 the estimated incidence in the UK was 262 varicella cases per 100,000 nulliparous women aged 15-44 years, with 10 of these cases occurring during pregnancy and resulting in nearly 0.06 cases of congenital varicella and 0.16 cases of neonatal varicella per

100,000 live births [25,28]. In 2002-2003, the majority of varicella cases in European countries were reported from Spain (28%), Poland (18%) and Italy (14%) [26,31].

In Italy, only 78% of 15 year-olds had antibodies to VZV between 1996 and 2003, and 18% of female teenagers were seronegative for VZV [25,30,31]. In the same period, nearly 90% of people in the UK had serological evidence of infection by the age of 20 years [28,30]. In Spain, the prevalence of VZV antibodies in the period from 1996 to 2003 was 94% in pregnant women aged 15-24 years, 95% in those aged 25-29 years and >95% in those aged 30-49 years [30-32]. The seroprevalence was 97.8% at the age of 10 years in Switzerland, and more than 90% at the age of nine years in Belgium, in the season 2000-1 [26,31].

In most European countries less than 5% of women of childbearing age (between 15 and 39 years-old) were seronegative for VZV in the period from 1996 to 2003, except in Italy (12.6%), Israel (7.6%), and Ireland (5.4%). In Finland, VZV seroprevalence was 96.2% in 2000 [31-33].

Prevention strategies

Safe and effective vaccines against varicella have been available in Europe for the last ten years. The increase in the age at onset, the burden of complications and the direct and indirect costs have prompted several countries to consider universal immunisation programmes for varicella.

Germany is the only country in Europe that has a routine universal childhood varicella immunisation programme, introduced in 2004, with a single dose administered to children at the age of 11-14 months and a catch-up dose for adolescents aged 9-17 years who have a negative history of chickenpox [34]. In April 2006, the combined MMR-varicella (MMR-V) vaccine was licensed in Europe, but it is as yet not available. However, a two-dose MMR-V

schedule is likely to replace the monovalent vaccine at least in Germany [25,34].

In Spain, varicella vaccine is recommended for all healthy susceptible adolescents (≤ 13 years), all children with chronic diseases, organ transplant recipients, seronegative households and health contacts of high-risk children [25]. The community of Madrid adopted universal infant vaccination in October 2006 [25]. Other countries including Cyprus, Italy, Latvia, Slovenia, Switzerland, and the UK recommend immunisation to high risk patients, seronegative healthcare workers, seronegative family members of high-risk patients, and adolescents with no recollection of having had the disease [25].

No specific programmes or initiatives have been endorsed so far by the WHO to promote varicella immunisation or prevention of congenital varicella.

How to enhance prevention strategies

Integration of preconception components into primary care can better serve women at various levels of risk across their lifespan [35]. Depending on the age group in which prevention strategies should be applied, prevention of CRS and CV require a strong integration of several activities which involve different professional levels.

Children and adolescents

Universal immunisation programmes targeted to children are already in place for rubella. The WHO Regional Office for Europe developed and implemented a strategic plan for the prevention of measles and congenital rubella infection in the WHO European Region in 2002 [3]. This plan targeted the elimination of measles and the prevention of congenital rubella infection for the year 2010.

TABLE

Vaccination policies for rubella in 16 countries as of 2003*

Country	Year of introduction of childhood rubella vaccination		Recommended age for second dose	Average vaccine coverage among infants (%)	Adolescent female vaccination (years)	Antenatal screening as of 2003	Average rubella incidence (per 100,000)
	One dose	Two dose					
Belgium	1985	1994	11	82 (1999)	1973-1994	Yes	0.2 (2001-2003)
Bulgaria	1992	2001	12	93 (1999-2003)	1988-2001	-	86.8 (1999-2003)
Cyprus	-	1989	4-6	86 (1999-2003)	1974-1989	Yes	0.2 (1999-2003)
Czech Republic	-	1986	2	97 (1999,2001,2003)	1982-1997	-	11.3 (1999-2003)
England and Wales	1988	1996	4-5	85 (1999-2003)	1970-1988	Yes	0.1 (1999-2003)
Hungary	1991	1999	11	100 (1999,2002,2003)	-	-	0.7 (1999-2003)
Ireland	1988	1992	4-5	76 (1999-2003)	1971-1988	Yes	1.6 (1999-2003)
Israel	1988	1995	6-7	95 (1999-2003)	1973-1999	-	0.2 (1999-2003)
Latvia	1993	2002	7	97 (1999-2003)	1993-2002	-	29.0 (1999-2003)
Lithuania	-	1992	6-7	97 (1999-2003)	1992-1996	-	20.2 (1999-2003)
Luxembourg	1986	1994	5-6	-	-	Yes	0.8 (2000-2001)
Malta	1989	1992	12	74 (1999-2003)	1976-1992	-	1.0 (1999-2003)
Romania	-	-	-	-	-	-	136.3 (1999-2003)
Slovakia	1985	1992	11	99 (1999-2003)	1985-1992	-	0.3 (1999-2003)
Slovenia	-	1990	6-7	93 (1999-2003)	1973-1990	-	0.5 (1999-2003)
Sweden	-	1982	12	92 (1999-2002)	1972-1982	-	< 0.1 (1999-2003)

* source: [17]

Nonetheless, the success in eliminating a transmissible disease depends mostly on the coverage level that is achieved. Measles elimination has already been achieved in some member states through routine immunisation programmes, which maintain high measles vaccine coverage using a two-dose schedule [2,36]. Since most European countries use combined measles vaccines including the rubella component, policies toward measles elimination should result in concurrent elimination of CRS [36]. Strategies for elimination of measles and CRS should be sustained in the entire WHO European Region maintaining coverage levels over 95% for rubella-containing vaccines. Catch-up programmes must also be maintained to avoid the accumulation of susceptibles in the general population. It should be considered that it is unlikely that universal programmes for varicella immunisation will be implemented in the short term in all countries of the WHO European Region. The recent licensure of MMR-V, however, may favour a link with measles and congenital rubella elimination strategy in the near future. Since patients reliably remember having had varicella, a minimal approach for prevention of varicella in pregnancy may consist of a verbal screening of adolescents to choose those eligible for immunisation. Some countries already actively offer varicella immunisation to high risk children. Although this strategy is directed to a small proportion of the general population, it is essential to monitor its impact. If susceptible individuals accumulate as an effect of targeted immunisation strategy, outbreaks may occur in this group at an older age, when varicella is more likely to be severe [37].

These potential strategies rely on the integration of roles of public health officers with those of family paediatricians and general practitioners [27].

Women of childbearing age

Information programmes should be in place to disseminate and to promote screening and immunisation against measles, mumps, rubella, and varicella in susceptible women of childbearing age. These programmes may be particularly effective if not limited to women who plan a pregnancy. Every visit of this target population to a gynaecologist or general practitioner may include counselling, screening, and oriented recommendations for immunisation. It is necessary to include rubella virus antibody screening in prenatal care even in countries with well-established vaccination programmes. One needs to keep in mind that people do not reliably recall a past rubella infection and that, in cases where it is not possible to determine the immunisation status or the presence of specific IgG antibodies, a woman must be considered susceptible. Vaccines against rubella and varicella infections should be offered to all women of childbearing age who do not have acceptable evidence of immunity [38,39].

Women during pregnancy

Screening and diagnosis of rubella and varicella infections during pregnancy pose particular problems. Communication of screening results to pregnant women may result in termination of pregnancy [38,39]. Besides the fact that the performance of commercial diagnostic tests is variable, it must be kept in mind that as the true incidence of a certain disease becomes low, the positive predictive value of diagnostic tests for confirming recent infection declines as well. This is particularly relevant for rubella infection in countries in which elimination has almost been achieved [22,39]. A woman identified as susceptible to rubella or varicella should be followed until the end of pregnancy to ensure that she will be immunised soon after delivery [22,39].

Women after delivery or abortion

Since delivery or abortion take place in medical facilities, this setting is particularly appropriate for administering due immunisations provided that information on previous screening is communicated. Cost-effectiveness analysis of antenatal varicella screening with post-partum vaccination of susceptibles suggests that the screening and vaccination strategies are more cost-effective in preventing cases in women than with the strategy to treat cases as they arise [27]. In case information on screening is not available, diagnostic tests may be offered to women with unknown susceptibility to rubella and varicella [27,39].

Women who have already had children are very likely to consult a family paediatrician before another pregnancy. For this reason, mothers can be verbally screened and provided with specific recommendations during paediatric consultation. This strategy could be added to that based on visits to general practitioners.

Surveillance and seroprevalence

The WHO Regional Office for Europe launched a strategic plan in 2005 to eliminate congenital rubella [3]. A European measles and rubella laboratory network was established in 2002 [3]. At present, 47 member states (90%) have a national measles/rubella laboratory, which is linked to one of three WHO European Region reference laboratories appointed in 2003 or to the specialised laboratory located in the European Region. The network has implemented standardised diagnostic methods and reagents, and a quality assessment programme, including proficiency testing and monthly online reporting of laboratory performance indicators; completeness of reporting from national laboratories was 70% in 2004 [3]. Seroprevalence studies should be encouraged periodically to precisely identify population groups that may be targeted for special prevention strategies. While surveillance of rubella is in place in all WHO European countries and many of them also have a system for varicella, much effort should still be devoted to surveillance of congenital rubella and congenital varicella [4,25]. Moreover, member states use different methods to collect measles and rubella data, including aggregate, case-based, and sentinel physician reporting, which require standardisation [2]. This activity could benefit from cooperation between public health professionals working in surveillance of transmissible infections and congenital defects registries regarding the sharing of data and the use of similar case definitions. Under-notification is a well recognised limitation of nationwide mandatory notification systems. It is therefore necessary to enhance the quality of surveillance systems and sero-epidemiology, particularly in countries in which the disease is under control [1,20,25].

Integration with other prevention strategies

Women of childbearing age should receive preconceptional counselling whenever they interact with medical facilities. General and hospital practitioners, gynaecologists and obstetricians, and possibly professionals in other specialties, should offer information for the prevention of adverse events in pregnancy advocating appropriate lifestyle habits, food and vitamin intake, and prudent use of drugs. Prevention of transmissible disease through immunisation, not only against rubella and varicella, should be one of the most important parts of preconceptional counselling.

Conclusions

Preconceptional screening and immunisation of pregnant women are not yet adequate in Europe. European countries should endorse common strategies to improve as much as possible the impact of recommendations for the prevention of rubella and varicella

in pregnancy. This is possible only through the coordination and integration of several activities and different actors who should share the final goal of preventing cases of these diseases.

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Research articles

FACTORS AFFECTING THE ADHERENCE TO AN ANTENATAL SCREENING PROGRAMME: AN EXPERIENCE WITH TOXOPLASMOSIS SCREENING IN FRANCE

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Monthly serological testing is mandatory in France for pregnant women not immune to toxoplasmosis. We assessed for the first time the adherence to this national programme, using data from antenatal tests for *Toxoplasma* antibodies collected by the Union of Health Insurance Services in the French Rhone-Alpes region. Data from 34,290 pregnancies was analysed. The first test was done late in 25% of women (8,430). Women had on average 5.7 tests during pregnancy, only 40 percent (13,774) were tested seven or more times as recommended. Young women were more likely to have a late first test, but age did not significantly influence regularity and number of tests. Free medical coverage favoured a late first test, fewer tests and longer between-test intervals. An early first test did not affect test numbers or between-test intervals. A re-useable prescription for several tests was associated with better adherence. Prescription by general practitioners was associated with an earlier first test, but fewer tests and longer between-test intervals. When prescribing physician(s) included a gynaecologist, the first test was more likely to be behind schedule, but the overall number of tests was higher and between-test intervals shorter. Because data was collected through private laboratories, our conclusions apply to the majority of French patients who need to schedule a separate visit for blood testing after prescription.

Introduction

Congenital *Toxoplasma* infection arises in 25% of acute maternal infections during pregnancy. The consequences for the foetus can be severe, most often ophthalmologic or affecting neurodevelopment [1,2], and are diagnosed immediately, at birth or later during childhood or adulthood [2]. In an attempt to decrease the number of children with severe infections, several countries have implemented mandatory or recommended antenatal testing programmes in order to promptly recognise and treat acute maternal *Toxoplasma* infections. In France, an antenatal screening programme was implemented in 1978. It has included, since 1985, detection of antibodies against *Toxoplasma* before the end

of the 12th week of gestation - the official deadline for registering a pregnancy - followed, since 1992, by a monthly testing until the time of delivery for patients who are not immune. There is a recommended minimum of seven tests. The preventive impact of this programme remains to be proven. Adherence to this programme is also relevant when debating its effectiveness, but has never been addressed. We present here an analysis of the adherence to the French screening programme for congenital toxoplasmosis. It is specifically targeted to women who are tested in private laboratories, which is common for outpatients in France. This feature of the French health care system requires an obligation on the patients' part to schedule the different appointments for blood sampling. Patients need to pay for the tests, but will be reimbursed, provided that the tests were prescribed by a physician (general practitioner (GP) or any specialist doctor) or a registered midwife.

The goals of our study were to assess adherence to the programme and to identify reasons for poor adherence, in order to develop a communication strategy specifically targeted to pregnant women and their physicians.

Patients and methods

Available data

We used data collected for reimbursement purposes by the Regional Union of Health Insurance Services (URCAM) of the French Rhone-Alpes region. They record the biological analyses performed at private laboratories and reimbursed for the part of the population (86%) insured by the main health insurance system. The national coding system for biological analyses allows differentiation between the first antenatal test, intended to determine the patient's immunity, and subsequent follow-up tests required to exclude later seroconversion. For each test, dates of issue of prescription and date of blood sampling were available, along with information on the professional who prescribed the test (GP, obstetrician-gynaecologist, other specialist or registered midwife, public or private practice). Patient data included age at delivery, dates of conception and

delivery, whether she delivered in a private or public hospital, and whether she was fully covered by the national health service - free medical coverage (FMC) being attributed to low income.

Study population

We selected all women living in the Rhone-Alpes Region who delivered between 1 July 2002 and 30 June 2003 and for whom at least two tests for *Toxoplasma* infection were reimbursed, including one follow-up test. The aim was to select women who were not immune to toxoplasmosis and who were supposed to undergo the mandatory monthly testing schedule. Data was extracted anonymously by the URCAM statistics department and analysed by the biostatistics department of the Lyon teaching hospital.

Criteria

The first studied criterion was whether or not the first test had been performed within the first 12 weeks of pregnancy. Factors included in the analysis were: age, FMC, delivery in a public or private hospital, profile and type of practice of prescribing physician.

Two additional follow-up criteria were the mean number of tests throughout pregnancy and the mean time interval (in days) between two consecutive tests.

Statistical methods

Continuous variables were described with mean, median, standard deviation (SD), minimum and maximum. The mean between-test interval for each patient was calculated as the mean of the intervals between two consecutive tests uncorrected for potential correlation between intervals. Binary variables were described with number and percentage. Association between outcome and independent predictors was studied through different types of regression models: Logistic regression was used for the binary dependant variable "late first test". All variables linked to the women (age, FMC, delivery in a public or private hospital) as well as the profile of the prescribing physician(s) during pregnancy

were divided into four categories - GP(s) only, obstetrician-gynaecologist(s) only, GP(s) plus obstetrician-gynaecologist(s), other specialists (including registered midwives) - and entered into the model.

For the other two criteria, three further predictors linked to the first test were added to the previous set of variables: "tests done on schedule (yes/no)", "time interval between prescription and testing" and "prescription for the initial test re-used on at least one follow-up test (yes/no)". Poisson regression was used for the ordinal variable "number of tests", with the number of weeks of pregnancy as offset. All independent covariates tested individually reached statistical significance ($p < 0.01$), except the items related to the patients' first test, which were nevertheless considered as important and retained in the final model.

A linear regression model was run for the continuous variable "time interval between two consecutive tests". All variables were individually associated with a p value under 0.01 and kept in the final model, except for the re-use of prescription. Nevertheless, this factor was considered to be important and retained in the model. The effect of age was modelled as a linear relation after verifying several multivariable fractional polynomials models.

Statistical significance was accepted for $p < 0.05$. Analyses were performed using STATA® release 9 (Stata Corporation 2005, College Station, Texas, United States).

Results

Study population

There were 41,086 deliveries during the study period. For 38,450 women, two *Toxoplasma* antibody tests, including at least one follow-up test, were reimbursed. After exclusion of 4,160 women, 34,290 remained in the final sample. The reasons for exclusion and their number are given in the Figure.

The characteristics of patients, prescribing physicians and tests are presented in Table 1.

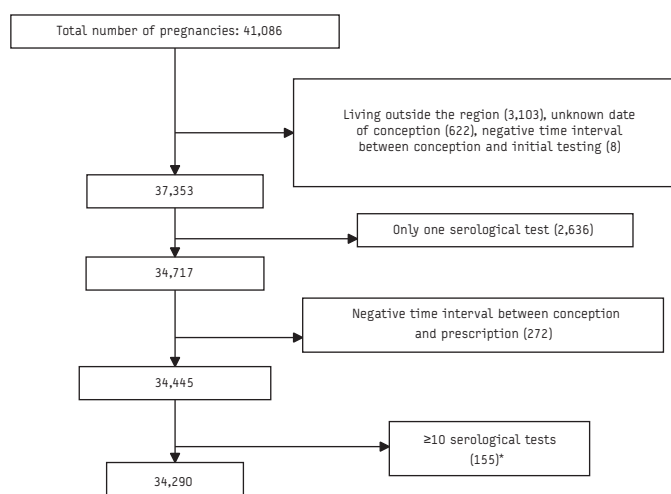
The mean age of the women was 29.5 years; 1,086 women (3.17%) were under 20 years-old and 467 (1.36%) over 40 years-old. Mean gestation was 37.6 weeks (SD 1.9). Most pregnancies lasted 37 weeks or more (24,882; 72.6%), very few lasted less than 34 weeks (1,164; 3.39%). A large proportion of women had one (15,068; 43.9%) or two (14,946; 43.6%) prescribing physician(s). The majority of women had all tests done in one (26,588; 77.4%) or two laboratories (6,599; 19.3%). Prescriptions for the first test were re-used for at least one more test by 2,832 (8.26%) patients. For 512 women (1.49%) there was a single prescription for the totality of the tests. The re-usable prescriptions were written by a GP for 707 (25.0%) of the 2,832 women, by an obstetrician-gynaecologist for 2,083 (73.6%), and by another specialist or a registered midwife for 37 (1.31%). Since almost all prescribing physicians (99.29%) were in private practices, this co-variable was disregarded in the following analyses.

Initial test

The first test was prescribed on average at 8.3 weeks of gestation (median 7.1; SD 5.0; min 0, max 36.6), in 60% of cases by a gynaecologist (Table 1). The mean time interval between prescription and testing was 7.9 days (median 3; SD 12.3, min 0, max 178); it was longer in younger women ($p < 0.0001$), in women with FMC ($p < 0.0001$), and when the test was prescribed by an obstetrician-gynaecologist rather than by a GP ($p < 0.0001$).

FIGURE

Exclusions from the data file with justification, antenatal toxoplasmosis screening programme, France, 2002/03



*Women who had more than ten tests reimbursed were excluded because this could suggest occurrence of a seroconversion.

The first test was performed at 9.5 weeks of gestation on average (median 8.4; SD 5.4, min 0, max 37.6). It was performed within the recommended schedule (in the first 12 weeks of pregnancy) in 75.4% of cases (25,860).

TABLE 1

Characteristics of women, prescribing physicians and tests, antenatal toxoplasmosis screening programme, France, 2002/03

Characteristics	Mean (SD; min-max)
Age of pregnant women	29.5 (4.9; 14-54)
Length of pregnancy (weeks)	37.6 (1.9; 21-44)
Number of prescribing physicians per patient	1.7 (0.74; 1-7)
Number of prescriptions per pregnancy	4.9 (2.0; 1-9)
Number of tests per pregnancy	5.7 (1.9; 2- 9)
Number of different laboratories used per pregnancy	1.3 (0.5; 1-6)
Number of weeks between first and last test	22.9 (7.7; 0-38)
	Number (%)
Free medical coverage	3,319 (9.7)
Delivery in a public hospital	23,537 (67.6)
Profile of physician who prescribed the first test	
General practitioner	13,431 (39.2)
Gynaecologist	20,569 (60.0)
Other	290 (0.8)
Profile of physician(s) who prescribed the follow-up tests	
General practitioner(s) only	6,596 (19.2)
Gynaecologist(s) only	15,864 (46.3)
General practitioner(s) and gynaecologist(s)	10,534 (30.7)
Other specialist(s)	1,296 (3.8)

SD: Standard deviation.

TABLE 2

Effects of the characteristics of women, physicians and the first toxoplasmosis test on the number of tests and mean between-test interval, antenatal screening programme, France, 2002/03

	Reference	Overall number of tests Risk ratio for one additional test [95% CI]	Mean between-test interval (days) Interval [95% CI]
Patient profile			
Age of pregnant women	Per year older	NS*	NS*
FMC	No FMC	0.84 [0.83;0.86]	6.02 [5.4;6.7]
Delivery in private hospital	In public hospital	1.04 [1.03;1.05]	-0.45 [-0.87;-0.03]
Testing profile			
First test late	Not late	0.70 [0.69;0.71]	-0.73 [-1.2;-0.2]
Interval between first test prescription and testing	Per 10 additional days	0.995 [0.994;0.995]	NS*
First prescription re-used for at least one test	Prescription not re-used	1.07 [1.29;1.47]	-4.7 [-5.4;-4.0]
Profile of prescribing physician(s)			
Gynaecologists(s) only	GP(s) only	1.08 [1.40;1.57]	-4.2 [-4.7;-3.6]
GP(s) + gynaecologist(s)	GP(s) only	1.16 [1.15;1.18]	-3.4 [-3.9;-2.8]
Other	GP(s) only	1.19 [1.16;1.22]	-4.3 [-5.4;-3.2]

*CI: confidence interval; FMC: free medical coverage; GP: general practitioner; NS: not significant.

Independent predictors for a delayed first test were: FMC (odds ratio (OR) 2.39; 95% confidence interval (CI) [2.22-2.58]), age (OR 1.03 95% CI [1.02-1.04] per year younger) and prescription by a obstetrician-gynaecologist (OR 1.29 95% CI [1.22-1.36]) or another specialist (OR 1.68 95% CI [1.30-2.18]) rather than a GP.

Test number and frequency

Number of tests

Women were tested on average 5.7 times (median 6; SD 1.9, min 2, max 9), an average adherence rate of 81% (see Table 1). 40.2 percent (13,774 women) were tested seven or more times, as recommended.

Independent predictors for a lower number of tests are summarised in Table 2: FMC ($p < 0.0001$) had the greatest impact (incidence-rate ratio (IRR)=0.84; 95% CI [0.83-0.85]), followed by delivery in a public hospital ($p < 0.0001$), GP(s) only as prescribing physician(s) ($p < 0.0001$), a first test performed late ($p < 0.0001$), a long time after prescription ($p < 0.0001$), and a test done with a prescription that was not re-used ($p < 0.001$).

Between-test intervals

The mean between-test interval was 37.6 days (median 32.7; SD17.9; min 0, max 229). Eighty percent (27,402) of women had at least one between-test interval exceeding 35 days, 22,954 women (66.9%) had two or fewer intervals exceeding 35 days. The intervals were significantly longer in women who had FMC ($p < 0.0001$), delivered in a public hospital ($p < 0.0001$), had only GPs as prescribing physicians, had a late first test ($p < 0.0001$) or used multiple prescriptions (one per test) rather than a re-usable prescription ($p < 0.001$) (Table 2).

Discussion

The goals of our study were to determine compliance with the screening programme for toxoplasmosis in pregnant women tested in private laboratories and to identify predictors for non-compliance. Compliance was unsatisfactory, with a quarter of the participants doing the first test too late, 80% of participants having at least one

between-test interval exceeding 35 days and 60% of participants completing fewer than the recommended seven tests.

These findings were based on a large dataset collected from the Rhone Alpes population which represented 9.7% of the total French population and 9.3 % of all births in 2003 [3]. It did not include women covered by the health care systems for agricultural or independent workers (14% of the population), but we have no reason to assume that the testing behaviour should be different in that subset of the population. Women who were not tested at all were also disregarded in the study, but these patients, whose number are impossible to estimate, are likely to have such a different profile that they would require a specific study to understand the reasons why they are not included in standard care. We can not rule out the possibility of a small proportion of women having a test and forgetting to apply for reimbursement, but considering the large amount of data in our file, it is unlikely that they significantly modified our conclusions. The number of pregnancies in our study was indeed consistent with the 76,349 births registered for 2003 in the Rhone Alpes region [3] and the estimated regional seroprevalence for *Toxoplasma* infection of 36.1% [4]. Furthermore, our data was in line with national estimates concerning mean age of pregnant women, rates of free medical coverage and of deliveries in a public hospital [5].

As no other study has been conducted since the French screening programme was implemented, it is unknown whether adherence has always been insufficient. Prenatal programmes for toxoplasmosis only exist in several other countries [6], although there are differences in the testing schedule and in how the sampling is organised. Data on compliance, however, have only been reported in one Brazilian study, which also found adherence to screening to be insufficient [7].

Compliance affects cost and effectiveness of screening [8], but the consequences of the substandard compliance observed in our study are difficult to measure. The earlier a patient at risk (i.e. a pregnant woman who has no immunity against toxoplasmosis) is identified, the more do they benefit from information on how to avoid infection. Consequently, late testing should be associated with a higher incidence of maternal infections. However, this cannot be measured in the absence of a notification system. There is also uncertainty regarding the effectiveness of health education [9]. Having a late first test that is positive for anti-*Toxoplasma* IgG makes it more difficult for the biologist to determine whether the infection was acquired before or after the beginning of pregnancy. This uncertainty generates additional costs for complementary testing as well as anxiety for the future parents.

In the event of seroconversion, long intervals between tests prevent prompt treatment and should theoretically increase the number of infected children and severity of infection. A study done by the Systematic Review on Congenital Toxoplasmosis (SYROCOT) study group found weak evidence that treatment started within three weeks, compared to treatment started after eight weeks of seroconversion, reduces mother to child transmission, which indirectly suggests that compliance with monthly testing is important. However, the study failed to demonstrate the preventive effect of antenatal treatment on clinical manifestations of congenital infection [10]. Compliance will have to be taken into account in any controlled studies conducted on the benefit of antenatal treatment, as well as in any "real life" applications of their findings.

Several studies will be necessary to understand the reasons for the insufficient testing observed in this study. They will have to take into account the use of other prenatal care programmes and additional socio-demographic and economic variables. The role of insufficient patient knowledge on *Toxoplasma* infection and on its consequences for the foetus should also be investigated. Previous data on primary prevention of toxoplasmosis suggested that French women at risk tend to neglect precautions regarding food and hygiene [11-12]. Linking the number and timing of *Toxoplasma* tests with the patients' daily efforts to avoid infection could help us understand if, or how, both types of prevention interact.

Meanwhile, our study provides several possible directions for improving preventive programmes, particularly those that require patients to make appointments for repetitive examinations. These efforts should ideally be directed towards all actors involved.

Two factors were associated with patients. Receiving free medical coverage was independently associated with a late first test, and with fewer tests overall and longer between-test intervals, indicating continued insufficient access to the health care system or a persistent lack of awareness regarding screening, already widely reported for instance in the 2003 French National Perinatal Survey [5]. Younger patients were also more likely to have a late first test which possibly reflects a lower awareness of standard care offered during pregnancy and a higher proportion of unwanted or belatedly recognised pregnancies. Interestingly, age did not affect the overall number of tests or their regularity, suggesting that factors responsible for the delayed first test were somehow overcome.

Efforts should be made to reach out towards patients who have the least access to information, in order to inform them of the measures to be taken in pregnancy in terms of hygiene and legal and administrative requirements. This information should ideally be given before conception [13-15]. Information on how to avoid *Toxoplasma* infection could be cost-effectively added to messages on other health issues related to young adults (i.e. use of alcohol and drugs, sexually transmitted diseases). Any message promoting an early first serological test would indirectly be a benefit for other areas of antenatal care. Subsequent reminders that testing for toxoplasmosis should be extended to the date of delivery could also be used to convey other information on second or third trimester issues, such as breastfeeding.

Secondly, actions need to be tailored to those who prescribe the tests. In our study, first tests were performed earlier when prescribed by a GP, but subsequent tests were more regular when prescribed by an obstetrician-gynaecologist. As these findings contrast with previous evidence [12-13], further studies are necessary and will need to take into account the adherence of physicians and midwives to recommendations for toxoplasmosis screening, as well as their sex, location and social context, which have been found to play a role in relation to health education and prevention [16-17]. Meanwhile, there is a need to remind GPs, obstetricians and registered midwives of their complementary roles [18]. The biologists performing the tests should also be encouraged to become involved and explain the importance of regular testing to professionals and patients.

The re-use of prescriptions had a positive impact on compliance. The principle of a single prescription covering the entire duration of pregnancy could be promoted as an easy measure. This could even be extended to other biological tests, appointments for medical visits or ultrasound examinations.

Interestingly, the French antenatal prevention programme for toxoplasmosis illustrates well the long-term natural limitations of a programme not supported by a specific campaign. Potential decisions to reinforce it will need to be associated with measures to monitor their effectiveness, and necessary corrections will need to be introduced promptly. However, before taking steps to increase compliance, it is necessary to address the uncertainty surrounding the impact of preventive measures for congenital toxoplasmosis.

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Review articles

EPIDEMIOLOGICAL IMPACT AND DISEASE BURDEN OF CONGENITAL CYTOMEGALOVIRUS INFECTION IN EUROPE

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In Europe, congenital cytomegalovirus (CMV) infection is the leading cause of neurological disabilities in children, causing severe sequelae such as sensorineural hearing loss, neurodevelopmental delay or blindness. The infection causes high disease burden and costs. Nevertheless, there is little awareness of CMV among medical officials and the general public. Although the individual risk of congenital CMV infection is greatest from a primary infection of the mother during pregnancy, maternal non-primary infections also account for a substantial disease burden associated with congenital CMV. Screening programmes for pregnant women and newborns are widely discussed, but have not been implemented by any public health authority in Europe so far. This article gives an overview about a variety of European and other relevant studies regarding CMV seroprevalence, congenital CMV infection and disease as well as screening strategies and preventive approaches.

Primary and non-primary maternal cytomegalovirus infection

Cytomegalovirus (CMV) is a beta-herpesvirus member of the family *Herpesviridae*. The virus spreads via excretion in nearly all body fluids, such as urine, saliva, vaginal secretions, semen or breast milk. Especially infants and toddlers shed high amounts of virus for months or even years and represent a substantial risk for transmitting the virus to pregnant women by saliva or urine [1]. Sexual transmission of the virus is a common way of infection in adults.

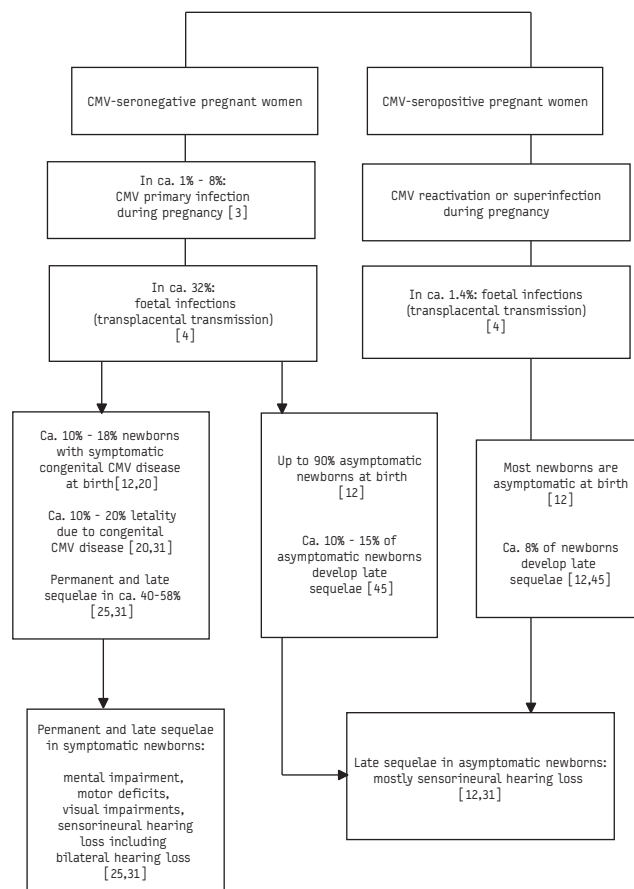
Because the infection in adult immunocompetent individuals is mostly mild or asymptomatic [2], primary CMV infection is rarely diagnosed during pregnancy. The risk of seronegative women to contract primary CMV infection during pregnancy has been reported to be between 1% and 8% [3,4] (see Figure). A force of CMV infection of ca. 0.03 per seronegative women per annum has been found in a British study by Griffiths *et al.* [5].

Viral transmission at the uterine-placental interface can result in congenital CMV infection [6,7] of the foetus or embryo, which can cause congenital CMV disease and permanent sequelae. The risk of CMV disease from intrauterine infection is highest in primary maternal infection. However, in non-primary maternal infections, which results from reactivation of latent CMV genomes or superinfection with new virus strains [8], permanent neurological disabilities or even death of the foetus have been observed [9-11]. In non-primary infection the foetus is thought to be partially protected by maternal immunity and transplacental transmission of immune IgG [12,13].

Multiple studies have determined the rate of vertical transmission in primary and non-primary maternal CMV infection and the development of subsequent CMV disease of the child [9,14-16]. The results of the studies are hampered by difficulties to distinguish between primary and non-primary maternal CMV infection. A metaanalysis by Kenneson *et al.* revealed a transmission rate of 32% in primary maternal infection and a transmission rate of

FIGURE

Frequency of maternal and foetal cytomegalovirus infection and morbidity of infected children



CMV: cytomegalovirus

1.4% in recurrent maternal infection [4]. The Figure shows the frequency of maternal and foetal CMV infection and morbidity of infected children.

Foetal CMV infection and the progression to congenital disease in children

The gold-standard method for prenatal diagnosis of foetal CMV infection is the detection of CMV in amniotic fluid by virus culture or PCR, which is as accurate as and even more sensitive than viral culture [17,18]. False negative results can occur when the test is performed too early after foetal infection, before the foetus sheds virus via the urine [17]. According to the European Congenital Cytomegalovirus Initiative (ECCI), the sensitivity of PCR used to detect viral DNA is very good if amniotic fluid is collected at least six weeks after seroconversion and around the 22nd week of pregnancy [3].

Diagnosis of congenital CMV infection does not necessarily predict later development of congenital CMV disease [19]. Systematic ultrasound is not sensitive enough to detect signs of foetal CMV disease, and most CMV complications can be observed only in the last trimester of pregnancy [3], when interruption of pregnancy is not legally possible in most European countries. Congenital CMV infection during the first trimester is more likely to cause CMV disease, since organogenesis takes place in this period [20,21].

CMV-damage in the foetus may cause spontaneous abortion or prematurity. Cases of congenital CMV syndrome present with an involvement of multiple organs including splenomegaly, hepatomegaly, prolonged neonatal jaundice, pneumonitis, thrombocytopenia, growth retardation, microcephaly and cerebral calcifications. Organ damage is thought to be caused by CMV replication in target organs like the central nervous system of the foetus and indirectly by CMV-induced placental dysfunction [19]. Permanent impairments mostly affect the central nervous system and include progressive hearing loss, spastic tetraplegia, mental retardation and visual impairments [21]. Nearly 14% of children with congenital CMV infection suffer from sensorineural hearing loss (SNHL), and 3-5% of children with congenital CMV infection suffer from bilateral moderate to profound SNHL [22]. About 15-20% of children with moderate to profound permanent bilateral hearing loss were associated with CMV infection, according to a publication by Grosse *et al.* [22].

The majority of congenitally infected children appear asymptomatic at birth, but neurological sequelae may develop after months or even years [23]. Fowler *et al.* report that after a mean follow-up of 4.7 years, 25% of children of mothers with primary CMV infection during pregnancy and 8% of children of mothers with recurrent CMV infection exhibit one or more sequelae [12]. Especially hearing loss may often not be present in the period immediately after birth [24,25]. In a longitudinal study by Dahle *et al.*, 7.4% of 651 children with asymptomatic CMV infection developed SNHL, compared to 40.7% of 85 children born with symptomatic CMV infection [25]. The development of late sequelae accounts for substantial disease burden associated with congenital CMV infection. According to Caroppo *et al.* the costs for prosthesis per child with SNHL that accrued for the Italian public health system in 2005 add up to 260,000 Euro [26].

Although there is evidence for mental retardation in symptomatic children congenitally infected with CMV, the intellectual development of the much larger group of asymptomatic CMV-infected children does not seem to be impaired [27]. A Swedish study failed to detect evidence for intellectual impairment at the age of seven years in a group of children with congenital CMV infection who had shown normal neurological development at the age of 12 months [27].

Seroprevalence of CMV and prevalence of CMV infection at birth in Europe

Prevalence in the mother

The prevalence of CMV infection at birth is related to the CMV seroprevalence in women of childbearing age, with a reported increase of 10% in maternal seroprevalence corresponding to a 0.26% increase in CMV birth prevalence [4]. Multiple studies have shown that the overall CMV seroprevalence in women of childbearing age depends on age, parity, ethnicity and social status, and differs between countries and regions [28,29,30]. A low socioeconomic status is a risk factor for CMV seroprevalence and congenital CMV infection [31,32]. The Table lists studies from several European countries, indicating factors that were found to influence CMV seroprevalence.

A Finnish study showed that the CMV seroprevalence was higher in Helsinki compared to a rural area in the southwest of the country (70.7% versus 56.3%, respectively) [33]. Often, the seroprevalence in immigrants differed from that of the native population: In a study in Ireland, a low seroprevalence of 30.4% was detected in 670 Irish women, whereas 359 non-Irish women living in Ireland showed a CMV seroprevalence of 89.7% [36]. The overall CMV seropositivity can also change over time. In Spain, 66.3% of 2,136 women were found to be seropositive for CMV in 1993, compared to 57.4% of 2,198 women in 1999 [37,38]. Between 1993 and 1999, the decrease in CMV seroprevalence has been significant in the age group of 31-41 year-olds in this study [37,38]. In pregnant women in Turkey, very high seroprevalences of up to 94.9% were reported [40,41]. In most European countries, a high socioeconomic status seemed to correlate with low CMV seroprevalence. The IgG antibody prevalence against CMV among pregnant women in Germany was highest among welfare recipients (93%), followed by those covered by statutory health insurance (56.2%), but was only 31.8% in the group of women with private health insurance [35].

Prevalence in the newborn

The prevalence of CMV infection in the newborn at birth depends on diagnostic criteria and the laboratory detection methods used. Some publications define CMV infection on the basis of a positive virus culture in urine or saliva [9,30,42]. In other studies, positive results of PCR assays are used for diagnosis of CMV infection at birth [16]. The sensitivity of CMV-IgM testing in the newborn as basis for birth prevalence estimates is about 25% and can not be recommended [4]. Diagnosis of CMV infection should be performed within two weeks after birth, since later diagnosis does not allow differentiation between congenital and sub- or postpartal CMV infection.

In a Dutch study, CMV infection was diagnosed by positive CMV PCR from throat samples or by CMV culture from urine samples. 7,793 newborns were tested, and the prevalence of CMV infection at birth was 0.9 per 1,000 newborns. None of seven congenitally infected children in this study showed any sequelae in a follow-up

period of 24 months [30]. However, a differentiation between primary and non-primary infection in the mothers of congenitally infected children was only available for two mothers, who suffered from a recurrent CMV infection during pregnancy. The overall CMV seroprevalence of mothers in this study was 41% [30].

A large Swedish study revealed 0.5% congenitally CMV-infected newborns by virus isolation testing. A total of 16,474 newborns were tested, and 29% of the infected children showed transient neonatal symptoms, whereas 18% of the infected children presented with neurological symptoms at the age of seven years [9].

In an Italian study, isolation of CMV from saliva led to diagnosis of congenital CMV infection [42]. Newborns were subdivided in two groups, a group of 185 children with suspected congenital CMV infection and a control group of 1,286 asymptomatic children. In the control group, overall prevalence of CMV in saliva was 0.47%, compared to 5% in the group of children with suspected CMV infection. Two of 15 neonates with congenital CMV infection developed sequelae in the two-year follow-up period and one further neonate died [42]. A meta-analysis by Kenneson *et al.* including 27 studies reported a birth prevalence of congenital CMV of 0.64% (95% confidence interval (CI): 0.60-0.69%) [4]. A further metaanalysis by Dollard *et al.* revealed a birth prevalence of 0.7% and a percentage of 12.7% symptomatic children at birth [31].

In an early African study from 1978, Schopfer *et al.* reported that 1.4 % of 2,032 newborns in Côte d'Ivoire had CMV viraemia,

when screened by viral culture [14]. Two studies recently performed in Gambia (West Africa), which defined CMV infection at birth on the basis of a sensitive nested PCR detection method and screening of urine samples within two weeks after birth, found prevalences of 5.4% and 3.9% [16,43]. Congenital CMV infection was associated with active placental malaria infection [16]. The prevalences of congenital CMV were higher in these studies compared to birth prevalences in industrialised countries [16,43]. Although these African studies may not be directly relevant for European societies in general, it is of interest that in populations with a presumably very high seroprevalence of CMV, about 1.4-5% of infants are shedding CMV at birth due to non-primary maternal infection. A considerable proportion of these children may develop late sequelae and thus contribute to the disease burden of congenital CMV infection. It is therefore important to consider vertical transmission of CMV due to non-primary maternal infection, and similar infection rates may be possible in immigrant communities living in Europe who originated in high-prevalence countries.

Prevention and treatment strategies against congenital CMV infection

Prevention strategies are classified as primary, secondary and tertiary prevention. Primary prevention strategies try to avoid an infection and are mostly accomplished by precautions against exposition to the virus, i.e. hygiene measures and change of behaviour. Secondary prevention strategies allow identifying infected patients at an early stage, with the aim of stopping progression of infection and disease. In the case of symptomatic disease, tertiary

TABLE

Seroprevalence of cytomegalovirus infection in different European countries and influential factors

Country and region	Study	Seroprevalence	Number of study participants	Factors influencing seroprevalence
Finland, Helsinki	[29]	70.7%	1,088 pregnant women	Social environment, low impact of age
Finland, southwestern (rural) Finland	[33]	56.3%	558 parturient women	Parity
France	[34]	51.5%	1,018 pregnant women	Age, parity, place of birth (seroprevalence increasing from north to south)
Germany	[2]	64.4%	9,870 men and women (aged 1 to > 60 years)	Age
Germany	[35]	43.3% in pregnant women with testing initiated by gynaecologist; 47.5% in randomly selected pregnant women	11,572 pregnant women with testing initiated by gynaecologist; 1,033 randomly selected pregnant women	-
Ireland	[36]	30.4% in Irish women 89.7% in non-Irish women	670 Irish woman 359 non-Irish women	Immigration
The Netherlands	[30]	41%	7,524 pregnant women (aged 16-47 years)	Ethnicity, socio-economic status, metropolitan area (connected to ethnicity)
Spain	[37]	1993: 66.3% 1999: 57.4%	1993: 2,136 women 1999: 2,198 women (aged two to 60 years)	Age
Spain	[38]	1993-1994: 62.8%	2,030 men and women (aged two to 60 years)	Age
Sweden, southern Stockholm	[39]	72 %	1000 pregnant women	-
Turkey, South	[40]	94.9%	1,652 pregnant women	-
Turkey, West	[41]	96.4%	1,972 pregnant women	-
United Kingdom, London	[28]	45.9% in white women 88.2% in Asian women 77.2% in black women	20,000 women	Ethnic group, parity, age, social class

prevention strategies try to prevent the development of severe sequelae after infection. Prenatal primary and secondary screening strategies as well as postnatal secondary and tertiary screening strategies are widely discussed for congenital CMV disease, but have not yet been implemented by any European country [44,45]. The implementation of screening programmes is hampered by obstacles such as lack of awareness, financial costs and possible deficits in the availability of detection methods.

Prenatal prevention

As a strategy for primary prevention, all pregnant women should be provided with information about the risk of CMV infection and the possible consequences an infection can have for the child. According to a study in the United States (US), not many women are well informed about the risk of CMV infection and congenital CMV disease. Of 643 women surveyed, only 22% had heard of congenital CMV and among a list of common causes of birth defects, women were least aware of congenital CMV [46]. In a national mail survey of the US population, only 14% of female respondents had heard of CMV [47]. Pregnant women, especially those who work with children, should be educated about behaviours that are associated with a high risk of CMV transmission [48].

Close contact with young children is a particular risk factor for CMV transmission, because infected children shed high concentrations of the virus over a long period of time in urine and salivary secretions. In a recent molecular epidemiological study, children were identified as the source of infection for the majority of pregnant women with primary CMV infection [1]. Preventive hygienic measures such as handwashing and avoiding direct contact with potentially contaminated body fluids, are likely to be effective to prevent CMV seroconversion in pregnant women when dealing with infants or toddlers [49]. Nevertheless, unambiguous results from intervention studies showing reduced rates of congenital infections are still lacking.

Another important route of CMV infection in adults is sexual transmission of the virus. A recent onset of sexual activity has been identified as an independent risk factor for congenital CMV infection in the offspring of young women [50]. However, precise data on the relative risk of CMV transmission during pregnancy by a serodiscordant partner are not yet available.

A safe and effective CMV vaccine for seronegative women is not available so far and remains a major public health priority in countries with a high proportion of seronegative women of childbearing age [51,52].

Prenatal screening

Different secondary prenatal screening strategies exist that rely on early detection of primary CMV infection in pregnant women. Most prenatal strategies are based on serological testing during pregnancy. Primary CMV infection may not be diagnosed on clinical grounds, since symptoms such as fever or flu-like symptoms are often mild or misinterpreted, which makes it important to do serological tests for definitive diagnosis. Evidence for primary infection is based on seroconversion of the mother during pregnancy and the detection of low avidity anti-CMV-IgG antibodies which indicate a recent primary immune response.

In a study in Belgium, Naessens *et al.* used a serologic strategy based on testing for CMV-specific antibodies during the first prenatal visit and at birth. This approach identified 82% of newborns at risk for congenital infection and neurosensory sequelae

[53]. Another screening strategy includes testing of maternal CMV antibodies at the beginning of pregnancy and at 20-22 weeks gestation to demonstrate seroconversion in pregnant women with primary infection. Screening during the first trimester allows to determine the approximate date of primary infection by using CMV-IgG avidity tests [3].

In a pilot study undertaken in several Italian regions, routine screening used CMV avidity testing following positive detection of CMV-IgM to detect primary CMV infections. A low avidity of CMV-IgG antibodies suggested a recently acquired primary CMV infection [54]. Nevertheless, positive CMV-IgG testing and the presence of high avidity IgG antibodies do not exclude the possibility of congenital CMV infection of the unborn, since non-primary infection during pregnancy and CMV transmission to the foetus can occur. The serologic screening models may therefore not be appropriate for all pregnant women, especially in populations with high seroprevalence for CMV as seen in some European countries.

Prenatal management and treatment

The management of the pregnancy in cases of primary CMV infection is a matter of debate [23]. Suspected foetal CMV infection most often results in amniocentesis, an invasive test that causes spontaneous miscarriages in about 1% of the cases [44]. The danger of amniocentesis for the foetus needs to be taken into consideration when planning strategies for prenatal diagnosis [44]. When a foetal CMV infection is diagnosed, a decision for elective termination of pregnancy is possible, but difficult because a majority of infected foetuses remain unaffected, i.e. asymptomatic after birth [19]. Diagnosis of CMV infection in the unborn will severely worry most women, and obstetricians might not be able to refuse the request of pregnancy terminations due to the inability of excluding all possible severe sequelae [3].

At present, there is no recommended treatment for pregnant women with CMV infection. The effect of passive immunisation on prevention of congenital CMV infection in clinical trials has been investigated by Nigro *et al.* [55,56]. In a non-randomised prospective study, pregnant women with primary CMV infection received a preparation of human hyperimmune IgG against CMV (Cytotect®). Cytotect® infusion was reported to be associated with a significantly lower risk of congenital CMV infection and disease at birth [55]. These findings remain controversial as the study was lacking a strict randomised protocol [57,58]. The site of action of CMV hyperimmunoglobulin is presumably the placenta, as manifestations of congenital CMV at birth are probably caused in part by virus replication in placental tissue, leading to placental insufficiency [6,7,59].

Nigro *et al.* further reported a regression of foetal CMV-associated cerebral abnormalities following therapy with Cytotect® in individual cases [56]. The sensorial, mental and motor development of these children was normal when evaluated at the age of three to seven years [56]. However, a publication bias favouring those cases in which hyperimmunoglobuline treatment had a protective effect cannot be excluded. Independent controlled studies are needed to evaluate the safety, effectiveness and cost-effectiveness of passive immunisation in women with primary CMV infection during pregnancy. Possible side effects of CMV immune globulin are mainly anaphylactic reactions [51].

Postnatal screening

Screening of all newborns for CMV infection is a postnatal tertiary screening approach. Universal hearing screening at birth by use of otoacoustic emission (OAE) is offered in most European countries and detects symptomatic hearing impairment at birth. However, more than two thirds of cases of hearing loss among children congenitally infected with CMV develop only months or years after birth and may therefore be missed by a hearing screening at birth [3,24]. Screening of all newborns for CMV shedding in the urine and monitoring of all congenitally CMV infected newborns in long-term audiologic follow-ups could improve the identification of children with progressive hearing loss which can become evident as late as at the age of five years or even later [24,60]. Early diagnosis and intervention such as speech therapy, sound amplification or cochlear implants are essential to improve the disease outcome in children with hearing loss. Newborns infected with CMV could also benefit from ophthalmological assessment and neuroimaging for documentation of central nervous system (CNS) disease in the neonatal period [48]. Postnatal screening strategies would allow the identification of risk factors for the development of severe sequelae and an assessment of the disease burden of congenital CMV disease.

The gold-standard to detect congenital CMV infection at birth is viral culture or PCR within the first two weeks of life from urine or saliva. Barbi *et al.* have implemented a nested-PCR test from neonatal dried blood spots on Guthrie cards as a convenient possibility for screening [42,61]. Most importantly, only this approach allows diagnosis of congenital CMV infection retrospectively. For this purpose, storage of Guthrie cards for a minimum of five years must be assured.

Postnatal treatment

Ganciclovir treatment of symptomatic newborns has been evaluated in several studies [62-65]. Kimberlin *et al.* investigated in a randomised controlled study the effect of a six-week therapy with intravenous ganciclovir in under 30 days-old neonates with symptomatic CMV disease involving the CNS [65]. At a follow-up hearing examination at the age of six months, 84% of the babies treated with ganciclovir had improved their hearing or maintained normal hearing between study entry and the age of six months, compared to 59% of controls. At the age of one year, the hearing had deteriorated in 21% of the treated children between study entry and the age of one year, compared to 68% in the control group [65]. According to Kimberlin *et al.* Ganciclovir therapy begun in the neonatal period in children with symptomatic CMV infection involving the CNS prevents hearing deterioration in the first six months of life and may prevent hearing deterioration in the first year of life [65]. Ganciclovir is toxic to the bone marrow, and two thirds of the treated infants in the study by Kimberlin *et al.* suffered from side effects such as significant neutropenia [65]. Recent studies in neonates with symptomatic congenital CMV infection reported that comparable plasma concentrations can be reached by oral administration of valganciclovir and intravenous administration of ganciclovir [66,67]. ECCI currently recommends the use of 6mg/kg intravenous ganciclovir twice daily for six weeks in babies born with CNS involvement and proven congenital CMV infection.

Disease burden and public health aspects

Based on the available data, congenital CMV infection is of major public health significance. Criteria for the prioritisation of infectious diseases in public health have been proposed, such as burden of disease, epidemiological dynamics, information need and health

gain opportunity [68]. Despite the fact that considerable knowledge gaps still exist to date, CMV has been added to a list of infectious pathogens selected for further evaluation of prioritisation [68], particularly in the context of congenital disease.

CMV infection is the leading non-genetic cause of hearing impairment in children. In France, it has been estimated that a number of 480 infants per year experience severe sequelae and a number of approximately 675 infants per year present with hearing loss due to congenital CMV infection [44]. Around 8,000 children with neurological sequelae related to congenital CMV infection per year have been reported in the US [69].

The disease burden of congenital CMV infection is high and similar to that for congenital rubella before the introduction of rubella vaccination [52]. Since congenital CMV affects the very young, it results in long-term morbidity. In the 1990s, the estimated costs associated with CMV disease for the US health care system amounted to at least 1.86 billion US dollars annually, with more than 300,000 US dollars per child [52]. To assess the socio-economic costs of congenital CMV infection and its impact expressed as quality-adjusted life-years in Europe, complete epidemiological knowledge of the prevalence of this disease is mandatory. Further research on preventive measures, therapeutic options and screening methods for congenital CMV infection and subsequent health impairment are worthwhile. The availability of evidence-based preventive and therapeutic options should predetermine the implementation of general screening programmes for congenital CMV infection in European countries.

Given the low awareness of the infection in the general public, the need for information on congenital CMV infection is great. Up-to-date information about congenital CMV infection for both healthcare professionals and the public are provided by ECCI. The ECCI provides recommendations by international and European virologists, epidemiologists, immunologists, obstetricians and paediatricians whose aim is to promote awareness of congenital CMV and support research initiatives into this important infection.

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Review articles

DRUG USE AND PREGNANCY – CHALLENGES FOR PUBLIC HEALTH

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Problem drug use in pregnancy affects a sizeable population in Europe. A literature review was carried out of articles in PubMed, European Monitoring Centre for Drugs and Drug Addiction publications, and related documents in order to assess public health challenges and possible intervention strategies related to problem drug use and pregnancy in Europe. It revealed the following: Involving pregnant drug users in drug treatment is likely to decrease the chances of pre- and perinatal complications related to drug use and to increase access to prenatal care. Timely medical intervention can effectively prevent vertical transmission of human immunodeficiency virus, hepatitis B virus as well as certain other sexually transmitted diseases, and would allow newborns infected with hepatitis C virus during birth to receive immediate treatment. Pregnancy may be a unique opportunity to also help women with dual diagnosis (substance use combined with mental illness) and enrol them into special treatment and support programmes. Issues related to homelessness and intimate partner violence can also be addressed with appropriate interventions. Treatment and care for pregnant drug users should offer coordinated interventions in several areas: drug use, infectious diseases, mental health, personal and social welfare, and gynaecological/obstetric care.

Background

Problem drug use (defined by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) as “injecting drug use or long duration/regular use of opioids, cocaine and/or amphetamines” [1]) and pregnancy affect a sizeable population in Europe. It is estimated that there are about 1.3-1.7 million problem opioid users in the European Union and Norway [2]. Furthermore, approximately 20% of drug users entering drug treatment and around 34% of opioid users are women (the great majority of whom are of childbearing age) [3], and every year, as many as 6.5-11% of female problem drug users may get pregnant or give birth [4,5]. This suggests that each year there may be as many as 30.000 pregnant women using opioids in Europe, and the number of pregnant women using drugs other than opioids may be equally high. The issue of pregnancy and drug use is important to address because of the associated personal and public health challenges regarding both the mother and the unborn child, especially regarding infections that are common among drug-using populations. In this article, we review some of these challenges: infection with blood-borne and sexually transmitted diseases (STIs, including infections with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), dual diagnosis (substance use combined with mental illness), social and personal welfare and drug treatment,; and assess and summarise possible solutions to alleviate these challenges.

Methods

A literature review of articles in PubMed published in or after 1990 was conducted using the keywords “pregnancy” and “drug use” / “substance abuse”, and specific keywords for each area of interest (e.g. “dual diagnosis”, “homeless” etc.). Articles discussing pregnancy and tobacco or alcohol use without the mention of other drugs were not considered. In addition, when articles were found that were especially relevant to this review, the “Related Articles for PubMed” links were also investigated. Furthermore, EMCDDA publications (annual reports, selected issues, statistical bulletin) with relevant information were included. When the original publication referenced other, non-PubMed or non-EMCDDA publications, those references were also included in this review. While our focus was on pregnant drug users in Europe, some non-European references were included when found relevant. In this paper, we use the terms “drug use” to refer to problem use of drugs other than alcohol or tobacco, and “pregnant drug users” to refer to pregnant women with problem use of drugs other than alcohol or tobacco.

Pregnancy complications linked to drug use

Continued drug use during pregnancy may lead to complications for the foetus, for the newborn, and later during childhood [6,7]. Complications for the foetus include spontaneous abortion, restricted foetal growth, incorrect maternal placentation, compromised foetal well-being and pre-term delivery. The newborn can be affected by low birth weight, postnatal growth deficiency, microcephaly, neurobehavioral problems and drug withdrawal syndrome [8,9]. In addition, behavioural and cognitive problems may arise later in childhood, and children may be affected by the mother’s ongoing drug use [7].

Drug treatment

Lack of appropriate obstetric and neonatal care has been associated with obstetric complications and with poor pregnancy outcomes among drug users [9-12]. Treatment of drug dependence of pregnant drug users therefore involves not only a stabilisation of their health and social situation as drug users, but also offers an opportunity for regular contact with health services, including standard pre-natal care [13]. It is thus important to improve pregnant drug users’ access to, and retention in, drug treatment. Since the 1970s, methadone maintenance has been recommended for opioid dependence in pregnancy [14], although some studies have shown that buprenorphine may offer an advantage over methadone with regard to lower intensity of neonatal abstinence syndrome [15-17]. New guidelines from the World Health Organization (WHO)

confirm the recommendation of agonist maintenance treatment for pregnant opioid users on the basis of the risks and poor outcomes associated with withdrawals [18]. However, the possibility of drug-drug interactions should be kept in mind, and dose adjustments of substitution treatment may be necessary in different stages of the pregnancy [19]. A recent systematic review of psychosocial interventions suggested that contingency management strategies are effective in improving retention of pregnant drug users in outpatient treatment, but failed to assess any effects on obstetrical and neonatal outcomes [20]. Evidence on the effects of home visits by nurses, counsellors or midwives to women with a drug problem is currently insufficient [21]. However, several decades of clinical management of pregnant drug users point to a need to consider the life circumstances of the individual women and apply a case management approach [9,10,13,14,17,19,22].

Infectious diseases

Certain infectious diseases such as HIV, HBV, hepatitis C virus (HCV), and some other STIs, are more common among illicit drug users (especially those who inject) than among the rest of the population, and their early detection is essential to reduce the risk of vertical transmission [2,3]. The prevalence of infectious diseases is also high among pregnant women who use illicit drugs [23]. For example, in a sample of 259 pregnant women enrolled in drug treatment in France in 1998, 63,3% were infected with HCV, 8,9% with HBV, 6,2% with HIV and 1,5% with syphilis [24,25]. While policies vary across countries, standard antenatal care in most European countries today include voluntary screening for infections, which can include HIV, HCV, HBV, syphilis, and STIs such as chlamydia infection, in order to provide early diagnosis and appropriate treatment for the mother and to reduce the risk of mother-to-child transmission [26]. Still, many pregnant drug users, especially those who have infectious diseases that are common among drug users (such as HIV or HCV), may receive suboptimal prenatal care due to difficulties accessing prenatal services [24,27,28]. This is worrying, since strong evidence supports the importance of early diagnosis and the effectiveness of interventions aimed at HIV infected pregnant women, with the reduction of vertical transmission rates to under 1% [29,30].

The risk of vertical transmission of HCV during birth is highly variable depending on HCV RNA viraemia and HIV co-infection: It is below 10% in HIV-negative study populations (1-3% among HCV RNA-negative women and 4-6% among HCV RNA-positive women) and up to 41% in study populations in which about half of the women were also infected with HIV [31-35]. Co-infection with HCV and HIV is also associated with an increased risk of vertical HIV transmission [36,37]. In contrast to preventing HIV infection of the child, no safe and effective prevention method exists to prevent perinatal transmission of HCV [31,34,38]. As no viral RNA is present in the breast milk or colostrum of infected mothers, there is no evidence of transmission of HCV through breastfeeding [32,33]. However, HCV viraemia has been found to be associated with active injection drug use among HIV-HCV co-infected female drug users, perhaps due to re-infection or reactivation of HCV [39]. HCV transmission does not occur through breastfeeding but only during pregnancy or birth. The likelihood of transmission increases with the viral load, which is higher during active injecting drug use. Preventing, reducing or stopping injecting (e.g. through opioid substitution therapy) may therefore be a way to reduce the probability of vertical HCV transmission. In addition, antiviral therapy is indicated for HIV-HCV co-infected women past

the first trimester in order to reduce the risk of both HIV and HCV transmission [40].

Infection with HBV is also common among drug users [2]. The current recommendation to prevent the transmission of HBV from mother to child is to administer to the newborn a combination of anti-HBV immunoglobulin followed by three doses of HBV vaccine [41,42]. WHO recommends the global implementation of childhood hepatitis B vaccination [43]. Still, many European countries (Denmark, Finland, Iceland, Ireland, the Netherlands, Norway, Sweden, and the UK) provide immunisation only for at-risk populations, a practice that is debated due to the difficulty of identifying all at-risk individuals [43,44]. Other STIs are also common among pregnant drug users [45]. As bacterial STIs (e.g. syphilis, gonorrhoea, chlamydia) can readily be treated with antibiotics, which also prevent vertical transmission [46,47], screening for STIs and treatment of those who are infected are recommended for pregnant drug users.

Psychiatric co-morbidity

Dual diagnosis, i.e. co-morbidity of substance abuse and mental illness, is common among both drug using and mentally ill populations [48]. In Europe, as many as 80% of clients enrolled in drug treatment report a mental health problem [2,49-52]. Psychiatric co-morbidity is complex because patients may suffer from more severe symptoms than people with only substance use or mental illness, they may not respond well to treatment, and, when in treatment, they may have higher rates of relapse and attrition [53,54]. While in the general population men report higher levels of drug use than women [55], women report higher rates of mental illnesses, especially depression and anxiety disorders [56]. However, levels of psychiatric co-morbidity among substance users seem to be similar in both sexes [57]. Little is known about pregnant women with a dual diagnosis. In a study in France, 22% of pregnant drug users in substitution treatment for opioid use reported moderate to severe psychiatric disorders, mostly depression, neuroticism and anxiety disorders [25]. Pregnant women suffering from psychiatric co-morbidity often report a history of emotional, physical and sexual abuse as well [57,58]. Pregnancy may be an opportunity of contact with care services for both conditions of co-morbidity. However, the fear of losing the custody of the child and the feeling of guilt about using drugs during pregnancy may often pose a barrier to seeking treatment [57]. Interventions among pregnant women with psychiatric co-morbidity should target the three problematic areas (mental health, drug related problems and pregnancy) in a co-ordinated and integrated way, taking into account the individual needs of these women [10,19,59].

Social and personal welfare

Issues related to the social and personal welfare of pregnant drug users include, among other things, homelessness and intimate partner violence. Overall, about one in ten drug users entering treatment in Europe lives in unstable conditions or is homeless [3]. Homelessness and drug use in pregnant women are associated with problematic perinatal events [11,12], inadequate access to health care, social isolation, and psychosocial and physical problems [60]. Among female drug users, those who are homeless more often face difficulties obtaining public assistance, and are afflicted by greater social isolation, a lack of family and social networks, higher rates of emotional, physical and sexual abuse as well as under-nutrition, and they are more likely to engage in survival sex [60]. Some homeless female drug users may be able to discontinue the

use of those drugs on which they are not dependent, but they may maintain the use of their main drug (most often crack cocaine or heroin) [57]. Homeless pregnant drug users are less likely to seek drug treatment than domiciled pregnant drug users, and, when in treatment, they are less likely to maintain abstinence and are more likely to leave treatment prematurely [60].

Many women are victims of intimate partner violence [61,62]. When compared to women who have not experienced assault, pregnant women who have been assaulted were more likely to drink alcohol or use drugs [63,64]. In a perinatal substance abuse treatment clinic, many pregnant drug users reported being abused during their pregnancy: 41% reported emotional abuse, 20% physical abuse and 7% sexual abuse [65]. Abused pregnant drug users often report that emotional abuse is more disturbing than physical abuse, and many report being subject to both emotional and sexual abuse [64,65]. The abuser in most of the cases is the partner, ex-partner or someone closely related to the victim [65-67]. The risk of increasing drug or alcohol use increases after experiencing violence [63,65,67]. Intimate partner violence among pregnant drug users is responsible for health problems such as depression, post-traumatic stress disorder, chronic pain in different parts of the body (e.g. in the abdomen), gastrointestinal and gynaecological problems [63,65-67]. Clinics, including prenatal clinics and drug treatment centres, may be the most appropriate place for pregnant drug users to receive interventions in order to prevent recurring partner violence and abuse [62,68-70].

Conclusions

Pregnant drug users are at a higher risk than pregnant women who do not use drugs of contracting blood-borne and sexually transmitted infections. In addition, they are also affected by a number of physical, mental and social health problems. Services geared towards the general population need to cater to pregnant drug users as well. Special services for problem drug users should use outreach methods to timely identify pregnant drug users not in contact with services and ensure referral and collaboration with pregnancy care givers, using integrated case management strategies. Treatment and care for pregnant drug users should offer coordinated, multidisciplinary interventions encompassing several areas: prevention, screening and treatment of infectious diseases; mental health; personal and social welfare; gynaecological/obstetric care; and drug use [20,21]. The aim of such treatment and care is to reduce risk through the integrated collaboration of obstetricians, addiction counsellors, social workers, general practitioners, and other health care specialists [71], and to link drug treatment with other interventions aimed to help pregnant drug users. In addition, to prevent parental neglect that may be the consequence of drug abuse, adequate parenting support services should be made available and easily accessible to pregnant drug users.

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Rapid communications

SURVEILLANCE FOR HEPATITIS B VIRUS INFECTION IN PREGNANT WOMEN IN GREECE SHOWS HIGH RATES OF CHRONIC INFECTION AMONG IMMIGRANTS AND LOW VACCINATION-INDUCED PROTECTION RATES: PRELIMINARY RESULTS OF A SINGLE CENTER STUDY

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Epidemiological data on the prevalence of serological markers of hepatitis B virus (HBV) infection in pregnant women in Greece are limited. We evaluated the prevalence of HBV serological markers in a multinational population of pregnant women in Athens, Greece. The overall prevalence of hepatitis B surface antigen (HBsAg) was 4.1% with the highest rates among Albanian immigrants (12%). Relatively low vaccination-induced protection rates (32.5%) were observed, a finding suggesting that surveillance and immunisation programmes targeted at pregnant women are necessary.

Background

Worldwide, about 350 million people are chronically infected with hepatitis B virus (HBV). Vertical (mother-to-infant) transmission of the infection occurs usually in perinatal period and is responsible for the majority of the disease burden in endemic areas. The risk of vertical transmission generally depends on the level of maternal infectivity during pregnancy, i.e. the presence of hepatitis B e-antigen (HBeAg) or HBV DNA levels [1,2].

Hepatitis B has long been a serious public health problem in Greece. Historically, Greece used to have the highest burden of HBV infection in the European Union, and an early hepatitis B prevention programme introduced in 1982 and aimed at high-risk groups had had little impact on disease incidence or prevalence [3]. More recent HBV vaccination programmes, demographic and socioeconomic changes, safer medical and nursing practices and screening of blood donors have resulted in a significant decline in chronic HBV infection in our country in the past decade [3,4]. However, the arrival of a great number of refugees, especially from countries with endemic HBV infection, is likely to have influenced this trend, requiring a reevaluation of epidemiological data. To date, epidemiological data on the prevalence of serological markers of HBV infection in pregnant women in Greece have been limited [5].

HBV prevalence in pregnant women

In our study we examined the current prevalence of HBV serological markers in a multinational population of pregnant

women in Athens, Greece. Between September 2008 and December 2008 a total of 749 pregnant women (mean age 28.5 years) who gave birth at the Department of Obstetric and Gynaecology of the Maternal and Perinatal Hospital 'Elena Venizelou' of Athens were prospectively evaluated. Hepatitis B surface antigen (HBsAg), hepatitis B e-antigen (HBeAg), antibody to hepatitis B e-antigen (anti-HBe), antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs) were detected using routine commercially available enzyme immunoassays (Abbott Laboratories, Abbott Park, Illinois, US). All women in the study population were screened for HBsAg, anti-HBc and anti-HBs, whereas HBeAg and anti-HBe were evaluated only in those who tested positive for HBsAg [HBsAg(+)].

The study was performed in accordance with the Helsinki Declaration and was reviewed and approved by the Hospital Ethics Committee.

Almost half of the study population was originally from Greece (370/749, 49.4%), 29% came from Albania (217/749), 12.8% (96/749) from Eastern European countries (Russia, Romania, Bulgaria), 5.2% (39/749) from Asian countries (Philippines, India and China) and 3.6% (27/749) from African countries (Egypt, Nigeria, Kenya). The place of origin of each woman included in the study population was determined on the basis of her and/or her parents' birth place (in case of second generation immigration), according to the medical records data. The proportion of each group in the study population is presented in Figure 1. It is important to note the small proportion of women from Asia and Africa in our study population and that the majority of these came from countries with intermediate HBV prevalence.

Overall, 4.1% (31/749) of women were HBsAg(+) and the vast majority of them (26/31, 83.87%) were Albanian. The prevalence of HBV serological markers in the study population, according to the place of origin, is presented in Figure 2. Among Albanian women the prevalence of HBsAg was 12% followed by 2.1% among women from Eastern European countries. The prevalence of HBsAg

among women of Greek origin (0.8%) was very low and significantly lower in comparison with the mean value of the studied population ($p < 0.001$). It is important to note that none of the women from countries of Asia and Africa were HBsAg(+). A significant proportion of young women from Asia and Africa who live and work in Greece are second generation immigrants and the majority of them were born in our country, in contrast to Albanian or Eastern European women. Moreover, as previously noticed, the majority of Asian and African women of our study population were from countries with intermediate HBV prevalence. Both factors could explain the discrepancy between the levels of HBV serological markers among Asian/African and Albanian/Eastern European women, in our study.

Overall, only 1.4% of HBsAg(+) women were also HBeAg(+) whereas the vast majority (98.6%) were HBeAg(-)/antiHBe(+). Despite that, it is well known that a significant proportion of HBeAg(-) chronic HBV infected women in our country exhibit high levels of viremia during the perinatal period, especially due to precore mutation of the HBV genome [6]. More than half (57.1%) of the Albanian women exhibited anti-HBc seropositivity followed by Eastern European women (28.1%), Asian women (17.9%) and African women (11.1%) whereas only 5.1% of Greek women presented serological markers of previous HBV exposure. Moreover, serological markers of past HBV infection with spontaneous recovery [antiHBc(+) and antiHBs(+)] were observed in 15.2% of the whole study population whereas 32.5% exhibited vaccination-induced protection [characterised by the presence of isolated antiHBs(+)].

Importantly, vaccination-induced protection rates were relatively highest and comparable among Albanian and Greek women (40.3% vs 33.8% respectively, $p = 0.115$) whereas significantly lower rates were found among Eastern European (22.9%), Asian (15.4%) and African (11.1%) women ($p < 0.05$, in all comparisons).

Conclusion

In the study described in this paper the overall prevalence of HBsAg among pregnant women in Greece was estimated to be 4.1% with highest rates among Albanian immigrants (12%). The HBeAg(-)/antiHBe(+) serological status was observed in the vast majority of HBsAg(+) women in our study population. Relatively low vaccination-induced protection rates (32.5%) were observed, a finding suggesting that surveillance and immunisation programmes targeted at pregnant women are necessary in order to avoid vertical transmission of HBV infection.

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FIGURE 1

Study on prevalence of serological markers of hepatitis B virus infection among pregnant women in Greece - distribution of the study population by place of origin (n=749)

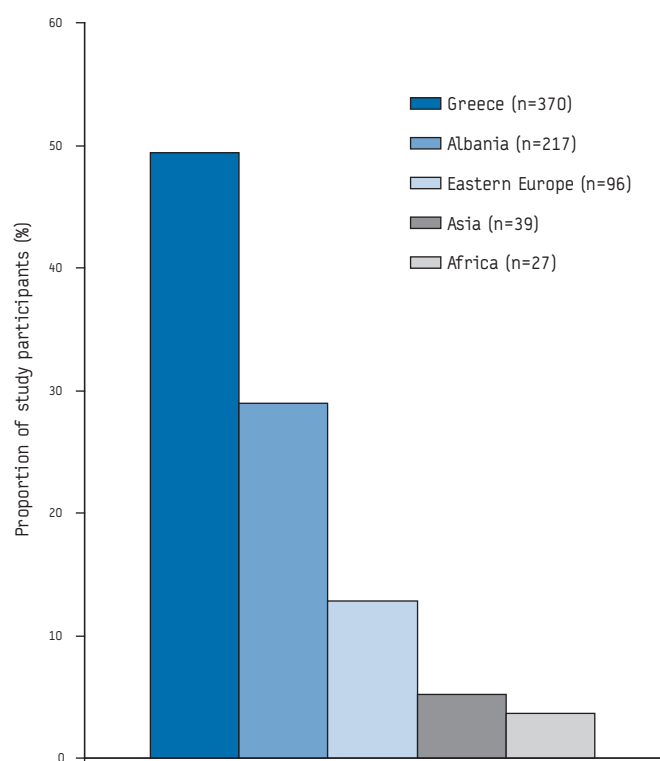
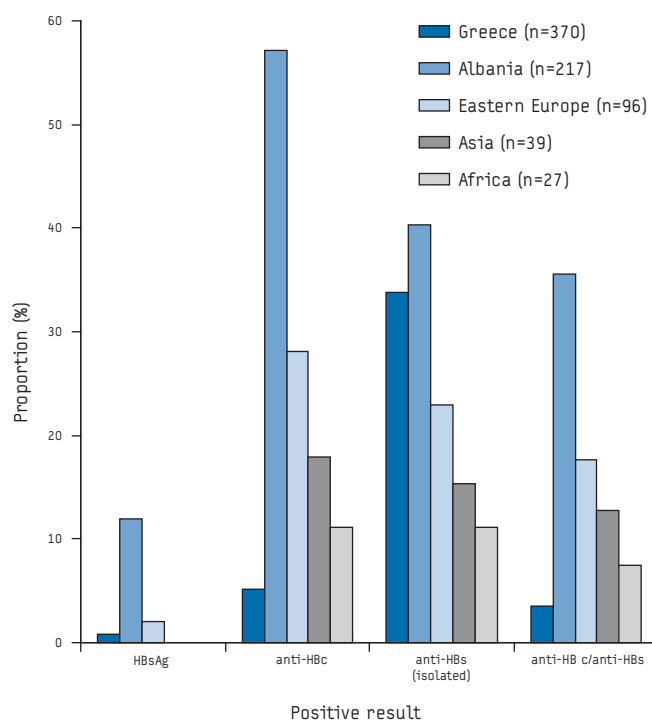


FIGURE 2

Prevalence of serological markers of hepatitis B virus infection among pregnant women in Greece, according to their place of origin (n=749)



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Meeting reports

THE 2008 CONGENITAL CYTOMEGALOVIRUS CONFERENCE, 5-7 NOVEMBER, CENTERS FOR DISEASE CONTROL AND PREVENTION, ATLANTA

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The theme of this conference was “public health action towards awareness, prevention, and treatment”. The purpose was to bring together researchers and clinicians from various fields to discuss the latest research on congenital cytomegalovirus (CMV) infection and how these findings can be translated into public health action for better health of women and children. In addition, families with children affected by congenital CMV participated in the conference, either in integrated sessions together with the experts or in separate sessions only for the families. These children were a testimony of the severe disabilities that congenital infections can cause.

More than 250 participants from all over the world attended the conference, which included about 50 oral presentations and 50 poster presentations. In this report the different topics of this conference will be briefly discussed, with a focus on disease burden and public health. Most presentations can be found at: <http://www.congenitalcmv.org/cmvsides2008.htm>

Epidemiology

Michael J. Cannon (United States (US) Centers for Disease Control and Prevention (CDC)), one of the organisers of this conference, described three areas of recent epidemiologic studies at the CDC.

- CMV awareness among women and obstetrician/gynaecologists,
- Seroprevalence data leading to an understanding of transmission modes on a population level,
- Studies on the overall burden of congenital CMV infection and disease and the particular burden due to permanent, bilateral hearing loss.

These studies are intended to identify women who are at high risk of giving birth to children with congenital CMV and likely to profit from antiviral treatment or other interventions, and to clarify what messages need to be communicated about congenital CMV prevention. Studies on the prevalence of CMV infections in general show that CMV seropositivity is highly determined by racial/ethnic factors and seropositivity of siblings and mother. CMV infections during pregnancy occur in about eight of 1,000 pregnancies. Most of these congenital CMV infections occur in previously seropositive mothers [1]. Between 17 and 20 % of these congenitally infected children will have permanent disabilities [2].

Karen B. Fowler (Department of Paediatrics, University of Alabama at Birmingham, US) showed that new developments in

diagnosing and treating CMV infections, as well as an emerging interest from the US Newborn Hearing Screening Community for the identification of CMV-related hearing loss, has resulted in a need to reconsider surveillance or screening programmes for congenital CMV infection.

Suzanne Luck (Royal Free and University College Medical School, London, United Kingdom (UK)) reported on a newly developed CMV-related treatment registry of pregnant women and infants in the UK that is currently being extended to the rest of the European Union (EU).

Postnatal treatment and follow-up

The benefits and risks of current antiviral treatments for children with congenital CMV were presented by David Kimberlin (University of Alabama at Birmingham, US). Data on the treatment of congenital CMV are only available for babies that are born symptomatic. In this group, administration of intravenous ganciclovir for six weeks protected against hearing deterioration. Recently, it has been demonstrated that administration of an oral solution of valganciclovir resulted in similar blood concentrations of ganciclovir as intravenous administration of ganciclovir. A new multicenter study conducted by the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Antiviral Study Group is now evaluating whether six months of oral valganciclovir therapy results in better hearing and neuro-developmental outcomes than six weeks of oral valganciclovir therapy.

The long-term sequelae of congenital CMV on hearing loss and brain development were discussed by John Eichwald (US CDC) and Ira Adams-Chapman (Emory University, Atlanta, US).

Pathogenesis and immunology

In this session, an up-date was given on the latest insight into the pathogenesis of congenital CMV and the immunological responses to this infection. Lenore Pereira summarised several studies on the pathogenesis of intra-uterine infection and the histopathological effects on the uterine-placental interface, such as villous inflammation, fibrosis and necrosis. Immunostaining revealed expression of proteins associated with hypoxia. These results suggest direct viral damage resulting in placental hypoxia. She also showed that treatment with intravenous hyperimmunoglobulin, a recently reported intervention in women with primary CMV infection, resulted in compensatory vascularisation of the placenta and villous

regeneration. Another presentation showed that a murine model that had previously been used to study the pathogenesis of CMV infection has now been used to study hearing loss.

Awareness and behavioural interventions

The main message of this session was that only few women have heard of congenital CMV, and although studies have shown that prevention is possible by adopting certain hygienic behaviours, most women were not informed by their obstetricians or gynaecologists about the risks of CMV infection and about possible hygienic measures. Information on the internet regarding CMV prevention is also lacking.

On the other hand, if counselling is applied, women are motivated to be screened for CMV IgG antibodies and to apply hygienic measures.

Prenatal diagnosis, prognostic indicators, correlates of immunity, and treatment

Maria Grazia Revello (Servizio di Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy) focused on recent developments in prenatal diagnosis. The main diagnostic methods are immunological detection of infection in the mother, detection of viral DNA and marker proteins in foetuses, and ultrasound examination. These methods have greatly improved the possibility of counselling pregnant women. Not only can infected foetuses at increased risk of congenital disease be identified more reliably, they also allow more efficient monitoring of the effect of newly described interventions, such as CMV hyperimmunoglobulins and valaciclovir.

Recently, administration of hCMV-specific immunoglobulin has been reported to spectacularly reverse the prognosis in severely affected foetuses [1]. Maria Grazia Revello announced that a multicenter randomised, double blind, placebo-controlled trial in pregnant women with primary CMV infection will start in Italy. The trial will concentrate on the prevention of mother-to-child transmission by administration of hCMV-specific immunoglobulin.

A comparable trial was presented by a second speaker, Mara Dinsmoor from Evanston Northwestern Healthcare.

Stuart Adler summarised in his lecture the findings from CMV hyperimmunoglobulin trials and proposed guidelines for treatment and monitoring. More treatment options may be underway, as an orally active analogue of cidofovir was shown to be effective in limiting CMV infection in a guinea pig model.

This session was concluded by an interesting discussion on the advantages and disadvantages of prenatal screening. The general opinion of the experts was that previous obstacles to prenatal screening, such as limited knowledge on the foetal outcome, the lack of reliable prenatal diagnostics and of intervention possibilities, have now been overcome. It is time to consider a well-designed prenatal screening programme.

Vaccines

For several years now, developing a vaccine for CMV has been regarded as a top public health priority for the US because of the frequency of congenital CMV infection and its impact on sensory, cognitive and motor disability in children. In this session, the many efforts towards vaccine development, the results in animal models and the first results in phase II trials were presented.

Robert Pass (University of Alabama at Birmingham, US) focused on a CMV glycoprotein B (gB) vaccine. A recent phase II clinical trial showed an overall vaccine efficacy of 50%. Rajiv Khanna (Australian Centre for Vaccine Development, Herston, Australia) showed the results of pre-clinical testing of a novel chimeric vaccine based on a replication-deficient adenovirus which encodes, as a contiguous polypeptide, the extracellular domain of the gB protein together with multiple major histocompatibility complex (MHC) class I and II-restricted T cell epitopes of CMV. CMV-specific CD8+ and CD4+ T-cellular as well as humoral immune responses were induced by this vaccine.

All speakers in this session and roundtable discussion emphasised that although pre-clinical and clinical vaccine studies show promising results, many questions still remain to be answered: What do we want to achieve with a vaccine? What will be the target population? What is the best immune correlate of protection? Which animal model can be used?

Newborn screening

While political and ethical questions remain to be resolved, this session showed that neonatal screening for congenital CMV on dried blood spots (DBS) is technically possible with high sensitivity and in a high throughput fashion. Several speakers emphasised that laboratory testing should be validated thoroughly in order to achieve this high sensitivity. Alternative materials (dried urine or saliva) were shown to be suitable for diagnosing congenital CMV with probably higher sensitivity than DBS testing. However, these materials are currently not usable on a routine basis.

Scott Grosse (National Center on Birth Defects and Developmental Disabilities, US CDC) pointed out that evidence of safe and efficacious treatment is probably crucial if a public health case is to be made for universal screening with DBS.

General conclusions

Considerable progress has been made in the field of congenital CMV. Knowledge is increasing on virus transmission and pathogenesis of congenital CMV, diagnostic algorithms are designed, and prenatal and postnatal intervention strategies are being evaluated.

However, despite the high disease burden of congenital CMV, public awareness is extremely low. Continued research in this field is needed for the development of preventive and therapeutic strategies that will have a high impact on the quality of life of many children worldwide.

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News

WEB-BASED INFORMATION ON INFECTIOUS DISEASES DURING PREGNANCY – INFPREG IN SWEDEN

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A non-commercial website containing multidisciplinary information on infectious diseases during pregnancy – INFPREG provides information in Swedish for experts and the general public on ante-/peri-/postnatal care [1]. The site, which has been running for 10 years now, intends to meet the need for up-dated information on the relevance of infectious diseases in pregnancy. It is divided into two sections, one for health professionals and one for the general public. Of 35 chapters, 33 provide specific information on various pathogens, and two chapters provide information on screening programmes and on vaccinations. Information presented in each chapter is the result of collaboration of experts from various fields: obstetrics, infectious medicine, paediatrics, clinical microbiology (virology, bacteriology and parasitology), neonatology, epidemiology, hospital hygiene, audiology, ophthalmology. For professionals, the website offers an interactive questions and answers facility. Questions are answered within three working days, and both are stored in a password-protected archive. The site adheres to AMA (American Medical Association) web site guidelines [2].

The chapters dedicated to health professionals include information on the nature of the causing agents, on contagiousness and transmission in society, on the clinical profile in general and in pregnant women, in the foetus and the newborn, on transmission risks, on laboratory methods, diagnosis of infection in the mother and in the foetus/child, on prophylaxis, on therapy, etc. The information available to the general public has essentially the same content but is presented in a more accessible form, and antenatal care centres in Sweden inform pregnant women about the INFPREG site.

INFPREG has so far been a success. The use of the site increased gradually among midwives, obstetricians and the public, with the number of visits increasing from 52,200 in 2002 to 265,000 in 2008. The website is also used in neighbouring Nordic countries, where the epidemiology, vaccination strategies and guidelines are similar to those in Sweden.

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Editorials

TUBERCULOSIS IN THE EUROPEAN UNION – ONGOING COMMITMENT NEEDED TO CONTROL THE DISEASE

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On 24 March 1882, the German microbiologist Robert Koch announced his discovery that the bacterium *Mycobacterium tuberculosis* caused tuberculosis (TB). He was awarded a Nobel prize for these findings in 1905. World TB day on 24 March commemorates this event and is an opportunity for a critical appraisal of the TB situation, for raising awareness and for joining forces in order to control the disease. It is estimated that every year, there are over nine million new cases of TB worldwide and around one and a half million people die from TB. Thus TB is still one of the most important infectious diseases causing death in humans.

In the European Union (EU), considerable progress has been made in preventing and controlling the disease: The number of newly diagnosed cases and the overall notification rate declined continuously in the past decade. The notification rate in 2007 was 12% lower than in 2003, which reflects a downward trend in 25 countries [1]. In spite of this decline, a total of 84,917 new cases of TB were registered in 2007 – half a century after the introduction of effective treatment – in the EU and the three countries in the European Economic Area/European Free Trade Association (EEA/EFTA) Iceland, Liechtenstein and Norway.

In March 2009, the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization Regional Office for Europe (WHO/Europe) publish their first joint report on the *Surveillance of tuberculosis in Europe – 2007* since the coordination of the former EuroTB network moved to ECDC on 1 January 2008 and the two organisations started to perform joint surveillance.

While the figures from this report for the EU and EEA/EFTA countries are encouraging, they also highlight a number of challenges that hamper the progress towards the elimination of TB.

Multidrug-resistant (MDR) TB, a form of TB which is difficult to cure and laborious for the patient, and which requires considerable human and financial resources, threatens the goal of eliminating TB in Europe. High proportions of MDR TB – up to 21% of all TB cases – are observed in some EU countries. In this issue of *Eurosurveillance* an article by Anderson et al. shows trends in MDR TB in Scotland, a low incidence country for TB, over eight years

and aims at identifying risk groups that need special attention [1]. A rapid communication based on the 2007 TB Surveillance report, shows a disparity in TB incidence within the EU [2], where the majority of countries are progressing towards elimination of the disease (defined as less than one case per 1,000,000 population per year) and where TB tends to accumulate in vulnerable populations with poor access to healthcare. An article by Mulder *et al.* illustrates relevant aspects of TB in individuals migrating from high incidence countries [3], and an article from Greece points out considerable underreporting for a particular region in this EU Member State [4].

The ECDC/WHO/Europe surveillance report also shows that some countries in the EU are still confronted with considerable numbers of newly diagnosed TB cases and notification rates from 36 to 118 cases per 100,000 population. These countries need particular support, and ECDC is closely collaborating with them to jointly face the challenges.

TB has been high on ECDC's agenda from start; the ECDC TB programme comprises a multi-disciplinary team of experts working together on all aspects of the disease to support the countries in their progress towards elimination of TB, a goal that requires sustained political commitment and equal access to early diagnosis, treatment and cure for all patients.

On request of the European Commission, ECDC will be supporting the follow-up of the *Framework Action Plan to Fight Tuberculosis in the EU* [5] in the coming months and will work closely with Member States and experts in the field to define the most effective manner of its implementation. We are certain that this will contribute to sustain the efforts towards TB elimination despite the obstacles that lie ahead.

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Rapid communications

TUBERCULOSIS IN THE EU AND EEA/EFTA COUNTRIES - WHAT IS THE LATEST DATA TELLING US?

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Since 1 January 2008, the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization Regional Office for Europe (WHO/Europe) jointly coordinate the tuberculosis (TB) surveillance activities in Europe. The data collected provides an opportunity for a comprehensive analysis of the TB situation. We aimed at analysing the EU and EEA/EFTA data to identify general TB trends and to provoke some discussion regarding the challenges and needs for monitoring the epidemic.

Background

Since 1 January 2008, the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization Regional Office for Europe (WHO/Europe) coordinate jointly the tuberculosis (TB) surveillance activities in Europe. The aim of this coordinated surveillance is to ensure a high quality of TB standardized data covering all 53 countries in the WHO European Region* and Liechtenstein. Designated national surveillance institutions are responsible for reporting the data. The surveillance data are submitted to and are validated in separate systems maintained by each organisation, which then feed into a joint database for the analysis.

The data provided by the 27 European Union (EU) Member States and three European Economic Area and European Free Trade Association (EEA/EFTA) countries (Iceland, Norway and Liechtenstein) provide an opportunity for assessing the TB epidemiological situation in more detail. In this paper we analyse in more detail the case-based data submitted to The European Surveillance System (TESSy) managed by the ECDC, including cases notified during 2007 and the updated data for treatment outcome monitoring for cases starting treatment in 2006.

The objective of this analysis is to identify general TB trends and to provoke some discussion regarding the challenges and needs for monitoring the epidemic that, despite a high level of heterogeneity among EU Member States, has shown a steadily declining trend over the past 10 years. To help understand better whether this decline is supported by other epidemiological indicators, a summary analysis of the case reporting, treatment outcomes and TB mortality, as well as trends in reported TB drug resistance, are presented.

TB case reporting and trends

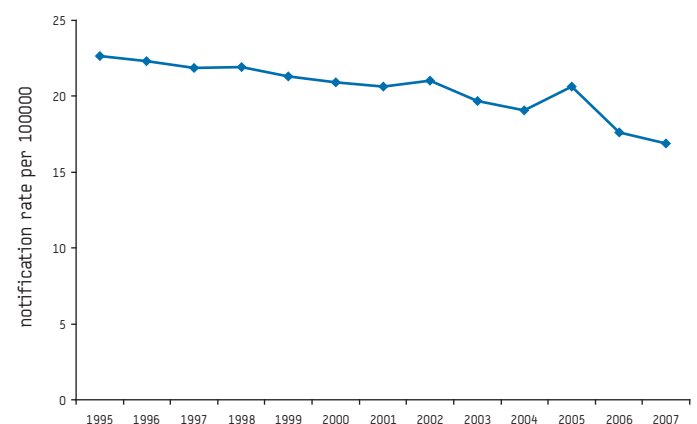
The 27 countries of the EU, plus Iceland, Norway and Liechtenstein reported 84,917 TB cases in 2007, representing

18% of the total number of cases in the WHO European Region (53 countries and Liechtenstein). TB notification rates were highest in Romania (118 per 100,000) and Bulgaria (40) – both countries joined the EU in 2007 – and in the Baltic States (range 36-71). The overall rate for the EU and EEA/EFTA countries was 17 per 100,000.

Between 2003 and 2007, the overall notification rates decreased by a mean of 3.8% annually (Figure 1). However, substantial increases were observed in Malta (+61% mean per year) and Iceland (+37% mean per year) while some increases were also reported in Ireland and Greece (+2%), United Kingdom (+3% mean per year) and Sweden (+5% mean per year), although in the latter two countries this trend has reversed in recent years.

Paediatric TB cases represented 4% of notified cases of both national and foreign origin. This proportion of TB cases in children has been stable for the last 10 years. In contrast, the middle-aged (45-64 years) and the elderly (>64 years) together represented

FIGURE 1
Trend of TB notification rate for the 27 EU and Iceland and Norway, 1995-2007



Source: EuroTB (up to 2006) and ECDC (from 2006). Population figures from EUROSTAT.
Note: LIECHTENSTEIN data not available for the whole period and therefore not included

more than half of the cases of national origin (natives) but only 28% of the cases of foreign origin.

Overall the trend of the proportion of TB cases attributable to foreign origin has remained stable over the period 2005, 2006 and 2007. However in the same period, a decrease of over 4% was recorded in Germany, Italy and Lithuania. In 2007, 21% of cases (range: 0-78% for all countries) were of foreign origin, two-thirds of whom originated from Asia or Africa and 6% from the former Soviet Union (FSU). Most cases of foreign origin were reported among younger adults, especially in the 25-44 year age group (53%).

Over the period 2003 to 2007, the rate of TB meningitis in children under 5 years remained below 1.0 per 10 million general population in most EU and EEA/EFTA countries. Rates above 1.0 for two consecutive years or more were reported by Austria (TB

case rate of 10.5/100,000 in 2007 for all forms of TB), as well as Lithuania, and Romania (total TB rates >30).

Treatment outcomes and mortality trends

In 21 countries with outcome data considered to be complete (for 2006), treatment success was reported in 80% of new definite pulmonary cases, representing a 7% increase from 2001 (Figure 2). The proportion of cases lost to follow up has decreased since 2001 but was more frequent among foreign-born cases than nationals (35% vs 16% respectively in 2006, all pulmonary cases) while death was less frequently reported (4% vs 8%).

In all EU countries, TB mortality rates have decreased or remained stable over recent years. A net decrease exceeding 10% per year over four consecutive years was observed in the Czech Republic, Finland, Greece, and Hungary.

TABLE 1

Tuberculosis cases, case rates per 100,000 population and mean annual change in rates, EU and EFTA/EEA countries, 2003-2007

Country	2003		2004		2005		2006		2007		Mean annual % change in rate, 2003-2007
	N	Rate	N	Rate	N	Rate	N	Rate	N	Rate	
Austria	980	12.1	1,061	13.0	999	12.1	873	10.5	874	10.5	-3.1%
Belgium	1,117	10.8	1,198	11.5	1,107	10.6	1,127	10.7	1,028	9.7	-2.4%
Bulgaria	3,263	41.7	3,232	41.5	3,302	42.7	3,232	42.0	3,052	39.8	-1.1%
Cyprus	35	4.8	30	4.1	37	4.9	37	4.8	42	5.3	3.4%
Czech Republic	1,162	11.4	1,057	10.3	1,007	9.8	973	9.5	871	8.4	-7.2%
Denmark*	393	7.3	385	7.1	422	7.8	377	6.9	391	7.2	-0.2%
Estonia	623	46.0	594	44.0	519	38.6	455	33.9	487	36.3	-5.4%
Finland	412	7.9	331	6.3	361	6.9	299	5.7	313	5.9	-6.1%
France	6,098	9.8	5,514	8.8	5,374	8.6	5,336	8.4	5,588	8.8	-2.6%
Germany	7,166	8.7	6,542	7.9	6,020	7.3	5,402	6.6	5,020	6.1	-8.4%
Greece	620	5.6	774	7.0	769	6.9	681	6.1	659	5.9	2.0%
Hungary	2,582	25.5	2,340	23.2	1,964	19.5	1,894	18.8	1,752	17.4	-9.0%
Iceland	5	1.7	12	4.1	11	3.7	13	4.3	14	4.5	37.2%
Ireland	407	10.2	432	10.6	450	10.8	458	10.7	478	10.9	1.8%
Italy	4,518	7.8	4,220	7.3	4,137	7.1	4,387	7.4	4,527	7.6	-0.6%
Latvia	1,726	74.2	1,610	69.6	1,443	62.7	1,328	58.0	1,255	55.1	-7.1%
Liechtenstein	0	-	0	-	0	-	0	-	5	14.2	-
Lithuania	2,821	81.7	2,514	73.2	2,574	75.4	2,559	75.4	2,408	71.3	-3.2%
Luxembourg	54	12.0	31	6.8	37	8.0	33	7.0	39	8.1	-5.3%
Malta	7	1.8	19	4.7	25	6.2	30	7.4	38	9.3	61.4%
Netherlands	1,321	8.1	1,344	8.3	1,155	7.1	1,021	6.2	960	5.9	-7.7%
Norway	337	7.4	302	6.6	288	6.2	294	6.3	307	6.5	-2.9%
Poland	10,124	26.5	9,493	24.9	9,280	24.3	8,593	22.5	8,616	22.6	-3.9%
Portugal	4,148	39.7	3,854	36.7	3,573	33.9	3,423	32.3	3,127	29.5	-7.2%
Romania	31,039	142.8	31,034	143.1	29,289	135.4	27,319	126.5	25,491	118.3	-4.5%
Slovakia	983	18.3	705	13.1	760	14.1	730	13.5	682	12.6	-7.8%
Slovenia	293	14.7	263	13.2	278	13.9	215	10.7	218	10.8	-6.7%
Spain	7,467	17.8	7,766	18.2	7,820	18.0	8,029	18.2	7,767	17.3	-0.6%
Sweden	408	4.6	461	5.1	559	6.2	497	5.5	491	5.4	4.9%
United Kingdom	7,220	12.1	7,609	12.7	8,317	13.8	8,498	14.0	8,417	13.8	3.4%
Total	97 329	19.8	94 727	19.1	91 877	18.5	88,113	17.7	84,917	16.9	-3.8%

Drug resistance

Twenty nine countries, (all except Poland) reported resistance data for cases notified in 2007. Data from 22 countries that performed culture and Drug Sensitivity Testing (DST) routinely in 2007, or provided DST results as part of a national case-linked dataset, were considered to be representative. Multi-drug resistance (MDR) remained more frequent in the Baltic States, with the proportion of combined MDR cases (all MDR cases regardless of previous treatment history) ranging from 10 to 21%, than in the other countries (range: 0-4%). Rates have remained relatively stable over the past years in the Baltic countries and it remains to be seen if the recent decreases observed in Estonia and Latvia are

TABLE 2
Characteristics of tuberculosis data in EU & EFTA/EEA, 2007

	EU & EFTA/EEA	
	N ^b	
Total population (millions)	30	496.3
Demographic and clinical features of TB cases, 2007		
Total number of cases	30	84 917
TB cases / 100 000 population	30	16.9
Mean annual % change in notification rate (2003-2007)	30	-3.8%
Foreign origin	30	21%
Sex ratio (male to female), nationals	30	2.0
Sex ratio (male to female), foreign born / citizens	30	1.4
Age over 64 years, nationals	30	21%
Age over 64 years, foreign born / citizens	30	9%
Pulmonary disease	30	80%
Pulmonary sputum smear-positive cases / 100 000 population	30	6.7
Previously untreated (diagnosed) for TB	30	79%
Culture positive	30	45%
HIV infection among TB cases (latest available data 2003-2007)	20	2.6%
TB deaths / 100 000 (median, latest available rates 2002-2006)	26	0.9
Multidrug resistance (MDR), 2006 ^c		
Primary MDR (median)	19	1.5%
Nationals, combined MDR (median)	21	0.6%
Foreign-born/citizens, combined MDR (median)	20	3.9%
Outcome, new definite pulmonary cases, 2005 ^{c,d}		
Success (cure or treatment completion)	21	80%
Death	21	7%
Failure	21	2%
Still on treatment	21	2%
Loss to follow-up (default, transfer, unknown)	21	9%

^a Mean value unless otherwise indicated; for definition of geographic areas see Technical Note

^b Number of countries with available data and included in the statistics. Liechtenstein is included in the report, but is only presented as EEA/EFTA country (it does not belong to WHO European Region)

^c Including only countries with complete/representative nationwide data (see Technical Note)

^d Among culture positive pulmonary cases in 21 EU/EEA/EFTA countries; in other countries defined by smear or combination of smear and culture Primary MDR: among previously untreated cases; Combined MDR: among all cases tested (see Technical Note)

sustained. However a decrease in the trends of MDR-TB among re-treated cases is starting to appear in this region.

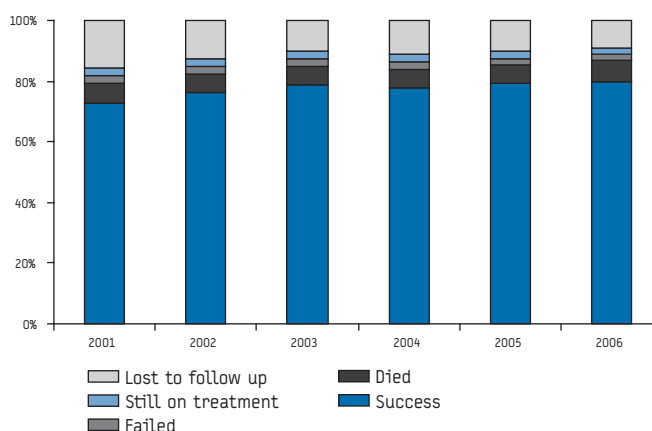
Conclusions

This data demonstrates that most countries of the EU and EEA/EFTA have continued to experience a steady decrease in the overall TB notification rate over the last few decades, even if this trend was briefly reversed in certain countries in the early 1990s. Several epidemiological indicators, such as age distribution, notifications of paediatric TB cases and paediatric TB meningitis trends suggest that the downward trend is real and sustained over the past five years. Additionally, TB mortality rates remain comparatively low.

However this picture should be interpreted with caution. It does not mean that TB is no longer a threat in this part of the world. A number of epidemiological challenges still exist and need to be addressed:

- Within the heterogeneous epidemiological setting described, the number of high/intermediate TB incidence countries remained the same. Serious attention to the evolution of the TB epidemic in these countries is needed.
- The quality of treatment monitoring and reporting remains quite poor and could hamper the effectiveness of TB control.
- Low incidence countries are experiencing a shift of the epidemic towards more vulnerable populations, particularly foreign-born.
- The quality of drug resistance testing and reporting needs to be assessed and further improved. As rates decline, the contribution of drug resistance in slowing down the declining trend of the epidemic will become increasingly important.

FIGURE 2
Treatment outcome in previously untreated laboratory confirmed pulmonary cases, EU and EFTA/EEA countries 2001 – 2006



Data source: country reports from: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Germany, Hungary, Iceland, Ireland, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia and United Kingdom

- Certain epidemiological and surveillance patterns in selected countries need to be evaluated in more detail. This would include further assessment of sustained increases in paediatric cases and/or overall notifications.

Finally, the trend suggesting a slow but sustained decline in the TB epidemic in the EU, highlights the need to identify a valid impact target to assess the epidemiological progress of the TB prevention and control work.

Outcome and impact indicators for Global TB control are well defined and supported by the existence of the Millennium Development Goals for TB and the Stop TB Partnership Targets. However this framework for measuring quantitative progress towards elimination of tuberculosis at a global level has not proven to be an effective stimulus in low/intermediate incidence settings [1]. Additionally some intermediate/high incidence countries will find it very hard to achieve these targets as they have experienced increasing rates since 1990. The current definition of TB elimination within a population is an incidence rate of less than 1 case per million population per year. This is different when compared with other infectious diseases, where elimination is defined as the lack of active transmission within a population, a definition that more accurately indicates the ability to prevent disease from spreading in a given population [2]. It has, however, been argued that it might be unrealistic to apply such a definition for TB elimination.

This discussion is unlikely to meet expert consensus in the near future. However, the wealth of quality epidemiological information being collected should be carefully analysed and monitored to better understand and predict the direction of the TB epidemic in the EU & EEA/EFTA setting.

Finally it should be remarked that the threat of drug resistance remains ever real. Progress made by the Baltic States in dealing with this problem may provide an example that can be of use for countries outside the EU to study and adopt in such forum as the forthcoming WHO ministerial meeting in Beijing [3].

*Countries of the WHO European Region: Andorra, Albania, Armenia, Austria, Azerbaïdjan, Belarus, Belgium, Bosnia & Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Macedonia FYR, Malta, Moldova, Republic of, Monaco, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, San Marino, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Turkey, Turkmenistan, Ukraine, United Kingdom and Uzbekistan.

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Surveillance and outbreak reports

TRENDS IN MULTIDRUG-RESISTANT TUBERCULOSIS IN SCOTLAND, 2000-7

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Overall numbers of multidrug-resistant (MDR) tuberculosis (TB) rose sharply in the United Kingdom and Scotland in 2007. Risk factors associated with MDR TB in the United Kingdom have been identified but there has been no previous report on risk factors associated with MDR TB in Scotland. Enhanced Surveillance of Mycobacterial Infections (ESMI) data were used to examine demographic and clinical characteristics and treatment outcome of MDR TB cases notified in Scotland between 2000-7. There was a total of 11 culture-positive cases of MDR TB, five of which were notified in 2007. The majority of patients were female, 15-44 years old and unemployed. All were born outside the United Kingdom and most had arrived within the past year from or frequently travelled to their home countries in China, the Indian subcontinent or Africa. Except for one individual, our patients did not self report a history of previous diagnosis of TB which was previously identified as a risk factor for MDR TB in the United Kingdom. Only three patients received directly observed treatment (DOT). Only two patients had completed treatment at 12 months, partially due to the inadequate length of follow-up under the current ESMI system. Our results suggest that most patients had primary resistance due to transmission of MDR TB in high incidence countries and thus point to the importance of international efforts to control MDR TB in these countries. In Scotland, national efforts should be made to increase the number of MDR TB patients receiving DOT and to extend follow-up to improve monitoring of treatment outcome. It is important to identify high risk groups for MDR TB infection in order to deliver effective community-based disease control measures.

Introduction

There are an estimated nine million new cases of tuberculosis (TB) worldwide each year and 5.3% of these are multidrug-resistant (MDR) [1]. In 2007, the highest incidence rates for MDR TB ever recorded were detected in 14 countries which comprised China and countries that were members of the former Soviet Union [1]. In the United Kingdom the proportions of MDR TB currently remain low at 1.2% and have been stable since 2000 [2]. However, the number of MDR TB cases per year has increased from 28 in 2000 to 55 in 2007 [2]. This rise is important in terms of future public health planning and resource allocation. It is important to prevent the transmission and emergence of MDR TB because the second line antibiotics that are necessary for treatment are less effective,

have toxic side effects and require extended treatment regimes for 18-24 months [3-5]. Treatment failure is therefore more common and subsequently leads to higher mortality rates and relapse [6-7]. It may also result in the emergence of extensively drug-resistant TB (XDR TB). Furthermore, due to increased drug, inpatient and directly observed therapy (DOT) costs, treatment of MDR TB is ten times more expensive than treatment of drug-sensitive TB. In the UK, the management of an MDR TB case has been estimated to cost the NHS £60,000 compared to £6,040 for a patient with drug-sensitive TB [8].

Effective community-based disease control measures, including contact tracing and optimal treatment outcome, rely on identification of patient groups at risk from MDR TB infection. For the United Kingdom specific risk factors associated with MDR TB include being male [9-10], although in 2008, more MDR TB patients were female [2], being 15-44 years old, or of younger age [2, 9-11], being born outside the United Kingdom [2, 10-12], having a history of previous diagnosis of TB [2, 9-13] and being of Black African, Indian-Pakistani-Bangladeshi or Chinese ethnicity [11]. Being HIV positive and living in London were associated with primary resistance [9-10, 12] whereas pulmonary disease and smear positivity were associated with secondary resistance [10, 12].

There has been no previous report on the risk factors associated with MDR TB in Scotland. The Enhanced Surveillance for Mycobacterial Infections (ESMI) scheme was introduced in 2000 by Health Protection Scotland as the routine surveillance system for the collection of demographic, clinical and laboratory data on patients notified with TB in Scotland. We have used data from the ESMI scheme to examine the demographic and clinical characteristics and treatment outcome of MDR TB cases notified in Scotland between 2000-7.

Methods

The Scottish Mycobacteria Reference Laboratory (SMRL) provided data on mycobacterial strains and drug resistance profiles of isolates obtained through culture and antibiotic susceptibility testing. An MDR TB case was defined as a culture positive case of Mycobacterium tuberculosis complex resistant to at least isoniazid and rifampicin. ESMI data were used to calculate the proportions

of TB cases that were MDR TB, over time, with 95% confidence intervals (95% CI) using Wilson's method [14]. Descriptive epidemiology of MDR TB cases, notified between 2000-7 inclusive, was carried out using a case series to examine: sex, age group, country of birth, years since entry into the United Kingdom, travel outside the United Kingdom in the last two years for at least one month, occupation, previous diagnosis of TB (used as a proxy for secondary resistance), pulmonary disease defined as TB infection in the lungs and/or tracheo-bronchial tree, site of disease and whether patients had received DOT. Patients' ethnicity, health board of residence (health boards are the geographical administrative units for the National Health Service in Scotland), sputum positivity for acid fast bacilli (AFB) and additional risk factors such as being immunosuppressed, a refugee/asylum seeker, an excess alcohol user, a drug user, homeless/hostel dweller/rough sleeper or a health care worker were also captured. Treatment outcome and patient status (alive/dead) were recorded 12 months after treatment start date.

Results

There were 2,199 culture confirmed cases of TB in Scotland between 2000-7 and 11 of these (0.5%; 95% CI 0.3-0.9) were MDR TB (Table 1). There were no differences in the numbers of MDR TB cases observed between 2000-6, with a range of 0-2 cases and proportions of 0-0.8%. However, in 2007 the number of cases increased to five which accounted for 1.7% of TB cases, although the increase was not significant due to the small numbers involved.

Demographic data are displayed in Table 2. Information was complete apart from single cases with missing values for number of years since entry into the UK, country travelled to and occupation. The majority of patients were female (8) and 15-44 years old (10). All were born outside the United Kingdom. Countries of birth included China (2), Pakistan (3), India (1), Zimbabwe (2), Somalia (1), Philippines (1) and Eastern Europe (1); ethnicities matched those of the native populations in the countries of origin. Most affected (8) had been resident in the United Kingdom for one year or less and all had been in the United Kingdom for less than five years. Seven had a history of travel abroad for at least one month in the past two years, as recorded by ESMI, and most had travelled to their home countries. The majority were unemployed (7); one of the only two employed patients was a health care worker. As additional risk factors immunosuppression was identified for one patient. Between 2000-4 the majority of MDR TB patients (4) were resident in urban areas with large cities which were covered by larger health boards. Smaller health boards covering rural areas have also had MDR TB patients which has impacted on their TB services (data not shown to preserve patients' anonymity).

Clinical data are shown in Table 3. Overall they were complete, apart from pending information regarding treatment outcome in three cases and one case with this information missing. One patient had a history of previous diagnosis of TB and reported having received at least one month of treatment in his home country. Three patients had pulmonary disease and two were also smear-positive. Extra-pulmonary sites of disease included intrathoracic and extrathoracic lymph nodes, pleura and spine. Only three patients were commenced on DOT. After isoniazid and rifampicin, the majority of isolates were resistant to streptomycin (7), rifabutin (7), ethambutol (5), pyrazinamide (4) and clarithromycin (4). Resistance to prothionamide (1) and clofazimine (1) was also detected in two different isolates. Only two patients had completed treatment 12

months after treatment start date. For three patients having started treatment at the end of 2007 information on treatment outcomes were pending. Only one patient died whilst under treatment.

Discussion

In 2007 there were five cases of MDR TB in Scotland and 55 in the UK representing the highest numbers ever recorded across the United Kingdom. The proportion of MDR TB in Scotland which was 1.7%, was higher than the UK national average [2]. However, it remained below the national guideline level of 2%, which was set to indicate adequate MDR TB control in the United Kingdom [15]. The increase in MDR TB cases in 2007 may indicate future trends and emphasizes the importance of maintaining or improving levels of control in high risk populations. The results of our study indicate that MDR TB cases in Scotland are imported into the country by young migrant populations recently arrived or returned from countries with high incidence rates of MDR TB. It is perhaps surprising that only one MDR TB patient was from Eastern Europe considering the recent influx of migrant workers from Eastern Europe into Scotland and the rest of the United Kingdom and the high incidence rates of MDR TB in this region [1]. It takes two to five years for the majority of new migrants, entering the UK with latent TB, to develop an active infection [2] and therefore it is possible that Scotland may experience a delay before detecting an increase in MDR TB patients from Eastern Europe. It is also possible that Eastern European patients from non-EU countries return home to seek health care and are thus not picked up by the United Kingdom notification systems.

MDR TB is often associated with a previous history of treatment [1-2, 16-17] and treatment failures or mismanagement leading to secondary resistance. However, in this case series, only one patient had a previous history of TB and treatment, suggesting that most patients had primary resistance, due to transmission of MDR-TB, in high incidence countries. However, previous history of TB diagnosis and treatment was self-reported, so these data may be biased.

The majority of MDR TB patients in our study had non-pulmonary infections with associated lower risks of transmission. Eight of the eleven patients were on self administered treatment, rather

TABLE 1
Sputum-positive multidrug-resistant (MDR) tuberculosis (TB) in Scotland, 2000-2007 (n=11)

Year	MDR TB			Total TB sputum-positive cases (n)
	n	%*	(95% CI**)	
2000	0	0	(0.0-1.4)	285
2001	2	0.8	(0.1-2.8)	248
2002	1	0.4	(0.01-2.2)	256
2003	1	0.4	(0.01-2.1)	265
2004	1	0.3	(0.01-1.8)	302
2005	0	0	(0.0-1.3)	270
2006	1	0.4	(0.01-1.9)	282
2007	5	1.7	(0.6-3.9)	291
Total	11	0.5	(0.3-0.9)	2,199

* proportion of total number of TB cases
** 95% confidence intervals (CI)

than on DOT which can aid treatment completion, especially with extended treatment regimes. Partially due to inadequate length of follow-up (12 months) under the current ESMI system, only two patients were recorded as completing treatment 12 months after treatment start date. As MDR TB usually requires treatment for at least 18 months, the recorded treatment completion at 12 months is likely to be an artifact. Both outcome forms for these patients were received with a delay of six months, which suggests that they also took 18 months to complete treatment. In Scotland, it has recently been agreed to introduce further follow-up of TB patients at 24 months, for those individuals who had not completed treatment at 12 months. In order to improve monitoring of MDR TB patients, the Health Protection Agency in England has similarly revised its system to monitor specific patients at 24 months [2].

This study is the first to describe the patterns and characteristics of MDR TB in Scotland. The results point to the importance of international efforts to improve treatment and control of MDR TB transmission in high incidence countries, as addressed by the World

Health Organization (WHO) Plan to stop TB in 18 High-Priority countries in the WHO European region [3]. This is essential not only to alleviate the associated morbidity and mortality in these countries, but also to prevent the spread of resistant TB strains to low incidence countries, which could impede all future hopes of global control of tuberculosis. National efforts should be made to encourage all new entrants to Scotland to register with general medical practices as soon as possible and research is being carried out to identify incentives which may help to increase NHS registration in these populations [18]. Clinicians should suspect MDR in TB patients from all regions of the world with a high incidence of MDR TB and should ensure timely susceptibility testing is carried out on isolates, that appropriate drug regimes are prescribed and contact tracing is carried out as required following appropriate guidance, including guidance on long haul air travel.

TABLE 2

Demographic characteristics of multidrug-resistant (MDR) tuberculosis (TB) cases in Scotland, 2000-2007 (n=11)

Patient	Year	Sex	Age group	UK born	Country of origin	Years since entry into the UK	Significant travel outside the UK	Occupation
1	2001	M	15-44	No	China	<1	Home country	Unemployed
2	2001	M	15-44	No	Pakistan	4	Unknown	Unemployed
3	2002	F	15-44	No	Pakistan	1	Home country	Unemployed
4	2003	F	45-65	No	Zimbabwe	<1	Home country	Health care worker
5	2004	F	15-44	No	Philippines	<1	Western Europe	Unemployed
6	2006	F	15-44	No	Zimbabwe	1	Home country	Unknown
7	2007	F	15-44	No	India	2	No	Employed
8	2007	F	15-44	No	Somalia	1	No	Unemployed
9	2007	F	15-44	No	Eastern Europe	<1	Unknown country	Unemployed
10	2007	M	15-44	No	Pakistan	Unknown	Home country	Unemployed
11	2007	F	15-44	No	China	<1	No	Further Education

M=Male, F=Female

TABLE 3

Clinical characteristics and treatment outcomes of multidrug-resistant (MDR) tuberculosis (TB) cases in Scotland, 2000-2007 (n=11)

Patient	Previous diagnosis	PuLmonary TB	Site of disease	Follow up at 12 months	DOT	Alive	Resistance
1	Yes	Yes	Lung	Still on treatment	Yes	Yes	INH,RMP,STM,RFB,CLR
2	No	No	Cervical LN	Treatment completed	Yes	Yes	INH,RMP,EMB,RFB,CLR
3	No	Yes	Lung	Missing	Yes	Yes	INH,RMP,EMB,PZA,STM
4	No	No	Axillary LN	Still on treatment	No	Yes	INH,RMP,EMB,RFB
5	No	No	Cervical LN	Still on treatment	No	Yes	INH,RMP,RFB,PTH
6	No	No	ITH and cervical LN's	Treatment completed	No	Yes	INH,RMP,EMB,PZA,STM
7	No	No	ITH, abdominal LN's, spine	Still on treatment	No	No	INH,RMP,EMB,RFB,CLR
8	No	No	Supraclavicular LN	Still on treatment	No	Yes	INH,RMP,PZA,STM,RFB,CFZ
9	No	No	Pleural	Pending	No	Pending	INH,RMP,PZA,STM,RFB
10	No	No	Spine	Pending	No	Pending	INH,RMP,STM
11	No	Yes	Lung	Pending	No	Pending	INH,RMP,STM, RFB,CLR

Key: INH=Isoniazid, RMP=Rifampicin, EMB=Ethambutol, PZA=Pyrazinamide, STM=Streptomycin, RFB=Rifabutin, CLR=Clarithromycin, CFZ=Clofazimine, PTH=Prothionamide, ITH=Intrathoracic and LN=lymph node.

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Research articles

SUBSTANTIAL UNDERREPORTING OF TUBERCULOSIS IN WEST GREECE - IMPLICATIONS FOR LOCAL AND NATIONAL SURVEILLANCE

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In order to estimate the reliability of the officially reported national tuberculosis (TB) incidence rates we performed a retrospective review of data collected in regional and national public health framework. TB notifications for the period 2000-2003 were obtained from two major hospitals and three relevant Public Health Departments (PHDs) in the region of West Greece, and subsequently compared with the data reported to the Hellenic Centre for Diseases Control (KEELPNO). During the four-year study period a total of 161 cases of TB were reported to the PHDs in West Greece; 70% of these cases were reported to the KEELPNO. Furthermore only 72 (38.7%) out of the 186 cases of TB identified in the two hospitals were notified to the PHDs. Assuming that the degree of undernotification observed for the two hospitals is the same throughout the region, we estimated that the case detection rate was 14 cases per 100,000 persons per year, i.e. 3.7 times higher than the rate officially reported for the period 2000-2003. Male predominance (2.1, male/female ratio) and an increased incidence in the elders (older than 60 years) and adolescents (10-14 years old) were also evident. The study demonstrated a substantial underestimation of TB burden in West Greece. In the face of the massive influx of immigrants and refugees coming from regions with high TB incidence and the increase of the number of drug-resistant cases a reliable and complete notification of TB is crucial in the planning of programs and development of appropriate control policies.

Introduction

The subject of underreporting is an important problem in tuberculosis (TB) care in many countries in the world including Europe. It is particularly important for Greece as its case detection rate, according to the World Health Organization (WHO) data, is one of the lowest in Europe [1,2]. In Greece as well as in other European countries the burden of disease is increasingly associated with immigrants from countries with a high prevalence of tuberculosis and other groups at higher risk of infection, such as the elderly (aged 60 years and older), homeless, drug users, and immunosuppressed patients [3,4]. It is widely acknowledged that these high risk groups should be the target of prevention and control strategies of tuberculosis in the European Union (EU) [5].

In Greece, during the period 1996-2005, the notification rates ranged between 5 and 11 cases per 100,000 population per year [6]. Between 2000 and 2003 only 5-6 cases per 100,000 population were reported annually through the national notification system, resulting in one of the lowest rates in the EU, comparable only with Sweden, Malta and Cyprus. It is worth mentioning that in the same period neighbouring countries reported notification rates between 19.3 (Albania) and 43.8 (Bulgaria) [6]. However, the Greek national data are not considered as complete due to various limitations in the notification system [7]. The massive influx of immigrants from the Balkans, Eastern Europe and Asia, i.e. regions with high TB incidence and increase in resistance of *Mycobacterium tuberculosis* does not correspond with the reported case detection rate [8-10]. Therefore, the necessity to report exhaustive and representative data in order to obtain reliable comparisons has been widely acknowledged not only in Greece [5].

TB has been a notifiable disease for many years, but completeness of notification varies among different countries. Despite a number of limitations, notification contributes to the monitoring and control of TB. The main drawbacks are insufficient data and incompleteness of notification which do not reflect the actual situation in the population [2,11,12,13]. In Greece, reporting of TB is obligatory and physicians make notification on a standardised notification form. TB cases are notifiable if they meet certain criteria: TB cases with culture-confirmed disease due to *M. tuberculosis* or culture-negative TB cases with clinical and/or radiological signs and/or symptoms treated with antituberculosis drugs [14]. At the level of the prefectures, the Public Health Departments (PHDs) are charged with the collection of data regarding all notifiable diseases. At the national level, the Hellenic Centre for Disease Control (KEELPNO) collects information from all PHDs for central epidemiological surveillance and trend analysis purposes.

The objective of this study was to examine the process of reporting TB cases between the local (two major hospitals) and regional levels (three public health departments) and subsequently between the regional and national levels (KEELPNO) in order to

evaluate the completeness of notification records held at the national level for the region of West Greece.

Study population and methods

The study took place in West Greece, one of the 13 peripheries of Greece, which is further divided into the prefectures of Aitolokarnania, Achaia and Elia, and covers an area of 11,350 square kilometres (8.6% of the total area of Greece). According to the 2001 census, the population of this region was 741,282 (7% of the country's total population).

For the study period of 2000-2003, the data on TB notifications were obtained both from the three prefectural PHDs and from the KEELPNO.

For the same period, all clinical records on TB cases were collected from the two major tertiary care hospitals in the municipality of Achaia (the Specialised Hospital for Pulmonary Diseases – Thorax Hospital and the University Hospital of Patras). Although in West Greece there are nine more small and medium-sized hospitals as well as 17 health centres, the two hospitals selected for the study are believed to cover a large proportion of TB cases in this region. For each TB patient, data were obtained regarding the date of diagnosis, the site of disease, the criteria used for the case's ascertainment and demographic characteristics (sex, age, profession, place of residence). These data were obtained mainly through the records kept by the hospital-based Committee

of Infectious Disease Control which is responsible by law for the continuous monitoring of all communicable diseases. In the next step, two researchers collected and confirmed all records of TB cases kept in handwritten form in a corresponding book of laboratory results in the Departments of Microbiology and Cytology. Furthermore we have traced additional cases through the patient discharge lists from the departments of internal medicine and pulmonology.

Incidence rates (per 100,000 population) were calculated according to 2001 census provided by the National Statistical Service of Greece. The study was approved by both the Board of Medical School of the University of Patras and the Regional Health Authority of West Greece.

Statistical Package for Social Sciences (SPSS) program-version 12.0 (SPSS Inc., USA) was used for data entry and descriptive analysis.

Results

Table 1 shows the TB cases documented in the two selected hospitals and the corresponding notifications to the PHDs. Based on the place of residence in West Greece, 186 notifiable TB cases were identified in the two hospitals in the four-year period. Of these, only 72 cases (38.7%) were reported to the PHDs. Specifically from the 144 TB cases identified in the Thorax-Hospital only 43 (30%) were reported to the corresponding PHDs whereas the notification rate for the University Hospital was significantly higher (69%). Consequently, at least 114 cases of TB were not notified to the PHDs of West Greece during 2000-2003, i.e. almost 30 cases per year. The combined undernotification rate of the two hospitals reached 61% (114/186) and it was significantly higher in 2002 and 2003 compared to 2000 and 2001.

During the study period (2000-2003), 161 cases were reported to the PHDs in West Greece by all sources (including 72 cases notified by the two hospitals), so that in total we identified 275 TB cases which would correspond to a mean annual notification rate of 9.5 per 100,000 (Figure). On the basis of demographical characteristics of the study population we observed a clear predominance of male patients (male/female ratio of 2.1) and an increased incidence in the elderly (over 60 years old) as well

TABLE 1

Tuberculosis cases documented and reported to the Public Health Departments by two large hospitals in West Greece during 2000-2003

Prefecture	Thorax and University Hospital identified cases	Reported cases to PHD	Notification rate (%)
Achaia	100	22	22
Aitolokarnania	39	20	51.3
Ilia	47	30	63.8
West Greece total	186	72	38.7

FIGURE

Mean notification rate of tuberculosis per 100,000 population, Greece, 2000-2003, by age and sex

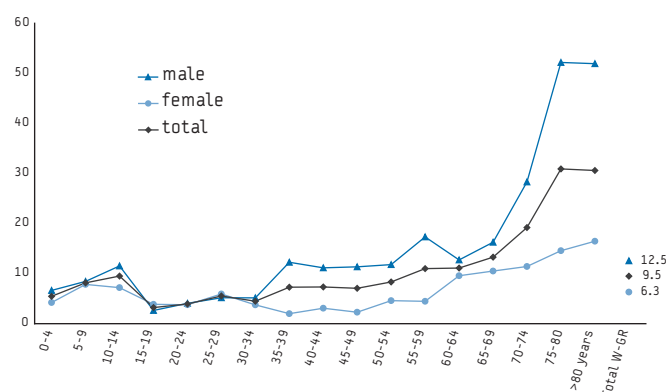


TABLE 2

Cases of tuberculosis registered in the Public Health Departments in three prefectures of West Greece and reported to the Hellenic Centre for Infectious Disease Control (KEELPNO)

Year	Registered cases of TB					Reported to KEELPNO	
	Public Health Departments				West Greece total	From West Greece n (%)	
	Achaia	Aitolokarnania	Ilia	West Greece total		From West Greece n (%)	Greece total
2000	12	26	23	61	56 (92%)	703	
2001	7	4	9	20	18 (90%)	617	
2002	18	13	10	41	27 (66%)	581	
2003	13	12	14	39	12 (31%)	621	
Total	50	55	56	161	113 (70%)	2,522	

as a clustering in the age-group of 10-14 years old (Figure). It is worth mentioning that eight paediatric cases of TB, including six boys and a girl from the urban area of Achaia, were reported from February to May 2002. Any other information was not possible to be obtained for these cases.

Finally, only 70% of the 161 cases notified to the three PHDs were further reported by the PHDs to the KEELPNO (Table 2). There were no significant differences in the proportion of cases reported from the PHDs to KEELPNO by prefecture, since the range was 68-74%.

Discussion

This study demonstrates a substantial underestimation of TB burden in West Greece and reflects an insufficient TB monitoring system in Greece. Assuming that the degree of undernotification observed is the same throughout the whole region of West Greece – which is more than probable considering that the Infectious Diseases Control Committees of the two large hospitals are relatively well organised – we estimate that the actual case detection rate could reach 14 cases per 100,000 persons per year, i.e. a value 3.7 times higher than the data officially reported by the KEELPNO for the period 2000-03. The reasons for this underreporting are not well studied. Although our results cannot necessarily be extrapolated to the whole national surveillance system, the few studies on completeness of tuberculosis notification in Greece have shown similar results [7,15,16]. Obviously the participation of physicians (in both primary and hospital care) in the obligatory (passive) reporting system is not efficient [17] perhaps because the reporting system has not been properly introduced to health professionals and other related stakeholders, and the forms as well as the procedures of reporting remain very complicated. As other studies mentioned, the inconsistency or incompleteness of data produce further difficulties in the data analysis [13,14,18].

A great challenge for TB control is posed by the fact that during the last decade there has been an uncontrolled illegal immigration from high TB endemic regions such as Balkans, Eastern Europe and Asia in many European countries including Greece. Between 1991 and 2004, the number of immigrants in Greece has raised from 270,000 to 1.1 million, accounting for the 10.3% of the total population. Immigrant population densities ranged between 0 and 25% in different areas, whereas in West Greece the density lies around the mean. Immigrants originated mainly from Albania (55%), Bulgaria (4.7%), Georgia (2.9%), Romania (2.2%), Russia (2.3%), Ukraine (1.9%), Poland (1.9%) and Asia (5.6%, mainly Pakistan, India, Iraq, Syria etc.) [8]. During the study period, many of the abovementioned countries showed very high mean annual TB incidence rates per 100,000 population, like Romania (140), Georgia (133), Russia (97), Ukraine (78), Bulgaria (44) [6]. The majority of these (in a great part illegal) immigrants and refugees usually do not undergo any tuberculosis control program [19,20]. Possible cases among the immigrants are less likely to be diagnosed which consequently contribute to further underestimation of the disease burden and facilitate further spread of TB in the country [16,19,20,21].

Another important finding is the observed peak in adolescents and the gender differences. This result is in line with well-established knowledge. During adolescence, higher prevalence of TB among males has been reported which may reflect a genuine sex difference in susceptibility to TB infection [22,23]. It is

probable that our results reflect the usual biphasic age-related TB incidence curve often found in low-incidence countries: the first peak mainly attributable to recent transmission and disease among young immigrants and the second peak reflecting reactivation of old infections among the native population in Western European countries [2,6]. Another possibility could be that undernotification is lesser in paediatric cases than in adult cases. However, the first peak in our curve is in a younger age group than in some other countries and cannot be explained by immigrant labour or marriage (usually 20–40 years age groups) [2,6]. Perhaps the peak in adolescents is due to a school outbreak but we lack data to support that. Gender differences in biological susceptibility may be one plausible reason but also socio-economic and cultural factors may play a role in determining sex differences in rates of infection and progression to disease. Also differences in the risk of exposure to infection between male and female adolescents play a role.

Our results indicate that in two specialised hospitals in West Greece physicians seem reluctant to notify TB cases and, in addition, the regional responsible authorities (PHDs) seem to fail in executing their professional duty of forwarding all surveillance data to the national level. This is partly due to delays in collecting all necessary supplementary administrative data from the hospitals which cause further delays in forwarding on time the data to KEELPNO. These problems should be investigated and addressed by, for example, the Ministry of Health or the Health Care Inspectorate. Effective disease control and prevention in Greece can be achieved only with a well organised surveillance of TB at the local, regional and national level in order to evaluate and plan programs, to target resources and to develop appropriate policies. In order to improve the accuracy of the notification system good understanding of the reasons for underreporting and proper and sincere cooperation with the physicians, the health centres and the hospitals are required. In the light of our findings, the following recommendations are made to increase the notification of TB and to target disadvantaged groups. On the national level KEELPNO must inform regularly all PHDs and physicians regarding the importance and usefulness of the TB notification as well as of the notifications for other infectious diseases. National training and consensus meetings should be organized in order to improve notification rates. On the regional level all necessary activities regarding notification should be centralized and coordinated by the local PHDs, given that offices and professionals at the local PHDs have to perform their duties. The quantity of information collected and reported must balance the need for simplicity, increased efficiency of the system and sufficient data. Cooperation should be strengthened among PHDs, health professionals and KEELPNO. Medical examination of immigrants (especially from countries with high TB incidence) should be enforced.

In the face of the massive influx of immigrants and refugees coming from regions with high TB incidence and the increase of the number of drug-resistant cases challenging the quality of the TB control system a reliable and complete notification of TB – including drug susceptibility testing for monitoring the occurrence of drug-resistant TB – is crucial in the planning of programs and development of appropriate control policies regarding early case finding and transmission control as well as treatment adherence and success.

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Review articles

EFFECTIVENESS OF TUBERCULOSIS CONTACT TRACING AMONG MIGRANTS AND THE FOREIGN-BORN POPULATION

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A literature review was performed to assess the effectiveness of tuberculosis (TB) contact tracing among migrants and the foreign-born population with emphasis on the European Union. Effectiveness of contact tracing was assessed using the following indicators: coverage, proportion of contacts with TB (TB yield), proportion of contacts with latent tuberculosis infection (LTBI yield) and number of investigated contacts per index case (contacts/index case ratio). The key findings from the literature review were: Among foreign-born contacts, a higher median LTBI yield was found compared with contacts born in the country, when exposed to the same foreign-born index cases. No clear differences were observed between TB and LTBI yield among contacts of foreign-born index cases compared with contacts of index cases from the general population (including the foreign-born) due to the large variation seen between the studies. The included non-European studies screened more contacts per foreign-born index case, used lower cut-off values to define a positive tuberculosis skin test and found higher LTBI yields among contacts. Although the high heterogeneity across the studies made the comparison challenging, several conclusions are made regarding contact tracing among migrants.

Introduction

Contact tracing is regarded as an effective strategy to identify recently infected individuals and has become an essential component of the tuberculosis (TB) control strategy in most low-incidence countries [1-4].

In most European countries, migrants and foreign-born account for a large proportion of TB patients, ranging from 9% to 76% [5]. Their risk of infection and progression to disease might differ from the local-born population (for the purpose of this paper, the term 'local-born' will be used in the sense of 'born in the country') due to increased exposure to TB in their country of origin [6]. Diagnostic results may need to be interpreted differently among migrants due to the high level of people in this group who are vaccinated with *Bacillus Calmette-Guérin* (BCG) and to the high prevalence of latent tuberculosis infection (LTBI) [6]. Some countries with a high prevalence of tuberculosis also have a high prevalence of human immunodeficiency virus (HIV) and high co-infection rates. Diagnosis of both latent TB infection and active TB are more complicated in this population. Particularly, interpretation of the results of the

tuberculosis skin test (TST) is often made difficult due to the high number of false negative results.

Regardless of the strategy used to detect TB and LTBI among migrants, it needs to be effective in the group that is targeted. Underwood et al. compared contact tracing with new entrant screening in East London and concluded that contact tracing was more effective in detecting and preventing tuberculosis than new entrant screening, mainly because contact tracing selects for families or communities at particularly high risk [7].

The above issues need careful evaluation when performing contact tracing among the migrants and foreign-born.

Contact tracing in general serves different purposes [4]:

- Identifying individuals with TB disease or LTBI among the contacts of a TB patient and providing adequate treatment or follow-up;
- Reducing morbidity and mortality due to TB among newly infected individuals;
- Reducing further transmission.
- The objective of this review is to assess the effectiveness of TB contact tracing among migrants and the foreign-born population, hereafter referred to as foreign-born, with emphasis on the European Union (EU).

Methods

Literature search

The online reference databases PubMed and Cochrane were searched using keywords combinations of TUBERCUL(OSIS) and IMMIGRANT(S) (or MIGRANT(S) or ASYLUM SEEKER(S) or REFUGEE(S) or FOREIGN-BORN or NEW ENTRANTS) and CONTACT (TRACING or INVESTIGATION or EXAMINATION). The search was limited to publications in English from the last 10 years. Additional references were obtained via the reference lists of the articles found through the search engines. Articles published up to June 2008 were included. Titles and abstracts were screened to sort the relevant papers from the non-relevant ones. Abstracts and where available full text of relevant papers were thoroughly screened and classified as A, B, C or D:

A: Randomised comparative research, where intervention and control are randomly assigned to receive a certain screening programme/test;

B: Studies reporting yield and/or coverage of contact tracing among the foreign-born by any given strategy (i.e. stone-in-the-pond principle, workplace contacts only) or any given method (chest X-ray (CXR), tuberculin skin test (TST), interferon gamma release assays (IGRA), symptom screening);

C: Studies reporting on contact tracing among the foreign-born but not reporting yield or coverage data;

D: Studies discussing policies and strategies of contact tracing at country or regional level in relation to public health/epidemiology as well as studies on the cost-effectiveness of contact tracing. Studies that reported contact tracing but did not relate to foreign-born people were also included in this group.

This classification was adapted for contact tracing studies from the classification used by Klinkenberg et al. for studies into the effectiveness of TB screening strategies for migrants [8].

Data Extraction

A datasheet was designed to extract data from articles classified A and B. We did not attempt to obtain original data. Articles classified C and D were used for discussion of the findings. In some studies, no differentiation was made between foreign-born and local-born index cases and therefore the term “index cases from the general population” was used.

Definitions

Index case: the initial patient diagnosed with TB.

Contact: a person who may have been exposed to the index case during the infectious phase.

LTBI yield: the proportion of LTBI cases detected among the total number of fully investigated contacts.

TB yield: the proportion of TB cases detected among the total number of fully investigated contacts.

Coverage: the proportion of investigated contacts (for LTBI) relative to the total number of listed contacts.

Contacts/index case ratio: the number of fully investigated contacts (for LTBI and TB) per index case.

LTBI treatment rate: the proportion of infected contacts that started LTBI treatment relative to the total number of eligible infected contacts.

LTBI treatment completion rate: proportion of contacts that completed LTBI treatment relative to the total number of infected contacts that started LTBI treatment.

Stone-in-the-pond or ring principle: a strategy wherein contacts are identified in concentric circles around the index case, depending on the frequency and intimacy of their contact [9].

Definitions for the expressions *migrant*, *asylum seeker*, *foreign-born* and *illegal migrant* were adapted from Rieder et al. [10].

Definitions of closeness of contacts were adapted from Kamphorst et al. [4].

Effectiveness of contact tracing

The following indicators, based on recommendations by the United States Centers for Disease Control and Prevention (CDC) in 2005, were used to assess the effectiveness of contact tracing [11]: coverage, TB yield, LTBI yield and contacts/index case ratio. The higher the values of these indicators, the more effective they were considered to be. For the sake of consistency the different

indicators were recalculated where possible using the same definition across all studies.

Because the strategy and the context of contact tracing across the studies differed considerably (depending on setting, infectiousness of the index case, media interest etc.), five analytical approaches were identified and followed:

1. Assessment of studies describing contact tracing for one foreign-born index case.

2. Assessment of studies reporting pooled results of smaller contact investigations exercises. For these studies, outcomes for foreign-born index cases were compared with outcomes for index cases from the general population (including foreign-born index cases) to assess differences in outcomes.

3. Assessment of differences in transmission of TB infection from foreign-born index cases to foreign-born contacts and local-born contacts.

4. Evaluation of whether the closeness of contacts affected the effectiveness of contact tracing.

5. Comparison between European and non-European studies with regards to the effectiveness of contact tracing.

Because only few studies reported yield among contacts by sputum status of the index case, data were not sufficient to present stratified results for this.

The results of three contact investigations described by Kim et al. were pooled to be included under approach 2, as all three were large scale investigations in a similar setting using a comparable strategy [12].

Results

Literature search

A total of 112 (non-duplicate) references were found using the search terms. A further six studies were found via the references of relevant articles. In addition, one study was found when PubMed was searched for studies not written in English, making it a total of 119 studies. After thorough screening of abstract and, where available, full paper, 70 papers were considered relevant and given a classification of A, B, C or D. No papers were classified as category A. Eighteen papers were classified B, of which six were from EU countries [13-18] and twelve from non-EU countries [12,19-29]. Table 1 provides an overview of the key parameters extracted from the eighteen B-classified studies.

Contact tracing strategies

No uniform contact tracing strategy was used across the selected studies. In six studies, the stone-in-the-pond principle was used

TABLE 1

Overview of contacts/index case ratio, coverage, TB yield and LTBI yield reported in the 18 B-classified studies

Parameter	Proportion (interquartile range) [range]
No. of papers	18
Contacts/index case ratio	7.5 (4.4–71.5) [3.0–475]
Coverage	73.5% (39.3–82.3) [29.1–93.3]
TB yield	0.44% (0.00–2.15) [0.00–14.08]
LTBI yield	31.9% (16.9–36.9) [0.0–44.4]
LTBI treatment started	83.1% (72.9–94.6) [69.1–100]
LTBI treatment completed	63.6% (56.4–67.2) [43.5–78.6]

[14,16-19,21]. In three studies, only workplace contacts were investigated. In the study by Gulati et al., the workplace contact investigation consisted of four components [22]: 1) interview with the index case; 2) a qualitative evaluation of the buildings and their ventilation systems; 3) screening of the co-workers; and 4) interviews with co-workers. The other two studies focused on workplace contacts because the index cases were foreign-born healthcare workers [13,26]. In two studies, only close contacts were screened [24,27] and in one study only household contacts [20]. In the remaining six studies, the contact tracing strategy was not clearly described, mainly because these were retrospective studies that used pooled data of various contact investigations.

With regard to the five analytical approaches described in the methods section, the following was found:

1. Studies with one index case of active tuberculosis

Five studies reported contact tracing activities around one foreign-born index case (Table 2). The median TB yield reported was 0.0% (interquartile range (IQR) 0.0–3.52). The median LTBI yield reported was 28.9% (IQR 12.7–37.1). A median of 89.2% (IQR 81.3–94.8) of the eligible LTBI identified contacts started preventive treatment, of whom a median of 66.7% (IQR 55.1–72.6) completed the preventive treatment.

2. Studies with pooled results of contact investigations

In Table 3, studies with pooled results of different contact investigations are presented.

TB yields among contacts of exclusively foreign-born index cases were in the same range as among contacts of index cases

from the general population (median TB yield of 0.63% (IQR 0.5–1.3%) versus 0.46% (IQR 0.0–2.2%)). The median LTBI yield seemed slightly higher among contacts of foreign-born index cases compared with contacts of index cases from the general population, being 39.1% (IQR 20.6–43.7%) and 33.7% (IQR 28.5–36.2%), respectively.

3. Foreign-born and local-born contacts from the same index case

Four studies reported separately on LTBI (but not TB disease) detected among foreign-born contacts and local-born contacts, with both groups exposed to the same foreign-born index cases (Table 4). The LTBI yield among foreign-born contacts was notably higher than among local-born contacts (median 48.9% versus 12.1%) except in one study: Verver et al. reported a slightly higher LTBI yield among local-born contacts than among foreign-born contacts [17]. The contacts/index case ratio found in foreign-born contacts and local-born contacts in these studies was similar (medians of 44.0 and 43.0, respectively).

4. Yield in close contacts and non-close contacts

The effect of closeness of contacts was assessed by comparing findings among close and non-close contacts from foreign-born index cases and index cases from the general population (Table 5).

The results indicate a slightly higher median LTBI yield in close contacts of foreign-born index cases than of index cases from the general population (median 43.7% and 37.0%, respectively), although the interquartile ranges are overlapping. In non-close contacts, the median LTBI yield is higher among contacts of index cases from the general population than among contacts of foreign-born index cases (median 29.0% versus 15.4%). However, the

TABLE 2

Variables of effectiveness in studies reporting contact tracing in studies with one foreign-born index case

Country	Country/region of origin index case	Contacts/ index case ratio (n)	Coverage (%)	TB yield (%)	LTBI yield (%)	Study
The Netherlands	Algeria	n.r.	n.r.	0.00	12.7 ^a	[18]
United States	The Philippines	475	29.1	0.00	5.3 ^b	[26]
United States	n.r. (foreign-born)	63	n.r.	14.1	44.4 ^b	[25]
United States	El Salvador	97	93.3	n.r.	37.1 ^b	[22]
United States	Central America	218	82.6	0.00	28.9 ^b	[23]
-	-	157.5 (88.5–282.3)	82.6 (55.9–87.9)	0.0 (0.0–3.52)	28.9 (12.7–37.1)	Median (IQR)

IQR= interquartile range; n.r.= not reported.

^a TST+ was defined as an induration of >10 mm; ^b TST+ was defined as an induration of ≥5 mm.

Note: In one of the five studies, TB yield was not reported as the paper focused on risk factors for LTBI. In three studies, no contacts with TB were detected [18,23,26]. The fourth study found 10 cases among 71 contacts [25].

TABLE 3

Median with interquartile range of effectiveness indicators for studies with pooled results of contact investigations

No. of studies	Median proportion of index cases that are foreign-born	Contacts/index case ratio (n)	Coverage (%)	TB yield (%)	LTBI yield (%)	References
6	100%	5.7 (4.8–12.3)	79.7 (76.0–81.9)	0.63 (0.48–1.31)	39.1 (20.6–43.7)	[12,14,17,19,21,24]
9	56.0 (51.5–60.0)	5.1 (4.1–6.9)	79.2 (75.3–83.1)	0.46 (0.04–2.15)	33.7 (28.5–36.2)	[13,15,16,20,21,24,27–29]

interquartile ranges are similar. The large difference reported in the contacts/index case ratio between non-close contacts of foreign-born index cases and those derived from index cases from the general population (48.0 and 2.6, respectively) is due to the fact that the data for the first group were mainly obtained in studies reporting on one large contact investigation. The high contacts/index case ratio may also explain the lower LTBI yield found in non-close contacts of foreign-born index cases.

Interestingly, the median TB yield found among close contacts of index cases from the general population was higher than among close contacts of foreign-born index cases (median 2.2% and 0.0%, respectively). However, results should be interpreted with care as only three studies were available of which two had a TB yield of 0.0%. The close contacts included local-born individuals as well as foreign-born individuals, although it is reasonable to assume that a higher proportion of close contacts of foreign-born index cases were themselves foreign-born (e.g. household contacts).

5. EU studies versus non-EU studies

Three EU studies and eight non-EU studies were found which reported specifically on contact tracing among foreign-born index cases. Five of the non-EU studies were reports of a single large contact investigation, which explains the high contacts/index case ratio. In the EU-studies a median LTBI yield of 11.6% (IQR 11.1–12.2%) was found; in non-EU studies it was 38.1% (IQR 26.8–43.9%). This large difference in LTBI yield is likely to be (at least partly) due to the lower TST cut-off values used in the non-EU studies (i.e. a positive TST defined as an induration of ≥ 5 mm). The

median TB yield was comparable between EU studies (0.44%, IQR 0.2–1.5%) and non-EU studies (0.60%, IQR 0.0–1.1%).

6. Sputum smear status of the index case

Sixteen of the 18 studies included in this review reported sputum smear status of the index case. However, only six of them compared outcomes by sputum smear status [15-17,24,27,29]. For these, a higher LTBI yield was found among contacts of sputum smear-positive index cases than among smear-negative index cases. An interesting difference regarding smear status was reported by Golub *et al.* [24]. Among contacts of sputum smear-positive index cases, similar LTBI rates were found in contacts of foreign-born and local-born index cases (46% and 43%, respectively). However, for sputum smear-negative index cases there was a difference. Among the contacts of local-born index cases, only 15% were infected, compared to 44% among the contacts of foreign-born index cases.

Discussion

The main findings resulting from this literature review were:

- When exposed to the same foreign-born index cases, a higher median LTBI yield was found among foreign-born contacts compared to local-born contacts.
- Large variation was seen between studies and no differences were observed between TB or LTBI yield among contacts of foreign-born index cases compared with contacts of index cases from the general population (including the foreign-born).

TABLE 4

The transmission of LTBI: foreign-born index cases to foreign-born contacts and foreign-born index cases to local-born contacts*

Transmission: foreign-born index cases to foreign-born contacts		Transmission: foreign-born index cases to local-born/low prevalence		Study
Contacts/index case ratio (n)	LTBI yield (%)	Contacts/index case ratio (n)	LTBI yield (%)	
2.0	9.7 ^a	0.9	12.6	[17]
52.0	67.3 ^b	25	44.0	[25]
36.0	80.6 ^b	61	11.5	[22]
82.0	30.5 ^b	124	8.9	[12]
44.0 (27.5–59.5)	48.9 (25.3–70.6)	43.0 (19.0–76.8)	12.1 (10.8–20.5)	Median (IQR)

IQR=interquartile range.

* The results in both parts of the table are from the same studies; ^a TST+ was defined as an induration of ≥ 10 mm for non BCG-vaccinated children and an induration of ≥ 16 mm for BCG-vaccinated children; ^b TST+ was defined as an induration of ≥ 5 mm; ^c TST+ was defined as an induration of >10 mm.

TABLE 5

Median with interquartile range of effectiveness indicators for contact tracing in close contacts and non-close contacts in foreign-born index cases and index cases of the general population

Group	No. of studies	Contacts/index case ratio (n)	Coverage (%)	TB yield (%)	LTBI yield (%)	References
Foreign-born index cases						
with close contacts	6	5.8 (5.4–12.8)	88.0 (86.3–91.1)	0.00 (0.00–0.77)	43.7 (25.5–48.9)	[12,17–19,23,24]
with non-close contacts	4	48.0 (5.5–93.4)	71.8 (70.8–83.8)	Insufficient data	15.4 (9.4–22.5)	[12,17,19,23]
General population index cases						
with close contacts	7	3.8 (3.2–5.1)	82.1 (80.3–84.2)	2.15 (2.07–2.28)	37.0 (32.9–40.2)	[15,16,20,21,24,27,29]
with non-close contacts	4	2.6 (1.8–4.1)	n.a.	0.40 (0.20–1.80)	29.0 (14.5–29.7)	[13,15,16,21]

n.a.=not applicable

- In non-EU studies, more contacts per foreign-born index case were screened, lower TST cut-off values were used to define a positive TST, and higher LTBI yields were found.

Of the nine studies with pooled results of contact investigations, three studies reported remarkably higher TB yields than the rest [16,24,27]. The study by Solsona *et al.* was conducted in the inner city district of Barcelona, where high risk groups (HIV-infected individuals, drug users, immigrants and homeless) represent a large proportion of the population [16]. In these high risk groups, higher TB rates can be expected regardless of recent infection. In the studies by Golub *et al.* and Marks *et al.*, only close contacts were included and a high proportion of the contacts were foreign-born, which might explain the high TB yield found [24,27]. However, Soren *et al.* also included only close contacts, but found no active TB cases among 659 contacts investigated [20]. The study by Anderson *et al.* did not detect LTBI in any of the contacts, possibly due to underreporting and/or incomplete test results [13].

The high TB and LTBI yield found in the study by Dewan *et al.*, a study with one foreign-born index case, might be due to transmission by another adult with TB living at the same place as the presumed index case [25]. The high number (n=475) of contacts screened per index case in the CDC study is likely related to the fact that the index case worked in the newborn nursery and maternity ward and therefore large scale contact tracing was conducted [26].

Limitations of the study

Although the focus of this study was on the effectiveness of contact tracing among the foreign-born population in EU countries, only six relevant EU studies were found from which data could be extracted. This highlights the lack of reported evidence from EU countries and indicates that more data reports are needed. The collection and reporting of data showed a high level of heterogeneity across the studies, which made the results difficult to compare and no firm conclusions could be drawn. For instance different cut-off values for a positive TST were used, i.e. $\geq 5\text{mm}$ and $\geq 10\text{mm}$. In addition, some studies used adapted cut-off values for TST testing in BCG-vaccinated individuals [17,28] whereas others did not [25]. Not all studies mentioned if and how persons with prior positive TST results were included. Slightly different definitions were used across the studies, for instance for close and non-close contacts. In the included studies among contacts of the index cases from the general population, close contacts included more often only household contacts than in studies reporting contacts of foreign-born index cases, which more often included workplace contacts. The broader definition used by the latter studies could explain why they found a lower TB yield among contacts in this groups because of less proximity to the index case. The characteristics of the index cases differed in terms of sputum and culture status. Not all studies accounted for or reported people lost to follow-up, and the duration of contact tracing differed between studies. Some studies used a three months follow-up period, while others used a few years.

Challenges of contact tracing among foreign-born individuals Sputum smear status of the index case

As mentioned, only six studies compared outcomes by sputum smear status. As expected, a higher LTBI yield was found among contacts of smear-positive cases compared to contacts of smear-negative patients. The yield was almost three-fold higher in foreign-born contacts.

It should be noted that this higher yield among foreign-born contacts could be due to the higher background rate of LTBI in

this part of the population who acquired infection in their country of origin. It is evident that if this hypothesis holds true, contact tracing in this group of individuals should possibly be considered as a form of screening to identify latent infections not related to the index case

Standardisation of methods used to diagnose TB and LTBI in contacts

In the studies included that reported TB yield, a large variety of methods was used to detect TB. While the gold standard to detect TB disease is a positive culture of *Mycobacterium tuberculosis*, not all studies used this. Most studies used CXR in combination with symptom screening. In most studies, CXR was used after a positive TST was found.

For many years, LTBI has been identified using the TST. Despite its widespread use, the TST has proved to be less specific among individuals born in high-incidence countries due to cross-reaction with the BCG vaccine (see below) and with atypical mycobacteria, both of which are present in individuals from high-incidence countries [30]. In some studies, CXR was used besides the TST to assess infection. Langenskiold *et al.* and MacIntyre *et al.*, for example, used both TST and CXR to define LTBI [15,28]. CXR was also used to find evidence of prior TB.

Recently, interferon gamma release assays (IGRAs) have become commercially available for the detection of LTBI. These tests have characteristics that seem to make them more suitable for screening among migrants: they do not cross-react with BCG vaccination and less frequently with atypical mycobacteria [31,32], and they seem to give a better indication of the time of infection [4]. However, there is a need to assess if the test is equally effective in people from high- versus low-incidence countries [33].

BCG vaccination status

Only four of 18 studies provided information on BCG vaccination status. This is a major drawback, as most foreign-born index cases and foreign-born contacts described in this study were from countries with a high TB incidence that have high BCG vaccination rates. Because of the possible cross-reaction induced by BCG, LTBI yield among foreign-born contacts needs to be interpreted with care for the studies that did not adjust the TST cut-off values for BCG-vaccination status, since the number of cases may have been overestimated due to false positives.

DNA fingerprinting and epidemiological linkage

The assumption underlying contact tracing is that contacts have been infected by the index case around whom the investigation is centred. However, it has been demonstrated through DNA fingerprinting that contacts can be infected by another strain of *M. tuberculosis* than the one that infected the presumed index case [21,34]. Identical DNA fingerprints between contact and index case suggest that transmission has occurred [35]. Thus, not all contacts have been infected by the presumed index case, but some have been infected by another source. Genetic characterisation of the pathogen can therefore have important implications for source finding.

In most low-incidence countries, foreign-born cases have a lower rate of clustering than local-born cases [36,37]. This is often interpreted to mean that foreign-born people develop TB as a consequence of reactivation of prior infection, the likelihood of which is related to country of origin, age at migration, socio-demographic factors, and duration of stay in the new country [5]. Moreover, a foreign-born person could have been recently infected or reinfected when visiting their country of origin, rather than by

transmission from the source case [5]. Similarly, clustering among local-born people could be due to specific sociological factors.

These findings suggest that the use of molecular typing and cluster analysis in support of traditional contact tracing should be further explored.

Stigma of TB and fear of naming contacts

Social stigma is recognised as an important barrier for successful care of people affected by TB [38]. Stigma might also prevent foreign-born index cases from naming (all of) their contacts. Fear might play a significant role in naming contacts when these are staying illegally in the country of residence. The number of exposed contacts can therefore be underreported, which can result in a bias. However, few data are available on the effect of stigma in contact tracing.

Treatment compliance

Only eight of the studies reviewed here reported the proportion of contacts who started LTBI treatment and only six studies reported treatment completion rates. These limited results did not indicate a difference in adherence between foreign-born contacts and contacts from the general population (including foreign-born). The overall adherence was 63.6%, suggesting preventive treatment can be effective. However, the benefits of treatment should be carefully balanced against the side effects such as drug-induced hepatitis [3] as well as against treating people unlikely to develop TB.

Cost-effectiveness of contact tracing

Although this was not the scope of this literature review, research indicated that contact tracing was highly cost-effective and resulted in net savings [39]. Dasgupta *et al.* reported that close-contact investigation was more cost-effective than screening of immigration applicants and surveillance programmes [39]. The latter two ways of case detection were less cost-effective largely because of substantial operational problems such as additional visits for education and reassurance, evaluation of side effects or new medical problems, or assistance with social problems, all of which are common in newly arrived immigrants.

Conclusions

From this review several conclusions can be drawn to address the challenges facing contact tracing among migrants.

Uniform contact tracing strategy

According to this study and that done by the Tuberculosis Network European Trials Group (TbNET) [40] a high variety of contact tracing strategies are being applied across and even within countries. Not every contact investigation can reasonably be conducted with the same strategy, uniform decisions about who needs to be assessed and why a certain strategy has been chosen should be agreed upon. It is therefore important to get more insight in decision making policies. Key questions to be answered are for example: which considerations are made to decide the initial size of the contact investigation? When do local health services expand the contact investigation to the next circle of contacts? Who is responsible for that decision?

Uniform data collection and reporting

To compare the effectiveness of the different contact tracing strategies used, data need to be collected and reported more uniformly. Definitions should be used uniformly throughout studies to be able to better compare results. Usage of standardised protocols might help to achieve this. International validated cut-off

values are needed to define a positive TST induration, and these should be adjusted for BCG-vaccination status.

Contact tracing as a screening strategy

The findings emphasise that foreign-born people from high-incidence countries are at high risk of acquiring or having LTBI. Contact tracing could be used as a screening strategy to identify cases in a high-prevalence population and could be seen as a 'high-risk screening' exercise [7].

Targeted screening

The objective of contact tracing is to find individuals recently infected with TB who are likely to develop active disease. Those at high risk of developing active TB need to be better targeted.

There is an urgent need for a diagnostic tool to identify people with recent latent infection that are at highest risk for developing active disease. This is especially relevant among foreign-born contacts due to the challenge of interpreting the currently available tests due to, for example, BCG, HIV status, nontuberculous mycobacteria and background TB prevalence. Additional research is needed to verify whether the promising IGRAs are reliable in detecting recent infection and are suitable for use in the migrant population.

In conclusion, it should be noted that finding of higher LTBI yields in contact investigations among foreign-born contacts is not unexpected given higher background infection prevalence in these populations. Identifying for which infected contacts close follow-up or preventive treatment should be offered remains a priority. This will be key in determining the role of extensive contact tracing in the context of enhanced TB control among high-risk populations and in establishing its cost-effectiveness.

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HEPATITIS A IN THE EUROPEAN UNION: RESPONDING TO CHALLENGES RELATED TO NEW EPIDEMIOLOGICAL PATTERNS

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Hepatitis A is a vaccine-preventable acute, usually self-limiting disease caused by infection with the hepatitis A virus (HAV). Transmission is usually by the faecal-oral route, including via person-to-person spread, contaminated water or food products. It has also been associated with injecting drug use and outbreaks among men having sex with men.

In the European Union (EU), though figures may vary among countries, the overall incidence of hepatitis A has decreased over the last 10 years from 15.1 per 100,000 population in 1996 to 3.9 per 100,000 in 2006 [1]. This decreasing trend has been attributed to continued improved sanitary and living conditions, with reduced exposure to infection, especially in early childhood. However, reduction in circulation of HAV leads to decreased acquisition of immunity and, in the absence of universal vaccination, an accumulation of susceptible individuals.

The impact of increasing susceptibility of the general population on the risk for outbreaks is clearly illustrated in independent outbreaks in Czech Republic, Latvia and Slovakia in 2008, described in three of the articles published in this week's issue of *Eurosurveillance* [2-4]. In these papers, the authors describe the extent to which hepatitis A can spread within at-risk susceptible populations and in the cases of Czech Republic and Latvia within the general population. In these reports, a significant proportion of cases are young adults, resulting in potentially more severe clinical presentation and posing a challenge to the health authorities in the area of safety of blood and tissue donation.

Experiences from the response to these outbreaks were the focus of a technical meeting organised by the European Centre for Disease Prevention and Control (ECDC) in collaboration with the Latvian Public Health Agency in Riga in November 2008, where the epidemiological pattern of hepatitis A outbreaks was reviewed, as well as the role of vaccination in outbreak settings. Discussions highlighted the fact that emergence of outbreaks in the EU generally follows the introduction of the virus from endemic countries through "seeding events". Non-immunised travellers to endemic areas are often at the origin of seeding events, as shown

in this week's issue of *Eurosurveillance* in the three articles from France, Belgium and Germany [5-7] which describe clusters of travel-related cases following visits to Egypt.

To prevent the introduction of HAV in the EU travellers to endemic countries need to be vaccinated, and indeed vaccination for travellers is recommended by the national guidelines of these three countries. However, as the authors point out, none of the cases reported in their articles had been vaccinated. These clusters therefore highlight the importance of effective travel medicine advice reaching EU travellers of all age groups.

Seeding events can be self-contained. However, when occurring in at-risk settings or communities, HAV transmission may be "amplified" and result in a wide spread of the disease, as described among injecting drug users in the reports by Czech Republic and Latvia, as well as among Roma

populations as reported by Slovakia. Similarly, infected food-handlers may contribute to transmission amplification. Introduction of HAV by children attending day care centres or primary schools represents another type of situations at risk of increased transmission. The Slovak experience shows that immunisation targeting at-risk communities following such introduction may prevent the spread to the general community. However, the Czech example shows that such a strategy for control may not be effective with hard-to-reach communities such as injecting drug users.

Once the outbreak spreads to the general population, vaccination of contacts, as carried out in Czech Republic, represents an option to complement isolation of cases and health education measures, if done within a few days from exposure. Currently, there is no evidence-based guidance available regarding the use of HAV vaccine in outbreak control.

Though the total number of cases may be decreasing yearly in the EU, the articles published in this edition of *Eurosurveillance* indicate that hepatitis A is still an important public health issue, and highlight the need for increased awareness of both the risk of infection to the individual and the possibility of community outbreaks within a changing EU epidemiology.

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As HAV vaccination is not included in universal immunisation schedules, the EU is likely to experience similar outbreaks in the future. This stresses the need to promote immunisation of all travellers to endemic areas to prevent return introduction and to develop evidence-based guidance for outbreak control strategies.

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A HUMAN CASE OF SWINE INFLUENZA VIRUS INFECTION IN EUROPE – IMPLICATIONS FOR HUMAN HEALTH AND RESEARCH

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Swine are susceptible to the same influenza A virus subtypes as humans – H1N1, H3N2 and H1N2 - and the histories of influenza in pigs and people are closely linked [1]. Many swine influenza viruses are a result of reassortment and their genes are composed of human and avian and/or swine virus genes. Indeed, it is known that both human and avian influenza viruses occasionally transmit to pigs, and that pigs can serve as “mixing vessels” for these viruses, meaning that viruses can exchange genetic material and lead to the production of a new “hybrid” virus [2]. This has led to the thinking that perhaps pandemic viruses could emerge following reassortment in pigs. However, since nobody has observed the start of a pandemic, there remains no direct evidence to make this more than a theory.

Influenza is one of the major causes of acute respiratory disease in pigs, but subclinical infections are also common. Unlike the non-zoonotic swine fevers it is not a disease that comes under the European Union’s harmonised Animal Disease Notification System and there are no routine European surveillance data. The symptoms and pathogenesis of influenza in pigs show remarkable similarities with those of seasonal influenza in humans, but the epidemiology is different. Part of this is due to the structure of the swine industry and the extremely rapid turnover of the swine population, with the constant introduction of immunologically naïve animals into swine herds. In swine-dense regions in particular, most pigs show serological evidence of having been infected with influenza by the end of the six-month-long fattening period, and many of them have undergone simultaneous or consecutive infections with two or even three swine influenza subtypes [3]. Unlike human viruses in temperate climates, swine influenza viruses circulate at comparable levels year round. Also, the viruses in Europe differ significantly in their antigenic and genetic make-up from those circulating in North America, even though they consist of the same H and N subtypes, and hence findings in the United States should not necessarily be extrapolated to Europe.

Humans in contact with pigs occasionally become infected by swine influenza viruses [4]. This issue of *Eurosurveillance* reports on a case of swine influenza in a middle-aged woman in Spain [5] which came to attention almost by chance. The woman worked

with pigs and suffered a mild self-limiting influenza-like illness for which few physicians would have taken a swab. However the general practitioner (GP) she consulted happened to be part of an active influenza surveillance scheme and a specimen was taken. This was passed on to the laboratory as a regular surveillance specimen and then recognised as being influenza A (H1N1) phylogenetically close to European H1N1 swine influenza viruses. Retrospective epidemiological investigations found no evidence of any further cases apart from the GP who had experienced similar symptoms but was not laboratory-confirmed [5].

Infection with swine influenza virus has been detected sporadically in humans since the 1950s and the human disease is usually clinically similar to disease caused by infections with human influenza viruses [4]. However, complications that include pneumonia and death have occasionally been reported in the literature in otherwise healthy adults without underlying disease [4]. On the whole, human infections with swine influenza virus, to date, have been different and much milder than those seen with avian influenza A (H5N1) [6] and more similar to infections with low pathogenic avian influenza viruses [7]. Single generation person to person transmission has been reported but appears to be rare and chains of transmission have not been observed in general [4]. Though it is not entirely clear what measures public health authorities should pursue when they discover such human infections, it seems reasonable to regard them as comparable to low pathogenic avian influenza and so deserving a similar approach [7].

There is one well-known exception to these generalisations. In 1976 an outbreak of swine influenza virus infections in humans was detected in recruits in a military camp in Fort Dix, New Jersey in the United States. The presumed link to pigs was never discovered but there was extensive human to human transmission, with over 200 infections resulting in 12 hospitalisations and one death [8]. This was human to human transmission of a novel influenza virus causing some significant human pathology, which today might be described as WHO Pandemic Phase 4 [9]. The unilateral decision was made by the national authorities to develop, produce and deploy a specific pandemic vaccine based on the new strain. However, the infections petered out and the vaccine was

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seemingly associated with occurrence of Guillain-Barré syndrome in a few recipients. Mass immunisation was terminated but the incident remains part of public health lore and has been reviewed extensively for its learning points [10,11].

While the reported case in this issue and other sporadic cases pose little direct threat to humans, they expose important gaps in knowledge about these zoonotic influenzas. The true incidence of swine influenza in humans, for example, is unknown. Recent serologic studies in the United States, where there has been more attention to zoonotic swine influenza than in Europe, have consistently found higher seroprevalence rates and higher antibody titres against all swine influenza viruses in those working with pigs than in non-swine-exposed controls [12-15]. This, and the fact that the current infection was detected by accident, suggests that the few reported cases of symptomatic swine influenza in humans represent a larger number of undetected infections among those in contact with pigs. However, there are no comparable data available for Europe and the prevalence of swine influenza in humans cannot be estimated from such studies because of the possibility of partial serologic cross-reactivity in the haemagglutination-inhibition test between human and swine influenza virus strains of the same subtype. Epidemiologists have tried to adjust for this by statistical methods, but they agree "it is possible that the elevated titers compared by proportional odds modeling do not correlate with infection" [13]. This stresses the need for combined serological and virological surveillance in humans exposed to pigs to gain this information. There have been recent developments in surveillance of influenza in European swine populations, which is an essential starting point for the monitoring of swine flu in humans. A fruitful initiative has been the "European Surveillance Network for Influenza in Pigs (ESNIP)" (2000-2009) a European Commission funded project that ends next month.

Even if the magnitude of the risk of swine influenza virus infections to human health is unknown, it seems unlikely to be high. Two factors are probably restricting infection of humans, though both are neglected research areas. Firstly, the host range of influenza viruses is generally very restricted by a limited fitness of a given virus in a different host species. Studies on the infectivity of animal influenza viruses for cells of the human respiratory tract, and the molecular determinants involved, have however so far focused almost exclusively on avian influenza viruses [16-18]. Secondly, immunity to human H1 or H3 influenza viruses may partially protect against infection with swine viruses. But animal model experiments on this issue are lacking. This type of research is needed if we want to understand the risk of zoonotic influenza based on scientifically proven facts rather than hypotheses.

The unknown element is the risk of reassortment to produce a novel virus, even a pandemic strain either in the pig "mixing vessel" or in a human dually infected with a human and pig strain. In the United States there have recently appeared triple reassortant swine influenza viruses with avian, human and swine genes and these have then transmitted to humans [19,20]. Fortunately, these and similar swine influenza viruses [21] that can infect humans have not yet met any of the criteria to cause a human pandemic. The true risk can only become clear if epidemiological investigations are combined with experimental research. Some scientists have advocated offering seasonal influenza vaccination to persons working with pigs to reduce their risk of getting infected [15]. However, experience with workers with domestic poultry on this

point is not encouraging [22]. In one audit attempt in Europe uptake of the vaccine was low and those offered immunisation were confused as to what they were being protected against. The possible efficacy of human influenza vaccines against swine influenza virus infection, on the other hand, also remains unknown.

Following the discovery in Spain it seems likely that more human infections will be detected and reported as has happened in North America. While such events will mean an improvement in surveillance rather than an increased risk, they highlight another area where closer human and animal surveillance is needed around a poorly understood zoonosis.

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Surveillance and outbreak reports

AN OUTBREAK OF NON-TYPEABLE MRSA WITHIN A RESIDENTIAL CARE FACILITY

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In a household setting within a residential care facility for visually and intellectually disabled people, a resident (index case) was diagnosed with dermal abscesses caused by a methicillin-resistant *Staphylococcus aureus* (MRSA) which was non-typeable by standard pulsed-field gel electrophoresis. In the process of 'search and destroy', all residents and staff in contact with the index case (a total of 200 people) were screened for MRSA. Five people (three personnel and two residents) carried non-typeable MRSA and were treated with antibiotics to eradicate the infection. The 'search and destroy' efforts did not result in the identification of a source. Goats and rabbits which were kept on the premises tested negative for MRSA. The condition of the index case is improving. Further restrictive measures were implemented within the facility to prevent wider spread of the MRSA. This discovery and spread within a residential care facility of a non-typeable MRSA which is often associated with livestock, is remarkable.

Introduction

A new methicillin-resistant *Staphylococcus aureus* (MRSA) isolate belonging to multi-locus sequence type ST398 was first described in a French study in 1998 [1]. No further reports concerning ST398 MRSA strains were mentioned until 2004, when a MRSA isolate belonging to ST398 was detected in the Netherlands [2]. This isolate could not be typed with *Sma*I pulsed-field gel electrophoresis (PFGE) and was termed non-typeable MRSA (NT-MRSA). All NT-MRSA isolated so far belong to ST398.

Voss and colleagues were the first to report the isolation of NT-MRSA strains from people taking care of pigs [2]. Since then, NT-MRSA has become increasingly common among Dutch MRSA isolates. In 2007, 29% of the MRSA isolates forwarded to the Dutch National Institute of Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) belonged to this group of MRSA. Publications about a connection between NT-MRSA in various animal species and NT-MRSA in humans soon followed. A French study reported increased NT-MRSA carriage rate in pig farmers caused by transmission of the strain ST398 [1]. A later retrospective case-control study showed a strong association between human NT-MRSA carriage and contact with pigs or calves [3]. New data revealed that family members living on pig farms can also be NT-MRSA carriers, even when they have not been in direct

contact with animals [4]. In reaction, screening of various animal species was performed. A survey of pigs in Dutch slaughterhouses showed that nearly 40% of the pigs were colonised with NT-MRSA ST398 [5]. NT-MRSA has also been isolated from horses and poultry [6,7].

On the basis of these results, it can be concluded that an NT-MRSA reservoir is established within a variety of animal species and could spread to humans. The emergence of NT-MRSA outside hospitals threatens the MRSA 'search and destroy' policy in Dutch healthcare facilities. It was considered only a matter of time before NT-MRSA would be transmitted from animals via farmers into healthcare settings. Indeed, both NT-MRSA colonisation of personnel and patients, and outbreaks within Dutch hospitals have recently been described [8-10]. It has been suggested that MRSA ST398 isolates are less virulent than other MRSA strains and have limited capacity to spread between humans, but recent reports have shown clinical manifestations of NT-MRSA such as wound infections [4] and endocarditis [11].

Here we describe an outbreak of NT-MRSA in a residential care facility for visually and intellectually disabled people.

One of the residents (index case) was diagnosed with abscessing acne and chronic hydradenitis in his armpits, loins, scrotum and between the buttocks. The index case was fully blind, had a severe intellectual disability and had been suffering from this skin condition since 2004. *S. aureus* isolated from wound swabs in the period between 2004 and 2007 were methicillin-sensitive. The patient was treated with several antibiotics (tetracycline, erythromycin, flucloxacillin, trimethoprim/sulfamethoxazole, clindamycin, minocycline, rifampicin), but this did not result in a significant clinical improvement. In October 2007, the abscesses were surgically treated, in combination with vitamin A therapy, but without success. All swabs taken at that time were suddenly positive for MRSA. Additional screening showed that nose, throat and perineum were colonised with MRSA.

The risk of MRSA transmission within the residential care facility to other residents and personnel was considered high because the index patient had already suffered from staphylococcal disease for

a long period. In the Netherlands, active 'search and destroy' efforts are taken to stop further transmission of MRSA within healthcare settings. The residential care facility therefore contacted the department of infectious diseases of the local municipal health service for advice. A multidisciplinary outbreak team was set up to assess all possible routes of MRSA transmission within the facility, and to identify all at-risk contacts of the index case.

Methodology and results

Assessment of the risk of MRSA transmission

The index patient lived in a household-like setting together with seven other residents and 15 staff members. Other contacts included staff members who also worked at various other units within the residential care facility, such as doctors and nurses, household, day care and facility personnel. The unit consisted of two groups living separately but sharing sanitation. The whole residential care facility has 35 units, situated in various buildings on the premises.

The outbreak team decided to screen all residents living and personnel working in the same unit as the index case, as well as doctors, nurses and family who had been in direct contact with the index case. A total of 43 people were identified as being at risk. Nose and throat cultures were collected from all those screened.

In addition, perineum and/or wound cultures were set up from samples from residents.

Preventive measures

In order to reduce the risk of further MRSA transmission, hygienic measures were implemented around the index case. His private room as well as the sanitation area he was using were disinfected daily, and nurses wore gloves, aprons and surgical masks during direct contact with the index case. He started using a private shower and toilet within the sanitary room. No other residents were allowed in the sanitary room while the index case was there, and the room was cleaned with hypochlorite after he used it. The index case's social contacts with other residents who lived in other units were restricted to a minimum, organised group day care was changed into private day care, and the whole unit is considered contaminated until the cultures of all included individuals are MRSA negative.

Screening results

Two other residents and three staff members from the same unit as the index case tested positive for MRSA. Three of them had positive nose cultures only, one had positive nose, perineum and skin cultures, and one person was MRSA-positive in nose and

TABLE 1

Antibiogram of isolates from residents and staff, NT-MRSA outbreak, the Netherlands, 2007

	Resident A (index)	Resident A (index)	Resident B	Resident C	Staff A	Staff B	Staff C
Date of sample	Nov 2005	Oct 2007	Oct 2007	Oct 2007	Oct 2007	Oct 2007	Oct 2007
Penicillin		R	R	R	R	R	R
Flucloxacillin	S	R	R	R	R	R	R
Gentamicin	S	R	R	R	R	I	R
Trimethoprim/Sulfamethoxazol	S	S	R	R	R	S	R
Doxycylin		R	R	R	R	R	R
Erythromycin	R	R	R	R	R	R	R
Clindamycin	R	R	R	R	R	R	R
Rifampicin		S	S	S	S	S	S
Fusidine acid		S					

S: sensitive; R: resistant; I: intermediate sensitive.

TABLE 2

Isolate typing, NT-MRSA outbreak, the Netherlands, 2007

	Resident A (index)	Resident A (index)	Resident B	Resident C	Staff A	Staff B	Staff C
Date of sample	Nov 2005	Oct 2007	Oct 2007	Oct 2007	Oct 2007	Oct 2007	Oct 2007
Spa-type	Not done	t2383	t011	t2383	t011	t2383	t2383
SSCmec	Not done	IV	IV	IV	IV	IV	IV*
PVL	Not done	Negative	Negative	Negative	Negative	Negative	Negative
MLST	Not done	ST398	ST398	ST398	ST398	ST398	ST398
PFGE SmaI	Not done	NT	NT	NT	NT	NT	NT
PFGE Crf9I	Not done	**	**	**	**	**	**

* SSCmec typing by multiplex PCR-typing according to the method of Kondo *et al.* [12] showed a PCR product for multiplex 1 and 3, but not for multiplex 2.

** PFGE Crf9I is shown in Figure 1.

PVL: Panton-Valentine leucocidin; MLST: multi-locus sequence typing; PFGE: pulsed-field gel electrophoresis.

throat. These results indicated considerable MRSA transmission within the unit and prompted the expansion of the ring for MRSA screening to the relevant direct contacts of all six MRSA-positive individuals. This resulted in the screening of a further 160 people. In this group, no new MRSA infections were detected; the outbreak seemed to be restricted to the unit of the index case. Personnel who work at various units, such as cleaning personnel and medical doctors, did not test positive for MRSA.

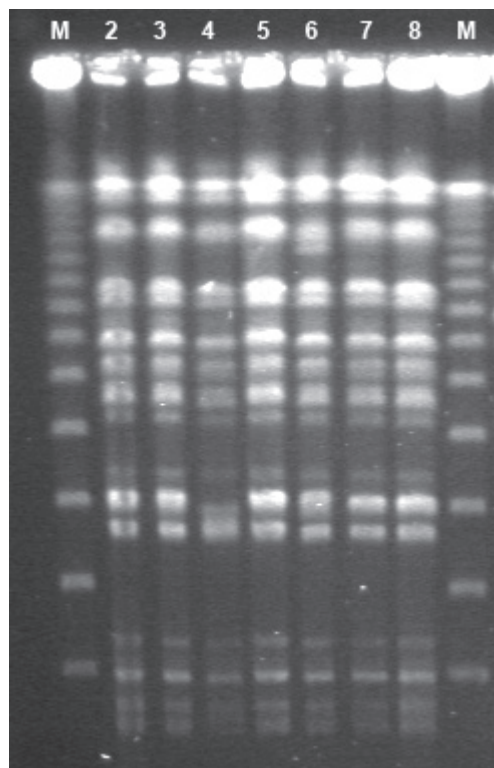
MRSA typing

In order to evaluate the transmission of MRSA strains, the bacterial isolates were typed. All six MRSA isolates had an almost identical antibiogram (see Table 1) and carried staphylococcal cassette chromosome *mec* (SSC*mec*) type IV* according to the method of Kondo *et al.* [12] (see Table 2).

All isolates were Panton-Valentine leucocidin (PVL)-negative and their genome did not contain the restriction site *Sma*I (Table 2). Therefore, they could not be typed by PFGE using *Sma*I and were considered NT-MRSA. In PFGE analyses using the restriction enzyme *Crf9I* (a neoschizomer of *Sma*I that is less sensitive to methylation), all isolates showed very similar banding patterns (Figure 1).

FIGURE 1

PFGE of *Crf9I* macro-restriction fragments of non-typeable (ST398) isolates, NT-MRSA outbreak, the Netherlands, 2007



Lane 2: resident A (index), lane 3: resident B, lane 4: resident B, lane 5: staff B, lane 6: staff A, lane 7: resident C, lane 8: staff C.
M: molecular length marker.
PFGE: pulsed field gel electrophoresis.

Spa-typing revealed two *spa*-types t011 and t2383, both belonging to the ST398 family, which in the Netherlands are primarily found among livestock (cattle and pigs) and people working with livestock (see www.spaserver.ridom.de). Two patients carried *spa*-type t011. The remaining four isolates, including the strain obtained from the index patient, had an uncommon *spa*-type t2383. Multi-locus sequence typing (MLST) confirmed that all strains belonged to the ST398 family (www.mlst.net).

Outbreak source and transmission

The index patient's lesions continued producing pus. The index could thus have functioned as a reservoir and may have maintained the outbreak. It is unclear if the index was the source of the outbreak.

This outbreak in a residential care setting indicates that NT-MRSA is also a public health issue. NT-MRSA is most often associated with direct contact with pigs or calves [4,13], but none of the MRSA-positive individuals had any contact with livestock. However, rabbits, chickens and goats were living on a farm on the premises of the residential care facility. The outbreak team decided to screen the goats and rabbits because various animals have been described as a source of MRSA and there had been sporadic contact between the residents and these animals. All cultures of the animals' anterior nares (three goats and four rabbits) were MRSA-negative.

A definite source for the NT-MRSA could not be traced. The outbreak of NT-MRSA was most probably caused by direct human to human transmission facilitated by the intensive contact between the residents and staff living and working in the unit. The contact between staff and clients is randomly organised, frequent and intense. An exact route of NT-MRSA transmission within the unit is therefore indistinct. Furthermore, there was no significant difference between MRSA-positive and negative staff regarding the intensity of physical contact with MRSA-positive residents.

MRSA eradication

To eradicate the MRSA, all MRSA-positive residents and staff (except the index case) were given oral and topical therapy (mupirocin nose gel and washing with chlorhexidine for five days), followed by three successive control cultures taken from the nose and throat. MRSA-positive residents were temporarily banned from group activities and MRSA-positive staff had to stay at home during the period of eradication. The residents' sanitary room and sleeping rooms were cleaned daily. Also hand-touch sites, such as door handles were thoroughly cleaned on a daily basis. All control cultures taken after completion of the eradication therapy tested MRSA-negative.

The preventive measurements were restricted to the unit of the index case. To date, the index patient is being treated with a combination therapy with rifampicin and trimethoprim/sulfamethoxazole and surgical incision of the abscesses. The skin lesions are slowly diminishing, and recent cultures taken from wounds, nose and throat in late December were MRSA-negative. Once his skin lesions have healed, eradication therapy will be started.

Discussion and conclusions

This MRSA outbreak in a residential care setting highlighted particular challenges. Firstly, the healthcare setting described in this article is not a hospital, but a permanent care facility for people with visual and intellectual disabilities. The outbreak

caused commotion among the staff members, and they had a lot of practical questions as they were unfamiliar with MRSA and an MRSA-outbreak in particular. Furthermore, it turned out that the use of gloves, surgical masks and aprons during washing and clothing was perceived as threatening by the clients.

The restriction of the index case's social contacts was difficult to implement. His wounds were resolving slowly, and hygiene measures were lifted to some extent after six months. In addition, follow-up samples of the wounds proved to be MRSA-negative under antibiotic treatment. It was therefore decided that after careful bandaging of the wounds, social contacts could be allowed within the unit.

To our surprise, two different *spa*-types were discovered by molecular typing. The rare *spa*-type t2383 only contains the first two repeats (08-16) of the seven repeats present in the t011 gene (08-16-02-25-34-24-25). Considering that the strains share the same antibiogram and have very similar PFGE patterns, it is tempting to speculate that the initial introduced strain had *spa*-type t011. It could very well be that one of the individuals carrying the t011 strain was the primary source for the other case. After a deletion of five repeats, this strain could then have colonised the cases infected with the t2383 strain. Alternatively, we can not exclude that both *spa*-types were introduced independently.

NT-MRSA is not only a Dutch problem, but has been discovered in a number of European countries, as well as in Canada, China and Singapore [14-16]. *Spa*-type t2383 (Figure 2) is a rare relative of t011 (Figure 3) (see <https://mrsa.rivm.nl/flash/flash.aspx>).

NT-MRSA transmission from human to human is relevant for the impact of NT-MRSA in public health care. Inter-human transmission of NT-MRSA has been described earlier within families of animal

farmers [2] and on a larger scale in patients and personnel of a Dutch hospital [9]. This outbreak within a non-hospital healthcare setting adds proof for the potential of NT-MRSA for inter-human transmission. Therefore, NT-MRSA might be able to gain a foothold in the human population.

Acknowledgements

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An earlier report of this outbreak was published in Dutch [17].

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FIGURE 2

NT-MRSA *spa*-type t2383 isolated in the Netherlands in 2007-2008

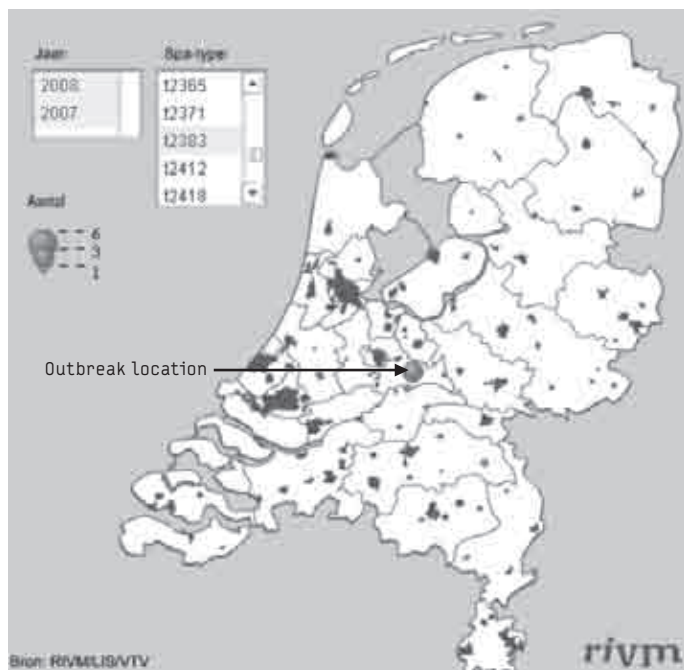
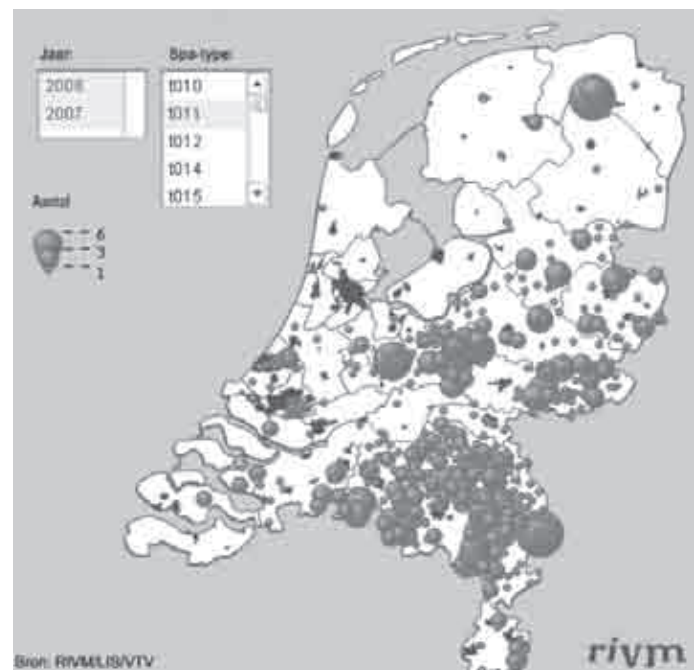


FIGURE 3

NT-MRSA *spa*-type t011 isolated in the Netherlands in 2007-2008



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LONG-TERM *CRYPTOSPORIDIUM* TYPING REVEALS THE AETIOLOGY AND SPECIES-SPECIFIC EPIDEMIOLOGY OF HUMAN CRYPTOSPORIDIOSIS IN ENGLAND AND WALES, 2000 TO 2003

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

Introduction

The aetiological agent of the diarrhoeal disease cryptosporidiosis in humans has been traditionally ascribed to the protozoan parasite *Cryptosporidium parvum*, on the basis of microscopical identification of oocysts or detection of oocyst wall antigens in faeces, and the assumption that all oocysts detected were monospecific [1]. However, *C. parvum* variants, recognised initially phenotypically (designated human or H and cattle or C types) and latterly through genomic studies, segregate into two genotypes (1 and 2), of which genotype 1 is host-adapted for humans [2]. This is now assigned species status and called *Cryptosporidium hominis* and genotype 2 remains *C. parvum* [3]. Although these are the most commonly found species in human cryptosporidiosis worldwide, the distribution varies temporally and geographically [1]. Six other *Cryptosporidium* species have also been found in this host (*Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium suis*, *Cryptosporidium muris* and *Cryptosporidium andersoni*), as have *C. hominis* monkey

genotype, *C. parvum* mouse genotype and the *Cryptosporidium* cervine, chipmunk genotype 1, skunk, horse and rabbit genotypes [4, 5, 6]. The site-specific occurrence and pathogenicity of these unusual *Cryptosporidium* species/genotypes in humans appears to depend on a combination of endemicity, exposure and parasite-related factors rather than host immune status [7].

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

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

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

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

Methods

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

Patient demographics (locality, date of birth or age, and sex), clinical details, specimen collection date, history of recent foreign travel and whether the case was considered to be part of a family or household cluster or a general outbreak, was collected from the diagnostic laboratory on a form submitted with each sample and outbreaks verified with the investigating authority. For specimens where the collection date was missing, a proxy date was calculated

by deducting from the date of receipt at the CRU the modal time delay for this interval (five days). Cases were geographically located using the Government Office Region of the submitting laboratory. Countries visited by patients reporting recent foreign travel were grouped according to the health advice provided in Health Information for Overseas Travel [18].

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

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

Results

Specimen submission

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

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

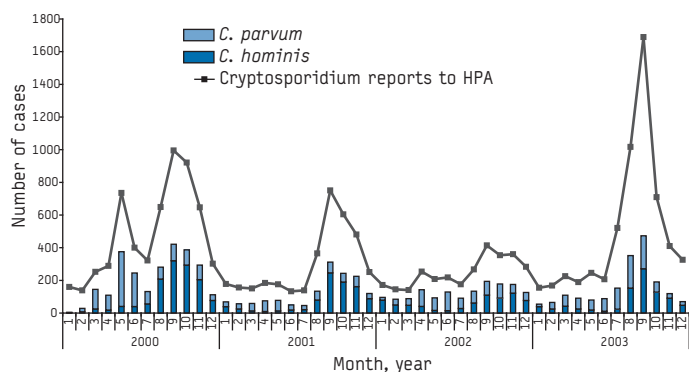
Of the 8,075 specimens received by the CRU, 3,965 (49.1%) specimens were from males, 4,072 (50.4%) were from females and for just 38 (0.5%) the sex of the patient was not known. This was not significantly different from the ratio of male to female cases (1:1.02) reported to national surveillance ($\chi^2=0.06$,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

Microbiological and genotyping results

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

FIGURE 1

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



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

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

Of the 7,758 first specimens from each patient, 3,817 (49.2%) were *C. hominis*, 3,564 (45.9%) were *C. parvum*, 40 (0.5%) were dual infections with *C. parvum* and *C. hominis*, other *Cryptosporidium* species/genotypes were identified in 67 (0.9%) and 270 (3.5%) were not typable. The unusual species/genotypes

were *C. meleagridis* (n=56), *C. felis* (n=4), *Cryptosporidium* cervine genotype (n=4), *C. canis* (n=1), *Cryptosporidium* horse genotype (n=1) and *Cryptosporidium* skunk genotype (n=1). The finding of the horse and skunk genotypes has been described by Robinson et al., [6] and the epidemiology of cases other than *C. parvum* and *C. hominis* is being prepared for publication elsewhere.

Demographics

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

TABLE 1

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

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

Source: United Kingdom Cryptosporidium Reference Unit data

p: significant values are indicated in bold

* Age not known for 60 cases

** Sex not known for 32 cases

*** Apart from baseline, ranked by decreasing population density per hectare

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

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

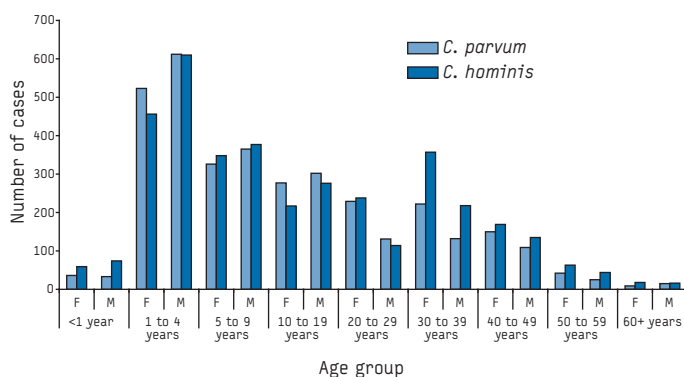
Travel history

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

Geographical distribution

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

FIGURE 2
Age and sex distribution of *Cryptosporidium parvum* and *Cryptosporidium hominis* cases in England and Wales over four years from 2000 to 2003 (n= 7,381)



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

Seasonality

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

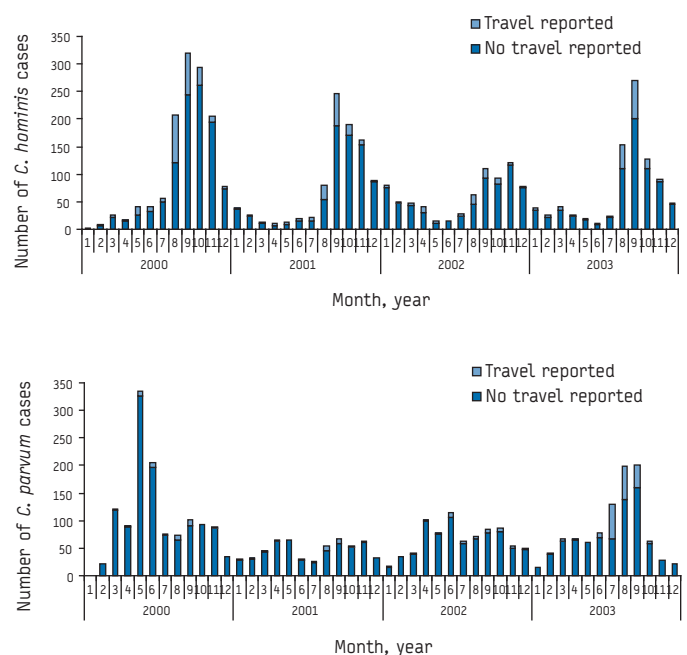
All ages were affected by the spring decline in *C. parvum* cases in 2001 (Figure 3), and the spring peak was only partially restored in 2002 and 2003 (Figure 1). During each of the four years most isolates were received during September, this peak being mainly composed of *C. hominis* and to a lesser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

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

Cryptosporidiosis outbreaks

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

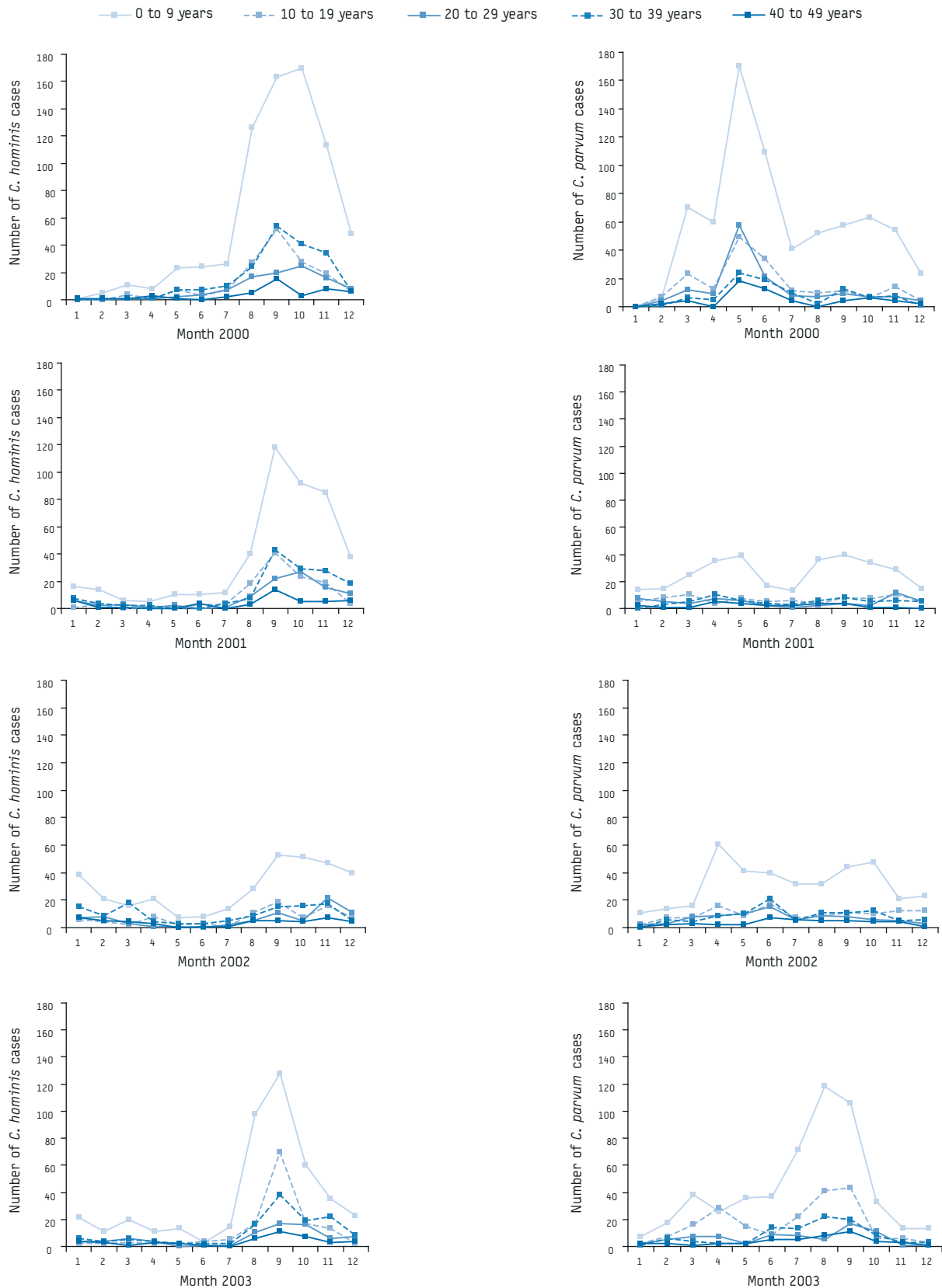
FIGURE 4
Distribution of *Cryptosporidium hominis* and *Cryptosporidium parvum* in cases reporting travel and not reporting travel outside the United Kingdom over four years from 2000 to 2003



Source: United Kingdom Cryptosporidium Reference Unit data

FIGURE 3

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



Source: United Kingdom Cryptosporidium Reference Unit data

TABLE 2

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

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

Source: United Kingdom *Cryptosporidium* Reference Unit, Health Protection Agency (HPA) and National Public Health Service for Wales data

~ Not recorded in the HPA outbreak database

* Concurrent community outbreaks of Norovirus may account for a proportion of cases.

NT = not typable

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

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

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

Discussion

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

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

The temporal distribution, with *C. parvum* predominating in the spring and *C. hominis* in the autumn, which has been reported in

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

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

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

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

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

Conclusion

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

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Surveillance and outbreak reports

AN OUTBREAK OF MEASLES IN ORTHODOX JEWISH COMMUNITIES IN ANTWERP, BELGIUM, 2007-2008: DIFFERENT REASONS FOR ACCUMULATION OF SUSCEPTIBLES

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

Introduction

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

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

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

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

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

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

Methods

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

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

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

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

Results

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

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

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

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

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

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

the 101 unvaccinated cases (according to the parents), 78 (77%) were eligible for vaccination according to their age.

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

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

Control measures

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

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

FIGURE 1

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

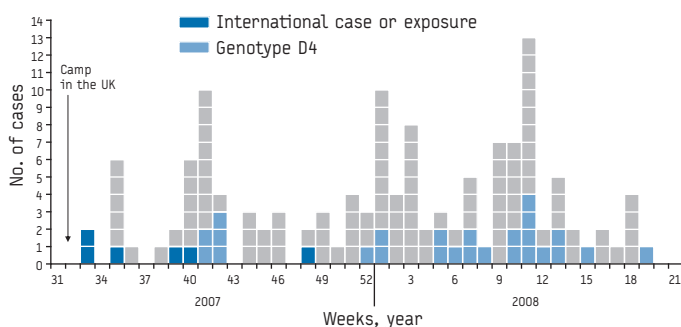
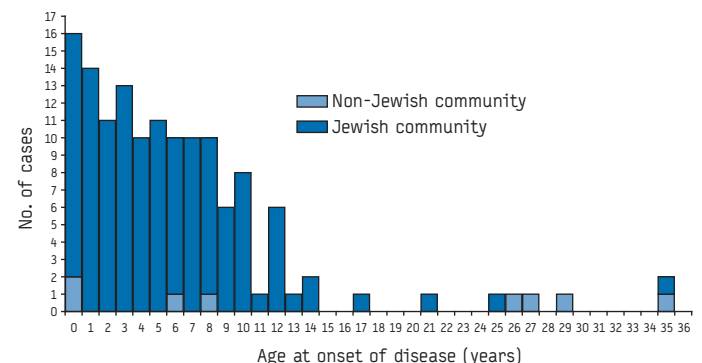


FIGURE 2

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



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

Discussion

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

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

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

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

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

the second dose of MMR, not only in subsidised schools but also in private schools might have avoided this outbreak.

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

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

Conclusion

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

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

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

Acknowledgements

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EPIDEMIOLOGY OF HUMAN CRYPTOSPORIDIOSIS IN IRELAND, 2004-2006: ANALYSIS OF NATIONAL NOTIFICATION DATA

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Cryptosporidium is a protozoal parasite which is of public health interest primarily due to its frequent association with drinking water. Since cryptosporidiosis became a notifiable human disease in 2004 in Ireland, evidence has been growing as to the national burden of illness caused by this pathogen. Nationally, crude incidence rates of between 8.7 and 13.4 per 100,000 were reported annually in the period 2004-2006. Rural areas reported more cases, with regional incidence rates as high as 31.4/100,000 per year. Over this time period, there has consistently been a peak in the number of notifications in springtime, contrasting with the reported seasonal distribution of cases elsewhere in Europe. Outbreak surveillance data suggest that drinking water is an important transmission route for general outbreaks, with person-to-person spread more common in family outbreaks. *Cryptosporidium* is an important gastrointestinal pathogen in Ireland, with much still to be learned about its epidemiology here.

Introduction

Cryptosporidium is a protozoal parasite which first came to prominence as a cause of chronic diarrhoea in acquired immunodeficiency syndrome (AIDS) patients in the United States in the early eighties, but is now recognised as a major cause of gastroenteritis in immunocompetent patients, leading to a spectrum of disease from asymptomatic shedding to watery non-bloody diarrhoea, sometimes accompanied by abdominal pain, nausea, anorexia, fever and weight loss.

A key feature of *Cryptosporidium* oocysts is their relative resistance to chlorination, and as a result, it has gained notoriety as a public health issue due to its association with municipal drinking water supplies and public swimming pools, both of which have been implicated as the vehicle of transmission in a number of outbreaks in developed countries [1-4].

Awareness of cryptosporidiosis as a cause of gastrointestinal illness has risen in Ireland in 2007 due in part to a large waterborne outbreak in a city in the west of Ireland [5], which resulted in an extended boil water notice for several months. Evidence as to the national burden of illness caused by this pathogen, however, has been growing since 2004, when cryptosporidiosis became a notifiable human disease. This paper outlines current knowledge of the national epidemiology of human cryptosporidiosis in Ireland, drawing on recent disease notification and outbreak surveillance data.

Materials and methods

Human cryptosporidiosis has been subject to mandatory notification in Ireland since 1 January 2004. As for all notifiable diseases, basic demographic data is reported routinely on all cases. The case definition adopted since 2004 is based on the European Union (EU) case definition [6]. Notification data are maintained in the Computerised Infectious Disease Reporting (CIDR) system, a central national repository for all infectious disease notification data in Ireland. The notification data used in this report is based on information retrieved from CIDR on cases of cryptosporidiosis reported from 2004 to 2006, as of 5 December 2007.

Reporting of infectious disease outbreaks has been mandatory in Ireland since 1 January 2004, and data on outbreaks of cryptosporidiosis between 2004 and 2006 were retrieved from CIDR for that time period. Prior to 2004, outbreak data had been collected on a non-statutory basis from July 2001 by the Health Protection Surveillance Center (HPSC). In this paper, we present data collected on outbreaks reported between July 2001 and December 2006.

The administration of public health activities in Ireland is divided into eight regional departments, referred to as Health Service Executive (HSE) areas. Regional incidence rates were calculated as crude incidence rates per 100,000 population using Central Statistics Office (CSO) population data from the 2006 census as denominator. For age-specific incidence rates, seven cases were omitted from the analyses, as the variable 'age' was not available.

Results and discussion

Incidence

In the three years from 2004 to 2006, between 367 and 568 cases of cryptosporidiosis were notified annually, resulting in a crude incidence rate (CIR) of between 8.7 and 13.4 per 100,000 population (Table 1).

To put this in perspective relative to other causes of intestinal infectious disease in Ireland, the reported incidence of cryptosporidiosis is similar to that of salmonellosis in the same time period. A recent study [7] compared the incidence of cryptosporidiosis in 16 countries in Europe in 2005, and reported an overall crude incidence rate of 1.9 per 100,000 in these countries, with Ireland having the highest CIR of the 16 countries included in the study. Even bearing in mind that comparison between surveillance data from different countries is difficult due to variation in diagnostic, investigative, and surveillance practices,

all of which influence reporting in each of the countries, it is clear that cryptosporidiosis is an important cause of gastrointestinal illness in Ireland.

Seasonal distribution

Between 2004 and 2006 in Ireland, there was a consistent pattern in the seasonal distribution of notifications (Figure 1), with the highest numbers of cases reported from April to June. Overall, 55% of cases occurred during the second quarter of the year in these three years.

This contrasts strongly with the seasonal distribution of cases reported in the United Kingdom (UK), Sweden and Germany [7], where the highest numbers of cases in 2005 occurred in autumn, and with Spain, where a seasonal peak was observed in June, suggesting that the epidemiology of cryptosporidiosis in Ireland differs appreciably from the current epidemiology of cryptosporidiosis in these countries. Spring peaks in incidence coincide with peak calving and lambing activities, and are believed to be associated primarily with transmission from animal sources [8-9]. Prior to the introduction of the 1999 UK water regulations, there had been both a spring and an autumn peak in the number of cases, whereas in the recent years a significant reduction in the number of spring cases has been noted and attributed to the effectiveness of these regulations [10].

It appears that the seasonal distribution of cases in Ireland more closely reflects that reported for New Zealand, which also displays a pronounced spring peak, albeit in addition to a smaller autumn peak [11].

Age-sex distribution

Figure 2 shows the mean annual age-specific incidence rate in Ireland 2004-2006. Notifications for children predominated with over three quarters of all reported cases being less than 10 years of age. There were more male (n=729) than female (n=634) cases notified.

It is widely accepted that there is a degree of bias in reporting of illness in young children for many diseases, as parents are more likely to seek medical attention and health personnel more

likely to take specimens for children than for adults. Moreover, higher incidences among younger children may reflect a lack of immunity as many older people may have already had exposure to *Cryptosporidium* during their lifetime.

However, selective criteria based on age are also commonly applied to samples for *Cryptosporidium* testing in diagnostic laboratories (a selection criterion probably not as frequently applied when testing for other common gastrointestinal pathogens such as *Salmonella* or *Campylobacter*), and this could have a significant impact on the reported age distribution. For example, although the HPSC report on waterborne cryptosporidiosis [12] recommends that all stool specimens received by laboratories from symptomatic individuals be tested for *Cryptosporidium*, it acknowledges that where resources are limited an age threshold of ten years can be applied, although this threshold is generally not employed during outbreaks. The effect on the reported incidence of disease in Ireland will be dependent on the number of laboratories that have opted to apply an age threshold as a selective criterion when examining specimens for *Cryptosporidium*. Anecdotally, we are aware that many laboratories do not test routinely for *Cryptosporidium* in adults, and a laboratory survey to investigate this further is underway. The recent outbreak in the west of Ireland provides some evidence of the potential effect of an age threshold selective criterion [5]. It was reported that more than 40% of cases in the outbreak were older than 15 years of age. On this basis, it is possible that in areas served by diagnostic laboratories where an age-threshold such as this is applied, around 40% of cases could remain undetected.

Regional distribution

There was a marked difference between the reported incidence of cryptosporidiosis in the HSE-East (which includes the capital city Dublin and thus has a larger proportion of urban dwellers than other HSE areas) and other more rural areas of Ireland (Table 1). The highest average crude incidence rate in 2004-2006 was reported in the HSE-West (22.5/100,000), which had a particularly high incidence in 2005 (31.4/100,000), followed by HSE-Midlands (18.2/100,000), where data were influenced by the occurrence of a community outbreak in 2004, which resulted in a higher than average incidence in that year.

TABLE 1

Crude incidence rates (CIR) of cryptosporidiosis by Health Service Executive (HSE) area and year, Ireland, 2004-2006

HSE area	CIR (95% confidence interval)			
	2004	2005	2006	Average 2004-2006
East	1.5 (0.9-2.2)	2.5 (1.7-3.3)	0.5 (0.1-0.8)	1.5 (0.9-2.1)
Midland	24.6 (18.5-30.8)	14.3 (9.6-19.0)	15.5 (10.6-20.4)	18.2 (12.9-23.4)
Mid-West	12.5 (8.8-16.1)	15.5 (11.5-19.6)	15.5 (11.5-19.6)	14.5 (10.6-18.4)
North-East	7.6 (4.9-10.3)	15.7 (11.8-19.7)	7.1 (4.5-9.7)	10.2 (7.0-13.3)
North-West	16.9 (11.6-22.1)	18.1 (12.7-23.6)	12.7 (8.1-17.2)	15.9 (10.8-21.0)
South-East	17.4 (13.6-21.2)	21.3 (17.1-25.5)	13.2 (9.9-16.6)	17.3 (13.5-21.1)
South	11.9 (9.2-14.6)	16.9 (13.7-20.1)	11.9 (9.2-14.6)	13.6 (10.7-16.5)
West	18.6 (14.4-22.7)	31.4 (26.0-36.8)	17.4 (13.4-21.4)	22.5 (17.9-27.0)
Ireland	10.2 (9.2-11.1)	13.4 (12.3-14.5)	8.7 (7.8-9.5)	10.7 (9.6-11.7)

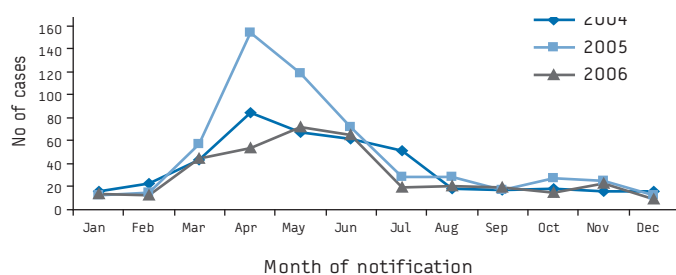
Data source: Computerised Infectious Disease Reporting (CIDR), Health Protection Surveillance Centre (HPSC), Ireland

Contact with farm animals and visiting farms are known risk factors for sporadic cryptosporidiosis. Living in an area with poorer water treatment has also been reported as a risk factor for cryptosporidiosis. Moreover, a high proportion of rural dwellers in Ireland are served by private wells, many of which would not have barriers against *Cryptosporidium*. We believe that the lower incidence reported from the HSE-East may reflect at least in part a true difference in risk between urban and rural dwellers.

There was also a noticeable difference between the age distribution of cases in different HSE areas. For example, the HSE-East reported a higher proportion of adult cases older than 15 years of age among their relatively small number of cases - almost three quarters of cases reported in the period 2004-2006 (Table 2). Interestingly, several of these cases were reported as travel-associated. In contrast, only 5-8% of cases in the HSE-West and HSE-Mid West during the same period were above the age of 15 years. Some of this variation is likely to be due to surveillance bias as discussed above.

FIGURE 1

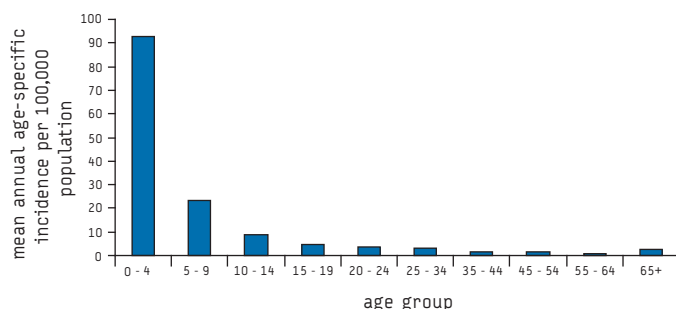
Seasonal distribution of cryptosporidiosis cases, Ireland, 2004-2006



Data source: Computerised Infectious Disease Reporting (CIDR), Health Protection Surveillance Centre (HPSC), Ireland

FIGURE 2

Mean annual age-specific incidence rates of cryptosporidiosis, Ireland, 2004-2006



Data source: Computerised Infectious Disease Reporting (CIDR), Health Protection Surveillance Centre (HPSC), Ireland

Transmission routes for cryptosporidiosis in Ireland

Outbreak surveillance data provide important information on disease transmission routes. Between July 2001 and December 2006, 23 outbreaks of cryptosporidiosis were reported to the HPSC, including nine family outbreaks and 14 general outbreaks (outbreaks involving cases who were not part of the same family) (Figure 3). Water was reported as a suspected source for 14 outbreaks - drinking water for twelve outbreaks (11 general and one family outbreak) and recreational water for two family outbreaks. For general outbreaks, drinking water was the most common suspected transmission route, while for family outbreaks person-to-person transmission appears more important (Figure 3). For one waterborne outbreak during this time period, there was analytical evidence demonstrating a statistically significant increase in the likelihood of disease in those who consumed tap water [13].

Some of the best available evidence internationally on the epidemiology of *Cryptosporidium* is from the United Kingdom [3,8,14-19]. Evidence has been gathered through a combination of outbreak surveillance, case control studies and speciation of positive human specimens from routine human surveillance. The most commonly reported outbreak transmission routes in England and Wales have been public water supplies and swimming pools [3,14,19]. Swimming pools have not been reported as a location for outbreaks for cryptosporidiosis in Ireland during this time period, although there were cases reported associated with an outbreak linked to a swimming pool in Spain in 2003 [20].

Since surveillance for human cryptosporidiosis began in 2004 following the revision of the list of notifiable diseases [21], much has been learned about the epidemiology of human cryptosporidiosis in Ireland. There remain, however, a number of issues on which further data would be advantageous. In the United Kingdom, speciation of human isolates has proved invaluable in elucidating the epidemiology of infection in conjunction with case control studies and other surveillance data [19]. In the time period 2004-2006 in Ireland, typing of positive human specimens was only rarely undertaken except in the event of outbreaks. A small number of hospital laboratories in Ireland have started to have *Cryptosporidium*-positive specimens typed on a routine basis

TABLE 2

Age distribution of cryptosporidiosis notifications, Ireland, 2004-2006, by Health Service Executive (HSE) area

HSE-area	<5 yrs	5-14 yrs	15+ yrs	Total
East	13 (19.4%)	5 (7.5%)	49 (73.1%)	67
Midland	99 (72.3%)	25 (18.2%)	13 (9.5%)	137
Mid-West	103 (66.0%)	41 (26.3%)	12 (7.7%)	156
North-East	68 (56.7%)	24 (20.0%)	28 (23.3%)	120
North-West	62 (55.9%)	33 (29.7%)	16 (14.4%)	111
South-East	134 (56.1%)	34 (14.2%)	71 (29.7%)	239
South	152 (60.1%)	59 (23.3%)	42 (16.6%)	253
West	208 (75.4%)	53 (19.2%)	15 (5.4%)	276
Total	839 (61.7%)	274 (20.2%)	246 (18.1%)	1359

Note: includes only cases where information on age was available
Data source: Computerised Infectious Disease Reporting (CIDR), Health Protection Surveillance Centre (HPSC), Ireland

since 2007, and the results of these studies will provide the first systematic evidence of the relative importance of different species in this country. Provisional results from these studies suggest that *C. parvum* is more common than *C. hominis* among sporadic cases in Ireland (unpublished data). A research study by Zintl *et al* [22] concurs with this.

Another issue that needs to be assessed quantitatively is the relative importance of travel-associated infection. In the United Kingdom, international travel is believed to play an important part in the epidemiology of *Cryptosporidium* in autumn months. The available data at national level in Ireland on 'country of infection' is limited but has been improving over time. Given that a number of community outbreaks have been reported in Ireland, it is likely that indigenous cases form the majority of cases, however, this would be important to verify, and hopefully can be achieved with time.

Increasingly, circumstantial evidence from outbreak surveillance data in Ireland suggests that drinking water and person-to-person spread are important transmission routes during outbreaks. Elsewhere, personal risk factors for sporadic cryptosporidiosis have variously included factors such as international travel, contact with cattle, visiting farms, contact with another person with diarrhoea, swimming in a public swimming pool, freshwater swimming, having a chronic medical condition, and drinking unboiled tap water [15-16, 23-26]. Socio-economic risk factors such as living in an area which has a high proportion of individuals of higher socio-economic status, living in an area with a high rate of manure application to land, or living in an area with poorer water treatment, were reported by Lake *et al* [27]. A key advantage of the Hunter study was that analyses of the case control study were undertaken separately for *C. parvum* and *C. hominis* cases permitting determination of the species-specific risk factors [15]. The only factor which significantly

increased the risk of *C. parvum* infection was touching or handling farm animals, while international travel, spending time sleeping or sitting on the ground and nappy-changing contact with a child less than five years of age were associated with *C. hominis* infection. No studies have been published in Ireland on the risk factors for sporadic cryptosporidiosis. Further research on this topic would be very valuable, in particular in the light of the seasonal distribution of cases and the likely difference in epidemiology that this suggests.

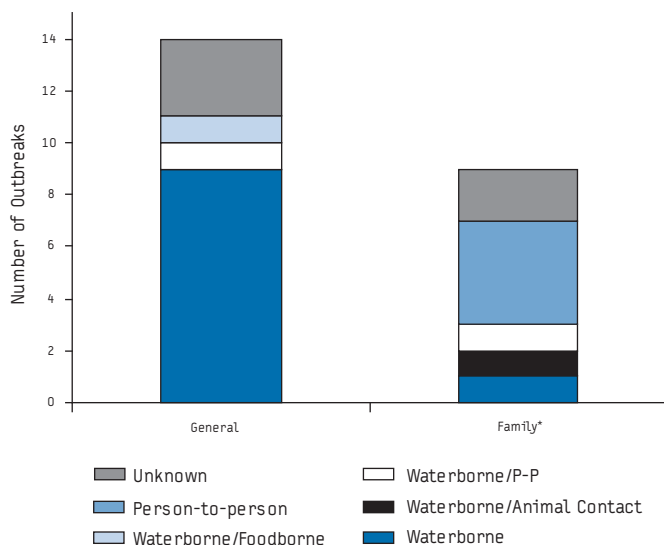
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FIGURE 3
Outbreaks of cryptosporidiosis in Ireland, July 2001 – December 2006, by suspected transmission route (n=23)



*Note: recreational water was suspected in two of the three family outbreaks where water was believed to have played a role in transmission
 Data source: Computerised Infectious Disease Reporting (CIDR), Health Protection Surveillance Centre (HPSC), Ireland

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Surveillance and outbreak reports

AN OUTBREAK OF SALMONELLA TYPHIMURIUM INFECTIONS IN DENMARK, NORWAY AND SWEDEN, 2008

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In November-December 2008, Norway and Denmark independently identified outbreaks of *Salmonella* Typhimurium infections characterised in the multiple-locus variable number of tandem repeats analysis (MLVA) by a distinct profile. Outbreak investigations were initiated independently in the two countries. In Denmark, a total of 37 cases were identified, and multiple findings of the outbreak strain in pork and pigs within the same supply chain led to the identification of pork in various forms as the source. In Norway, ten cases were identified, and the outbreak investigation quickly indicated meat bought in Sweden as the probable source and the Swedish authorities were alerted. Investigations in Sweden identified four human cases and two isolates from minced meat with the distinct profile. Subsequent trace-back of the meat showed that it most likely originated from Denmark. Through international alert from Norway on 19 December, it became clear that the Danish and Norwegian outbreak strains were identical and, later on, that the source of the outbreaks in all three countries could be traced back to Danish pork. MLVA was instrumental in linking the outbreaks in the different countries and tracing the source. This outbreak illustrates that good international communication channels, early alerting mechanisms, inter-sectoral collaboration between public health and food safety authorities and harmonised molecular typing tools are important for effective identification and management of cross-border outbreaks. Differences in legal requirements for food safety in neighbouring countries may be a challenge in terms of communication with consumers in areas where cross-border shopping is common.

Introduction

The endemic level of salmonellosis in three Nordic countries Norway, Sweden and Finland is low. The majority of cases are acquired during travel abroad, and among domestic cases, *Salmonella* Typhimurium is the most common serovar [1]. In Norway approximately 70-80% of notified cases of salmonellosis are acquired abroad, and among patients infected in Norway, *S.* Typhimurium is the most common serovar with generally 5-15

domestic cases reported monthly [2]. In Sweden the situation is similar, with the majority (70-80%) of human cases being travel-related and *S.* Typhimurium being the most common domestic serovar [3]. Norway, Sweden and Finland have special rules concerning trade of meat and meat-products within the European Union (EU), requiring that each consignment produced in another EU Member State and destined to be sold in these countries must be accompanied by a certificate stating that the product has been analysed for the presence of *Salmonella* according to a defined procedure [4]. In Denmark, both the epidemiological situation and legal requirements regarding *Salmonella* in meat is different. An estimated 40-50% of *Salmonella* cases are travel-related [5] and *S.* Typhimurium is traditionally the second most frequent serotype after *S.* Enteritidis. However, a series of large *S.* Typhimurium outbreaks occurred in Denmark in 2008 [6], making *S.* Typhimurium the most frequent serotype in 2008 [7].

In order to rapidly identify possible outbreaks, Norway and Denmark routinely genotype all *S.* Typhimurium patient isolates with the multiple-locus variable number of tandem repeats analysis (MLVA) method [8]. The reference laboratory in Norway receives isolates from human cases as well as from animals, foods and feed. The database on MLVA-typed isolates currently comprises MLVA profiles from more than 3,000 isolates from both human and non-human sources, collected since 2004. In Denmark, human *S.* Typhimurium isolates are routinely MLVA-typed and phage-typed, while food and animal isolates are only phage-typed. In outbreak investigations food isolates with matching antibiograms and matching or related phage types are MLVA-typed. The Danish database currently comprises more than 4,000 human and non-human isolates collected since December 2004. If clusters of cases with a specific MLVA profile are detected, an investigation is initiated to verify and control the outbreak. In Sweden, isolates of *S.* Typhimurium from human, animals and feed are routinely phage-typed, and food isolates are phage-typed upon request. Until early

2009, MLVA-typing was only performed on clusters of various phage types of particular interest from an epidemiological point of view.

Outbreak detection

On 7 November 2008, the Danish Statens Serum Institut (SSI) registered a cluster of eight recent cases of *Salmonella* Typhimurium with the same new distinct MLVA profile. On the same day the Zoonosis Laboratory at the Danish National Food Institute identified two isolates from pork products with the same MLVA profile.

On 4 December 2008, the Norwegian Institute of Public Health (NIPH) also registered a cluster of six cases with *S. Typhimurium*-infection with a new, distinct MLVA profile, all submitted during the previous month.

In both countries independent outbreak investigations were initiated in order to identify the source of these infections and prevent further spread. Investigations in Sweden were undertaken later, following information from Norway on the possible source of infection in meat purchased in Sweden.

Methods

Case definition

For the purpose of outbreak investigation, a common case definition was used in all three countries. A case was defined as having a laboratory-confirmed *Salmonella* Typhimurium infection with the distinct MLVA-outbreak profile, and with illness onset after 1 September 2008. The MLVA profile was assigned as 3-12-4-13-2 (using allele numbers suggested by Lindstedt et al.); sizes of fragments were STTR9: 181 bp, STTR5: 275 bp, STTR6: 319 bp, STTR10: 370 bp, STTR3: 490 bp [8].

Patient interviews

In Denmark, seven among the initial cases were interviewed using a trawling questionnaire with focus on consumption of pork and pork products, and remaining cases were interviewed using a short standard questionnaire. In Norway, all cases were interviewed with a detailed standard trawling questionnaire for food-borne outbreaks. In Sweden, the cases were interviewed regarding general risk exposure for *Salmonella* with additional questions on supermarkets visited and travel history to either Norway or Denmark, as well as a focus on pork products consumption.

Microbiological investigation

In Denmark, due to a large ongoing outbreak of *S. Typhimurium* U292 [6], a temporarily intensified programme for surveillance of *Salmonella* isolates from food production facilities was set up in September 2008. As a result of this programme, a number of isolates were referred to the Danish National Food Institute for analysis. Isolates were phage-typed, and all isolates with phage types matching the human isolates were MLVA typed.

In Norway, food products from patients' homes, identified to be at risk, were sampled and tested. When preliminary results indicated presence of *Salmonella*, the isolates were sent from the local microbiological laboratories to the reference laboratory at NIPH for verification and MLVA-typing.

In Sweden, *Salmonella* Typhimurium RDNC and later also U302 isolates (due to relatedness with the phage type reactions in the Norwegian and Danish isolates) collected from patients and food products during late 2008 and early 2009 were typed with MLVA.

Environmental investigation

In all countries, detailed information regarding place and date of purchase of suspected products was collected from the

patients in order to trace the contaminated consignment. In Denmark, the Food Safety Authority obtained detailed information regarding distribution of contaminated batches. In Sweden, the local environmental health authority visited the relevant shops, checked their hygiene routines, traced the origin of suspected meat products and checked the *Salmonella* certificates on imported meat consignments.

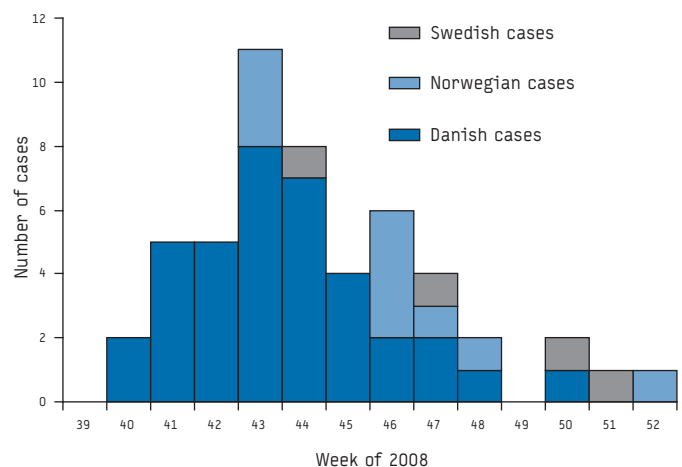
Results

The investigation in Denmark

A total of 37 cases were confirmed. The outbreak strain was fully sensitive to all antibiotics tested and determined to be phage type U288 or RDNC. The majority of patients became ill in October and November (Figure 1). The median age of the cases was 54 years (range 1-86 years) and 15 were female. Four patients died, all were older than 75 years, and suffered from underlying illnesses. The precise causes of death could not be established, and it remains unclear to what degree the *Salmonella* infection contributed as a cause of death.

Within two weeks following the detection of the outbreak (on 7 November), the outbreak strain was identified among *S. Typhimurium* isolates from Danish pork meat (6 times) and pork products (4 times; raw pork sausage, raw pork roulade and twice in minced pork). The pork and pork products originated from 6 different companies. Of these, one company (in which most samples with the outbreak strain were found) was a cutting plant that supplied meat to the other five companies all of which were wholesalers. In addition, the outbreak strain was found in samples from a sow herd in December 2008. In the period during which the outbreak took place, pigs originating from the sow herd, but reared at other farms, were mainly slaughtered at two different slaughterhouses. Subsequently, it was also recognized that during 2008 there was an increased *Salmonella* seroprevalence in some of these slaughter pig herds. This was detected through the Danish serological *Salmonella* surveillance programme [5,9]. One of the slaughterhouses supplied meat to the incriminated cutting plant.

FIGURE 1
Cases of *Salmonella* Typhimurium in an international outbreak affecting Denmark (n=37), Norway (n=10) and Sweden (n=4), October-December 2008, by week of onset of illness (n= 51)



The majority of cases (30) were from Zealand (Figure 2); relatively many from the less densely populated south-western part of the island. The culture-positive meat processing plant, the culture-positive sow herd, the majority of related slaughter pig herds in addition to the two slaughterhouses, were also located in the same part of the country.

The results of the patient interviews were compatible with the hypothesis that fresh pork meat and different pork products originating from the two above mentioned slaughterhouses were the source of the outbreak. The particular MLVA-pattern was found for the first time in Denmark in three patients with onset dates in June and July, 2008. They were not counted among the outbreak cases, though it remains possible that their infections also originated from the same pig herds.

The incriminated cutting plant and one of the two slaughterhouses had been selling pork and beef to a number of Swedish establishments. No direct trade link between the Danish

FIGURE 2
Place of residence of human cases of *Salmonella* Typhimurium (dark grey, n=51) and companies/shops (white, n=8) where meat was bought by cases or found positive with the outbreak strain of *Salmonella* Typhimurium, Denmark, Norway, Sweden, 2008



cutting plant and the slaughterhouse on one hand and the Swedish shops on the other was evident in the sales register from the Danish establishments. However intermediary establishments in Sweden were involved in distributing the meat. Links from Denmark to the Swedish shops were thereby established (Figure 3).

The investigation in Norway

Ten cases were verified with the outbreak strain. The outbreak strain was fully sensitive to all antibiotics tested and determined to be phage type RDNC. The patients were all adults (21-80 years) living in the south-eastern part of Norway and their illness onset was between the end of October and the end of December (Figure 1 and 2). Eight patients reported that during the week before illness onset, they had consumed minced meat purchased at shopping centres located across the border in Sweden. Four of them remembered having eaten raw, rare or undercooked minced meat. Several had tasted raw minced meat while preparing food. The minced meat was either a mix of pork/beef or only beef, but most said they were not sure about this. The outbreak strain, with the rare MLVA profile, was isolated from samples of minced meat from the homes of two patients, but since the product had been repacked in patients' households, the original wrapping with product information was not available. However, one of the patients provided a bank printout that confirmed the exact place (retail outlet) and date of purchase, thereby facilitating further traceback along the food chain.

International alerts

On 15 December, after receiving completed questionnaires from five Norwegian patients, all of whom reported consumption of meat bought in Sweden the week before illness onset, NIPH notified the Swedish Institute of Infectious Disease Control (SMI) about the outbreak and asked if they had seen similar isolates of *S. Typhimurium*. In reply, SMI reported no findings of the specific RDNC phage pattern. On 19 December, an urgent inquiry was sent through the Food- and Waterborne Diseases network at the European Centre for Disease Prevention and Control (ECDC), and in response, Denmark reported the ongoing phage type U288 outbreak with identical MLVA-profile.

The investigation in Sweden

In Sweden four cases were confirmed with the outbreak MLVA profile. The patients were all adults, and three were in their 50s. They fell ill between October and December (Figure 1) and were from three different counties in the south of Sweden (Figure 2). One patient had been living and working in Copenhagen before disease onset and was most likely infected there. These cases were identified following MLVA-typing of recent patient-isolates belonging to phage type U302.

On 23 December, the Swedish National Food Administration found that the shops the Norwegian patients had visited, were selling pork from three Danish companies, one of which was the cutting plant incriminated during the investigations in Denmark. The two positive minced meat samples from the Norwegian patients contained only beef according to information from the shops. However, cross-contamination from other sources could have occurred during mincing in the shops. The Swedish environmental health authority could not identify any faults or breaches in the routines of the shops, and there was no meat from the relevant time period available for sampling. They could also confirm that the companies had thoroughly checked the *Salmonella* certificates of all consignments from other countries.

Sweden found two isolates with the outbreak strain from minced meat. The first minced meat sample was taken in November from a grocery store in the south of Sweden (Figure 2). A follow-up sample taken from the meat-grinder in the shop one week after the first, was also positive for *S. Typhimurium*, and both had the outbreak MLVA profile. This indicates that there was a persistent contamination of the grinder. This grocery store had been selling pork from the incriminated Danish cutting plant on some occasions during October and November. None of the four Swedish patients had been to this store nor to the shops visited by the Norwegian patients. However one of them had bought meat in another Swedish shop receiving meat from the above mentioned Danish slaughterhouse.

Product tracing

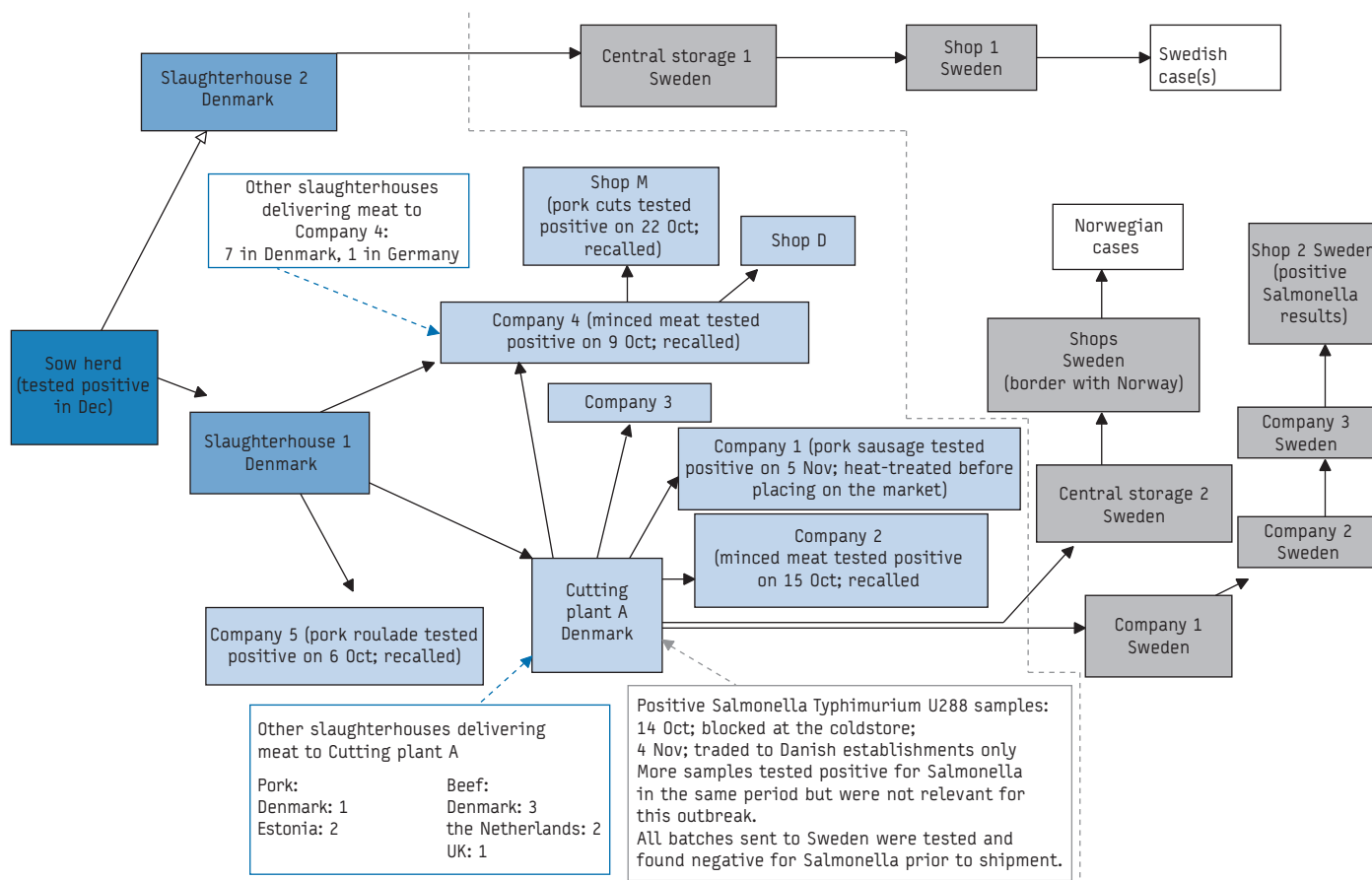
Product trace investigation revealed the trade route for meat from the Danish cutting plant to shops in Sweden, both to shops near the border where the Norwegian cases had bought meat and to another one where minced meat samples had tested positive for *Salmonella*, thus, confirming the link between Danish meat and positive findings of *Salmonella* in the environment and minced meat samples. Furthermore tracing of products from the second Danish slaughterhouse revealed a link to yet another Swedish shop indicating a possible second route of dissemination of contaminated meat to Sweden (Figure 3).

Control measures

In Denmark, following the multiple findings of the outbreak strain in food products, investigations were undertaken at the facility producing the raw pork sausage, as well as the cutting plant and the slaughterhouses that supplied meat to the cutting plant. Samples were taken for analysis from the cutting plant and the sausage-producing facility. The microbiological analysis did not identify *Salmonella*, which suggests that these facilities did not harbour persistent infections in their production environments. Furthermore, all analytical reports concerning batches of meat sent to Sweden from the cutting plant and one of the two slaughterhouses, were reviewed for sampling consistency according to the legal requirements. No known positive batches were put on the Swedish market, and the requirements concerning sampling and analyses were fulfilled.

In Norway, on 7 January, NIPH and Norwegian Food Safety Authority (NFSA) published an Internet update on the outbreak, in which the Norwegian public was informed that the *Salmonella* outbreak strain had been detected in minced meat bought in Sweden, and consumers were advised about safe handling of meat. This was followed by international alerts by the NFSA through the Rapid Alert System for Food and Feed (RASFF) [10] on 8 January, and by NIPH through the Early Warning and Response System (EWRS) on 9 January.

FIGURE 3
Trade route diagram for establishments involved in the outbreak of *Salmonella* Typhimurium in Denmark, Norway and Sweden, 2008



In Sweden, no further measures were taken, since the contaminated meat was not available in the shops anymore, and all environmental control samples from the shops were now negative.

Discussion

We report an outbreak of *S. Typhimurium* affecting three Nordic countries. The link between the outbreaks was established thanks to cross-border information exchange. Outbreaks that seem local may have international connections, and therefore early alerts are important for efficient investigation and management of such events. In the example described here, MLVA typing was instrumental in identifying and defining the outbreaks, in revealing the possible food/animal sources, and in establishing the link between the outbreaks in Denmark and Norway, and subsequently Sweden. We note with interest that the outbreak strain was communicated between the countries as belonging to three different phage type assignments: U288 in Denmark, RDNC in Norway and U302 in Sweden. Thus, in this outbreak, it would have been misleading if only phage type information had been exchanged between the countries. MLVA-typing has previously been successfully used in outbreak investigations in the Nordic countries [11-14]

In Sweden four human cases with the same MLVA profile were identified in addition to isolates from minced meat samples from a grocery store. No link between the cases and this grocery store or the shops identified by the Norwegian cases, could be established. However one of the Swedish patients reported buying meat in another Swedish shop, which was receiving meat from the incriminated Danish slaughterhouse. This shop belongs to a retail chain and is supplied from a central storage facility, and a possible connection to two more of the Swedish cases is likely. The fourth case was most probably infected during a stay in Denmark.

In Sweden, MLVA typing of human *S. Typhimurium* isolates had only been performed when epidemiologically relevant. However, as a result of this outbreak, as well as the Swedish experience with analysis of several clusters of other phage types of *S. Typhimurium* during 2008, it has now been decided to use MLVA to type all domestic human and other relevant *S. Typhimurium* isolates.

This outbreak also calls attention to some aspects concerning trade between countries with different endemic situation and regulations regarding *Salmonella*. Due to the endemic situation in Sweden and Norway (and also Finland) with a very low prevalence of *Salmonella* in domestic food, additional sampling for *Salmonella* is required on all fresh meat consignments sold to these countries from other EU/EEA countries [4].

The EC regulation on microbiological criteria for foodstuffs, says that when testing against set food safety criteria gives unsatisfactory results, the product or batch of foodstuffs shall be withdrawn or recalled [15]. However, products placed on the market, which are not yet at retail level and which do not fulfil the food safety criteria, may be submitted to further processing by a treatment eliminating the hazard in question provided that this use does not pose a risk for public or animal health and that this use has been authorised by the competent authority [16].

In Sweden, meat processing plants can get permission to heat-treat fresh meat contaminated with *Salmonella*, thereby eliminating the health hazard. However it is very uncommon that companies apply for this. Norway is due to implement EU harmonised

legislation in this area, thus in near future the same option to sanitize contaminated meat applies.

In this outbreak, the source of the Norwegian cases could be traced back to shops located in Sweden close to the border with Norway, selling meat from Danish producers. These shops target Norwegian consumers. Information about the outbreak and the source was made public in Norway, since several of the cases in Norway reported consuming raw meat. This illustrates some of the challenges regarding food safety advice to consumers in areas where cross-border shopping is common, as the consumers may not be aware of information about or recalls of products in neighbouring countries.

In conclusion, this outbreak illustrates that good international communication channels, early alerting mechanisms, inter-sectoral collaboration between public health and food safety authorities and harmonised molecular typing tools, are important for effective identification and management of cross-border outbreaks.

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Surveillance and outbreak reports

AN OYSTER-ASSOCIATED HEPATITIS A OUTBREAK IN FRANCE IN 2007

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Following the notification of nine hepatitis A cases clustered in the Côtes d'Armor district in northwestern France, epidemiological, environmental and microbiological investigations were set up in order to identify the source and vehicle of contamination and implement control measures. In total, 111 cases were identified in the outbreak, all of whom lived or had stayed as tourists in the Côtes d'Armor district. Of the cases, 87% had eaten raw shellfish, and 81% specifically oysters. Traceback investigations carried out on raw shellfish consumed by the cases showed that the raw shellfish originated from a single shellfish farm. The shellfish were probably contaminated either in the submersible tanks or in a depuration land-based tank where they were stored. The source of contamination was not identified but shellfish could have been tainted by sewage overflows or by wastewater releases from a polluted storm sewer close to the shellfish farm or from on-site sanitation facilities. To prevent future hepatitis A outbreaks due to shellfish consumption from this area, hazards specific to each farm should be analysed. Timely information on sewage overflows should also be part of communities' efforts regarding sewage collection and treatment.

Introduction

Hepatitis A virus (HAV) is transmitted via the faecal-oral route by either person-to-person contact or consumption of contaminated food or water. The incubation period ranges from 15 to 50 days with a mean of 30 days. The disease is usually diagnosed by detection of immunoglobulin M antibodies to hepatitis A (IgM anti-HAV) in the serum.

In France, surveillance of acute hepatitis A has been based on mandatory notification since November 2005. The notification form collects information on socio-demographic, clinical, biological characteristics and main at risk exposures to HAV infection. The incidence of reported cases of hepatitis A (notification rate) was 2.2/100,000 in 2006 and 1.6/100,000 in 2007 [1].

Between 14 and 21 August 2007, nine hepatitis A cases were notified to the district health services of the Côtes d'Armor (Brittany). Eight of them lived or had recently stayed in the northwestern area of the district, including four who were closely clustered near the same seaside resort - Paimpol bay - and seven who reported having eaten oysters. An investigation was carried out to confirm the outbreak, to assess its size, to identify the source and vehicle of contamination, and to implement appropriate control measures.

Methods

Epidemiological investigation

A case was defined as a person with IgM anti-HAV detected in the serum between 1 July and 15 October 2007 who had stayed in the Côtes d'Armor district in the six weeks before the onset of symptoms, whether as resident or as tourist.

The cases were identified through mandatory notification. In addition, in the Côtes d'Armor district, biologists, general practitioners, paediatricians, gastroenterologists and emergency physicians were informed about the current outbreak and asked to notify HAV cases.

The cases were interviewed by telephone using a standardised questionnaire about date and symptoms, place of residence, date and place of stay in the Côtes d'Armor district for tourists and possible exposures defined as food consumption and place of purchase, school or child care center attendance, travel, household contact with a case, participation in specific events or activities and bathing in recreational water.

Microbiological analysis

The biologists were asked to send the cases' sera to the National Reference Centre (CNR) for genotyping and phylogenetic analysis. A 452 base-pair fragment encompassing the VP1/2A junction was amplified and the phylogenetic tree was constructed with the MEGA software. The HAV genotype was determined using referent

sequences whose GenBank accession numbers were X75215, AB020264, AF357222 for genotype IA, M14707 and M20273 for genotype IB, AY644676 for genotype IIA, AY644670 for genotype IIB, AY644337 and AJ299464 for genotype IIIA, D00924 for genotype V. Two other sequences, published by the CNR in the Event (Enteric Virus Emergence, New Tools) database were added for genotype IIIA: 2004-AUV-SEF-GIII and 2004-PB-CL-GIII.

Traceback investigations

Traceback investigations were carried out on suspected contaminated food for cases who had stayed briefly (less than 15 days) in the Côtes d'Armor district during the estimated at risk period and for cases included in clusters with common meals (as at family events). For these cases, places and dates of purchase and consumption of the suspected food could be determined precisely.

Environmental investigation

In order to determine the origin of the contamination of the suspected shellfish, the functioning of the sewage system and wastewater treatment plants located around Paimpol bay during June and July 2007 was investigated.

Laboratory testing for HAV was performed on shellfish samples collected between 24 August and 24 October from shellfish beds of Paimpol bay and on storage tanks located on the foreshore. Wild oysters around the bay, storm sewage, sludge, raw and treated sewage were also sampled for microbiological analyses. Viruses were extracted from shellfish tissues or concentrated from 40 ml of water samples before extraction and purification of nucleic acids [2,3]. All steps were controlled by adding a mengovirus at the first step of the extraction (extraction efficiency control) or external control RNA (inhibitors removal controls) in the real-time RT-PCR mix. Real-time RT-PCR was done as described [4].

Results

Epidemiological investigation

One hundred and eleven cases were identified. The symptoms occurred between 25 July and 9 October (weeks 30 to 41), mainly during weeks 32 and 33 (Figure 1). One hundred and six cases were interviewed.

Fifty-seven cases were tourists, either French or foreigners, including six cases who were living abroad: in Germany (1), the Netherlands (1) and Switzerland (4). The date of stay was collected for 53 tourists: 39 (74%) were present in the Côtes d'Armor district on 13 July, 46 (87%) on 14 July and 43 (81%) on 15 July. Twenty-six tourists were present in the area exclusively during the 7 to 22 July period.

The fifty-four (51%) remaining cases were living in the Côtes d'Armor district (Figure 2). The places of residence or stay in the district were clustered in the northwestern area near the towns of Paimpol and Lannion (Figure 3).

Among the 106 interviewed cases, 54 were men and the median age of cases was 40 years (range: 4 to 82 years). Eighty-eight cases (83%) reported jaundice and 28 (26%) were hospitalised. No death was reported.

At risk exposures were documented for 89 cases that occurred between 25 July and 2 September (weeks 30 to 35). All cases had eaten molluscan shellfish in the Côtes d'Armor district. Seventy-seven (87%) had eaten bivalve molluscs that are usually eaten

raw (oysters, warty venus, carpet shells, european bittersweets); 72 (81%) cases including the 26 cases who stayed briefly in the Côtes d'Armor district had eaten oysters (Table). Moreover, three clusters with common meals were identified among seven cases:

FIGURE 1
Cases of hepatitis A among residents and tourists in the Côtes d'Armor district outbreak, France, 2007, by week of onset (n=108)

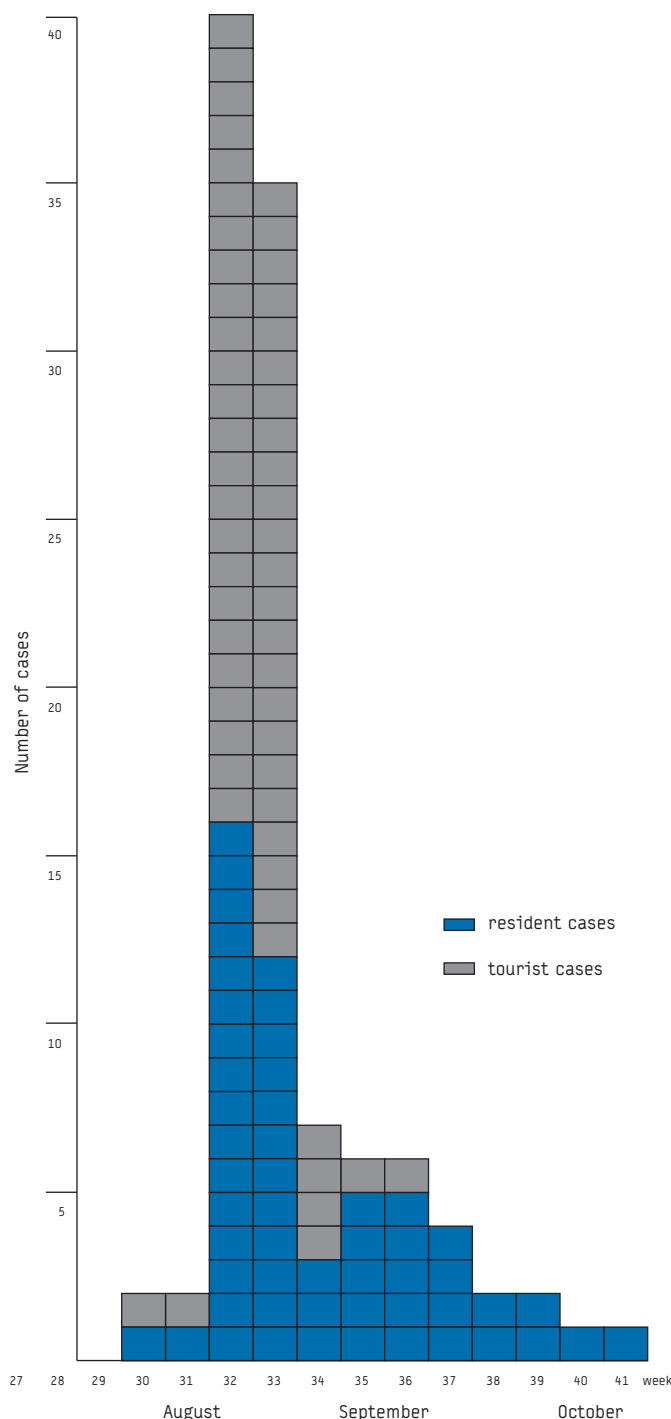


FIGURE 2

Place of permanent residence for cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007 (N=111)

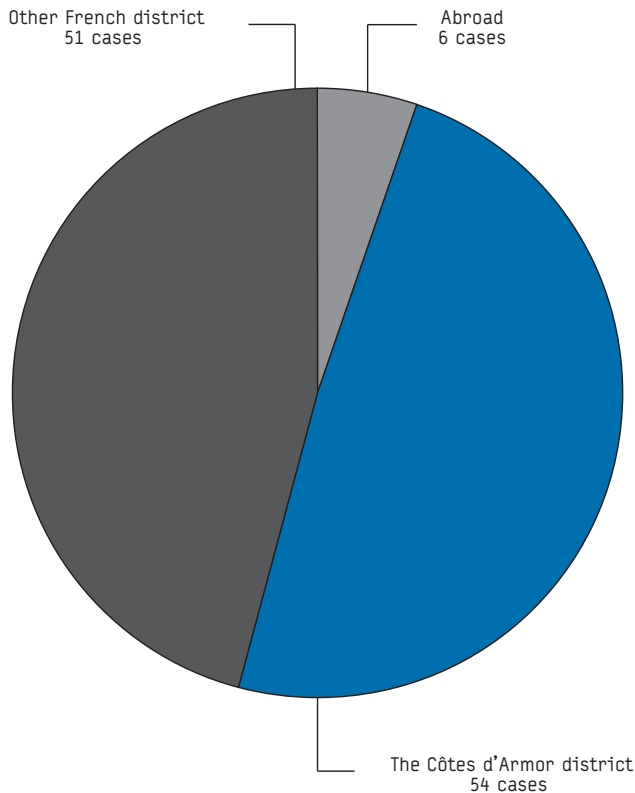
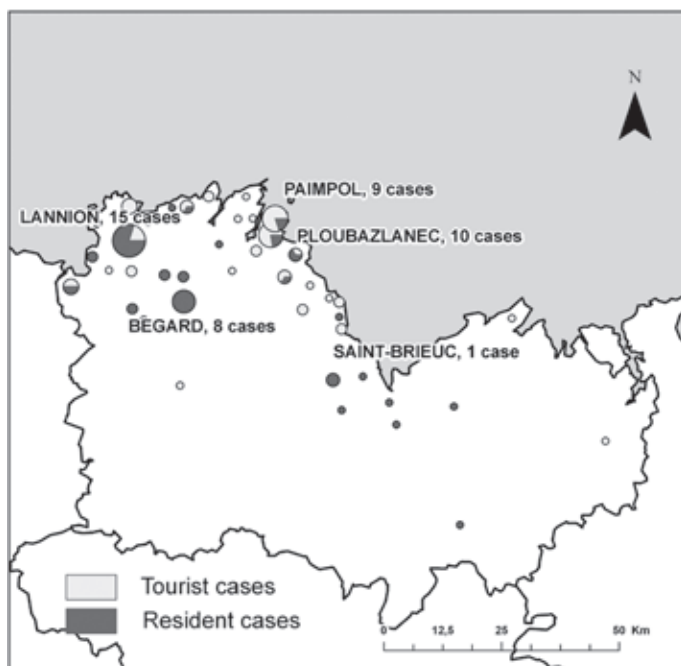


FIGURE 3

Place of residence or stay for cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007, by municipality (n=107)



two cases were linked to a meal on 13 July, two cases to a meal on 15 July and three cases to another meal on 15 July. Six of the seven cases had eaten raw shellfish.

The consumptions of raw vegetables, herbs and unpeeled fruits were documented for 80 cases. Tomatoes and lettuce had been consumed by 74 (92%) and 72 (90%) cases respectively. The other at risk exposures concerned less than 60% of the cases.

Microbiological analysis

Among the 71 sera received at the CNR, viral RNA was detected for 68 sera; 66 sequences were identical over an analysable 425 base-pair fragment and were clustered with genotype IIIA strains. The two other sequences differed only by one nucleotide change.

Traceback investigations

Considering the epidemic curve, the incubation period and the dates of stay of the affected tourists, it was estimated that the contaminated shellfish were probably consumed between 7 and 22 July. Traceback investigations were carried out for 20 of the 26 cases who had eaten oysters and stayed in the Côtes d'Armor district exclusively during this period. Seventeen cases had bought oysters from one farm located at the north of Paimpol bay, partly on the farm itself and partly through restaurants, supermarkets or fish shops. Although there were seven farms in the bay at the time of the outbreak, 13 of the 17 cases had exclusively consumed oysters originating from this particular farm. Among the three cases who had not consumed the oysters from the suspected farm, two had eaten other raw shellfish from the same farm and the last one had consumed wild oysters picked up near the farm. The raw shellfish consumed by six of the cases linked to the three clusters were exclusively originating from the previously mentioned suspected farm. On this farm, the shellfish from different production areas had been stored in submersible storage tanks up to 10 days and then depurated during 48 hours in a land-based tank before being sold. The farm was located near a storm sewer outlet at the north of the bay.

Environmental investigation

Sanitation of the bay

A separate sewage system with 16 pumping stations collects the wastewaters of Paimpol and Ploubazlanec in the north and northwest areas of the bay. The treatment plant is an activated sludge plant; a buffer tank is used to regulate and adapt the sewage inflow to the plant's capacity (22,000 inhabitant equivalents). There is no disinfection treatment. The disposal is located near Paimpol harbor entrance at the very far end of the bay. Local streams disperse the treated effluents towards the north seashore of the bay. This seems to have an impact on the bathing water quality at the two beaches closest to the disposal and each has once been classified C (water liable to be temporarily polluted) during the 2001 and 2006 summers. The months of June and July 2007 were much rainier than the same months of the 1997-2006 period: 85.4 mm vs 40.2 mm in June and 88.2 mm vs 51.5 mm in July. The monitoring of the sewage collecting and treatment installations revealed sewage overflows due to heavy rains: 300 m³ of diluted raw sewage discharged from the buffer tank on 24 June which was a neap tide day, and overflows from eight different pumping stations on 23 July. At the north of the bay, 40 houses, whose connection to the sewerage system is scheduled in the next few years, were served by on-site sanitation systems. Whether the facilities were working or not was not known at the time of the

investigation. A storm sewer outlet was also identified in the vicinity of the suspected shellfish farm.

Microbiological results

For viral investigations, a total of eight shellfish samples, four sludge and 24 water samples were analysed. All these samples were negatives for HAV RNA using the primer set and probe located in the 5'NC region. All the controls such as extraction efficiency and absence of inhibitors were verified, eliminating false negative result option.

Discussion and conclusion

We described a large hepatitis A outbreak which was the largest reported since the beginning of the mandatory notification in November 2005. Previously only two larger outbreaks had been reported in France, in 1992 and 1997 [5,6].

The results of the investigations indicated that it was a common point source outbreak due to the consumption of raw shellfish between 7 and 22 July 2007. The shellfish - mainly oysters - originated from a single farm located at the north of Paimpol.

The consumption of raw shellfish and especially oysters was frequently reported among the cases. The proportion of the cases who consumed oysters (81%) was much higher than in the CALIPSO

study carried out on a population selected for its heavy sea product consumption (61%) [7]. The consumption of raw shellfish among the cases was similar to those observed in previous hepatitis A outbreaks that occurred in Brittany in the Côtes d'Armor district in 1999 (oyster consumption: 88%) and in the Morbihan district in 1992-1993 (raw shellfish consumption: 81%) [6].

Raw seafood, and oysters in particular, are a well-known source of HAV outbreaks in France [5,6,8] and abroad [9,10].

Although more than 90% of the cases reported having eaten lettuce and tomatoes, the diversity of the purchasing places, the ban on using sewage sludge for market gardening, and the absence of wastewater reuse in French agriculture ruled out the hypothesis that raw vegetables might have been the vehicle for HAV in the outbreak described here.

Trace-back investigations revealed that the raw shellfish consumed by cases originated from only one farm and one site of Paimpol bay. During the period of suspected contamination, the French Research Institute for Exploitation of the Sea (Institut français de recherche pour l'exploitation de la mer - Ifremer) shellfish surveillance network (Réseau de Contrôle Microbiologique - Remi) had no evidence of faecal contamination of the shellfish ground areas (data not shown). The investigations suggested that the shellfish were probably contaminated on the farm, in the

TABLE

Molluscan shellfish consumptions during six weeks prior to symptoms onset in cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007 (n=89)

	Cases who stayed exclusively in the Côtes d'Armor district during the period at-risk (7-22 July, 2007), n (%)	Other cases, n (%)	All cases, n (%)
Raw bivalve molluscs:	26 (100)	51 (81)	77 (87)
Japanese oysters (<i>Crassostrea gigas</i>)	26 (100)	46 (73)	72 (81)
Warty venus (<i>Venus verrucosa</i>)	8 (31)	17 (27)	25 (28)
Grooved carpet shells (<i>Ruditapes decussates</i>), Japanese carpet shells (<i>Ruditapes philippinarum</i>)	9 (35)	13 (21)	22 (25)
Common european bittersweets (<i>Glycymeris glycymeris</i>)	4 (15)	8 (13)	12 (13)
Gastropod and other bivalve molluscs			
Mussels (<i>Mytilus edulis</i>)	11 (42)	42 (67)	53 (60)
Periwinkles (<i>Littorina littorea</i>)	9 (35)	25 (40)	34 (38)
Whelks (<i>Buccinum undatum</i>)	10 (38)	22 (35)	32 (36)
Common scallops (<i>Pecten maximus</i>)	6 (23)	14 (22)	20 (22)
Limpets (<i>Patella vulgata</i>)	0 (0)	2 (3)	2 (2)
Common cockles (<i>Cerastoderma edule</i>)	2 (8)	7 (11)	9 (10)
Hard shell clams (<i>Merceneria merceneria</i>)	1 (4)	0 (0)	1 (1)
All gastropod and bivalve molluscs	26 (100)	63 (100)	89 (100)

submersible tanks or in a depuration land-based tank where they had been stored temporarily before being sold. The date of the shellfish contamination was difficult to assess precisely because of the storage periods. It could have occurred anytime between mid-June and the second week of July.

We did not identify the source of the shellfish contamination. Shellfish could have been tainted by sewage overflows or by wastewater releases from polluted storm sewers or from on-site sanitation facilities. The location of the farm near a storm sewer outlet increased the vulnerability of the farm's shellfish storage tanks and of its depuration tank's water supply. Heavy rains may also have contributed to the shellfish contamination as well as the neap tides which hinder the dispersion of effluents.

HAV was not isolated from any of the different samples (shellfish, sewage, storm sewage, sludge, raw and treated sewage). However, the environmental contamination was limited in time and possibly restricted to a small area. Indeed, the sampling was late with respect to the date of the estimated shellfish contamination. The epidemic curve indicated a common point source. However its right skewed shape suggested a person-to-person transmission for the few cases occurring from week 36 onwards.

The number of persons affected by the outbreak was probably higher than reported due to asymptomatic or unrecognised infections. Under-notification of hepatitis A especially outside the Côtes d'Armor district and lack of information on foreign tourists diagnosed abroad could have also contributed to underestimating the outbreak burden.

The phylogenetic analysis attributed the outbreak to a strain belonging to HAV genotype IIIA. This genotype is endemic in South-East and Central Asia (India, Nepal, Sri Lanka and Malaysia) [11], and has also been associated with outbreaks among intravenous drug users (IDUs) in Nordic countries [12]. Before 2004, this was a rare genotype in France though it had been detected in a single patient in a previous outbreak in the Côtes d'Armor district in 1999 [13]. The strain identified in the present outbreak is closely related to a strain responsible for an outbreak in a primary school in Auvergne in 2004 [14] and distinct from previously published sequences.

The previous hepatitis A outbreak (33 cases) that occurred in the Côtes d'Armor district in the winter of 1999 was also linked to the consumption of raw oysters from the Paimpol bay. Raw sewage discharged from the treatment plant and sewage overflows were suspected as the source of contamination of oysters. Two additional outbreaks of hepatitis A due to shellfish consumption have been reported in other French regions [5,8]. The outbreak we investigated occurred during the summer contrary to the other French outbreaks that occurred in winter after the Christmas holidays when raw shellfish is heavily consumed.

Control measures taken by the district authorities included prohibition of recreational shellfish harvest in the bay from 24 August to 4 September. In order to prevent further outbreaks, measures should be implemented to improve the quality of the shellfish. The general improvement of the sewage collecting and treatment installations that has been implemented since the 1999 outbreak should be continued. The monitoring of these facilities should also be used to timely alert shellfish farmers, district health and veterinary services about sewage overflows. We also recommend

assessing specific risks on each farm of the bay to identify specific hazards and possible control measures. These recommendations may contribute to preventing not only hepatitis A [15] but also other food-borne infections.

Our results highlight the fact that in a country with low HAV endemicity, such as France, consumption of raw shellfish can cause a large community outbreak. Increasing susceptibility of the European general population either from low endemic countries or from countries in transition (from moderate to low) is an important public health issue as illustrated in 2008 by reported outbreaks in several European countries [16,17].

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Research articles

HIGH RATES OF COMMUNITY-ACQUIRED, PANTON-VALENTINE LEUKOCIDIN (PVL) - POSITIVE METHICILLIN-RESISTANT *S. AUREUS* (MRSA) INFECTIONS IN ADULT OUTPATIENTS IN GREECE

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Staphylococcus aureus was isolated in 88 (30.8%) of 286 adult patients suffering from various skin and soft-tissue infections examined in the outpatient department of a 650 bed tertiary-care hospital of Athens, Greece between January 2006 and December 2007. Twenty-seven (30.7%) of the *S. aureus* infections were caused by methicillin-resistant *S. aureus* (MRSA). All MRSA isolates were also resistant to tetracycline, fucidic acid and kanamycin, but were sensitive to gentamicin and tobramycin, as well as to cotrimoxazole, chloramphenicol, quinolones, clindamycin and erythromycin. All isolates belonged to staphylococcal cassette chromosome *mec* elements (SCC*mec*) type IV, and were found to carry the *lukF-PV* and *lukS* genes coding for Pantone-Valentine leukocidin (PVL). Pulsed-field gel electrophoresis (PFGE) and *spa*-typing revealed high genetic similarity among all MRSA isolates and with the PFGE pattern of the well-described ST80 clone that seems to be spreading through Europe. The high prevalence of MRSA among *S. aureus* infections in the community signify that empiric therapy in Greece, when clinically indicated, should exclude β -lactam antibiotics. Moreover, the establishment of an active screening for PVL-positive community-acquired (CA)-MRSA carriage and the adoption of a search and destroy strategy for CA-MRSA in all patients admitted with purulent skin and soft-tissue is of high priority in Greece as well as in all European countries which face high rates of CA-MRSA infection.

Introduction

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

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

Materials and methods

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

Laboratory testing

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

Molecular testing

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

Results

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

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

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

Discussion

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

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

In Europe although CA-MRSA skin and soft-tissue infections have been reported from most countries, the prevalence of infections due to CA-MRSA appear to vary across the continent [17-27]. However, reports of prevalence rates of MRSA among *S. aureus* infections are, to the best of our knowledge, lacking. The currently prevailing genetic type among CA-MRSA in Europe is the PVL-positive, t044/ST80-SCC*mec* type IV [12]. In a recent Danish study, travel to or residing in countries abroad, especially in the Mediterranean region, the Balkans (Serbia) and the Middle East, where there is a high prevalence of CA-MRSA infections caused by t044/ST80-SCC*mec* type IV, have been associated with infections with this type

[17]. Moreover, in some European countries strains with USA300 genotype are starting to be isolated with increasing frequency: The emergence of clones that are related to the USA300 has been associated with increasing rates of CA-MRSA in Spain. These clones were primarily isolated from immigrants from South America [25]. Further increasing isolation rates of the USA300 clone have been reported in Germany [26].

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

TABLE

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

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

* Note: All stains were sensitive to tobramycin and gentamicin, cotrimoxazole, chloramphenicol, quinolones, clindamycin, erythromycin.

** M=male; F=female

*** Oxa=Oxacetylcln; Tet=Tetracyclin; Km=Kanamycin; FA=Fucidic acid

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

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

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

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ANTIVIRAL RESISTANCE OF INFLUENZA A (H3N2) STRAINS ISOLATED IN NORTHERN GREECE BETWEEN 2004 AND 2007

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The prevention and control of influenza with vaccines and antiviral drugs is of great importance. M2 inhibitors, amantadine and rimantadine have been extensively used in some countries. The next generation of antiviral drugs, neuraminidase (NA) inhibitors oseltamivir and zanamivir, are being stockpiled for a potential influenza pandemic. The emergence of resistant strains is thus an important issue. The purpose of this study was to examine the sensitivity to M2 and NA inhibitors of Greek influenza A(H3N2) strains isolated during three influenza seasons between 2004 and 2008 and to determine the phylogenetic clades of those strains. M2 and NA sequences of 34 patient isolates were checked for known resistance mutations. In addition, haemagglutinin (HA) sequences were used to determine the phylogenetic relationship between resistant and sensitive strains. All influenza A(H3N2) strains isolated during the season 2004-5 were found susceptible to adamantanes, bearing the S31N mutation, compared to 88% of the strains isolated in 2005-6 and 75% of the strains isolated in 2006-7. Molecular analysis of the HA gene showed a correlation of the mutants with specific phylogenetic clades. No known mutations in the NA or HA gene that have been implicated in resistance to NA inhibitors were found in the A(H3N2) strains isolated in the three influenza seasons. Despite the fact that amantadine is the only drug approved for prophylaxis in Greece, it has not been extensively used. So it seems that resistant strains circulating in the area after 2005 followed the global trend of replacement of susceptible strains by resistant ones. Oseltamivir and zanamivir are currently approved only for therapeutic use in Greece and has not been extensively used either.

Introduction

The prevention and control of influenza through the use of vaccines and antiviral drugs is of great importance. Adamantanes, amantadine and rimantadine, are inhibitors of influenza A virus M2 protein. They were the first antiviral drugs licensed for treatment and prophylaxis of influenza A infections and have been extensively used in some countries. Since the emergence of viruses resistant to M2 inhibitors, many countries have begun to stockpile also the next generation of anti-influenza drugs, the neuraminidase (NA) inhibitors oseltamivir and zanamivir. The emergence of resistant strains is thus an important issue.

Adamantanes inhibit viral replication during the early stage of infection by blocking the ion channel that is formed in the

envelope of influenza virus particles by the M2 protein. Five amino acid substitutions at positions 26, 27, 30, 31 or 34 within the transmembrane domain of the M2 protein have been implicated in loss of sensitivity to M2 inhibitors [1,2]. In many countries like Canada, China, Japan, or the United States (US), adamantanes have been extensively used the past years. The emergence of resistant viral strains is now a major concern, as viral resistance to adamantanes occurs rapidly *in vivo* and *in vitro*. Moreover, the resistant strains that are becoming increasingly common in communities in Asia and the US appear to be virulent, genetically stable and capable of competing with wild-type drug-sensitive strains [3].

Oseltamivir and zanamivir block the function of NA, thus inhibiting the spread of newly formed viral particles. Various mutations have been implicated in the resistance to oseltamivir and/or zanamivir, the most common being amino acid substitutions at positions 119, 222, 274, 292 and 294, and a deletion at positions 244-247 of the NA gene. Further, it is under investigation whether specific mutations at positions 198, 229 and 262 in the haemagglutinin (HA) gene could correlate with the resistance of influenza viruses to NA inhibitors [4-6].

The purpose of this study was to examine the sensitivity to M2 and NA inhibitors of Greek influenza A(H3N2) strains isolated during the influenza seasons between 2004 and 2008, and to determine the phylogenetic relationship between those strains.

Methods

This study included molecular analysis of the M2, NA and HA genes of influenza A(H3N2) strains that were isolated in northern Greece during the last three influenza seasons. Samples from patients with influenza-like illness (ILI) were collected by sentinel general practitioners and outpatient hospital clinics and sent to the National Influenza Centre for Northern Greece for the seasonal influenza surveillance of the four influenza seasons 2004-5, 2005-6, 2006-7 and 2007-8 (December to March). The sentinel network is organised by KEELPNO, the Hellenic Center for Diseases Control and Prevention (HCDCP), and covers the whole of northern Greece.

Clinical samples, which were nasopharyngeal swabs in 2SP (sucrose-phosphate) medium were first cultured in Madine Darby Canine Kidney (MDCK) cells and embryonated chicken eggs. Virus detection was done by haemagglutination test. RNA extraction,

TABLE 1

Primer sequences, used for RT-PCR amplification of regions of the M2, NA and HA genes

Primer	Gene	Binding site (nucleotide position)	Sequence
MF8 forward	M2	8-30	5'- GCAGGTAGATATTGAAAGATGAG - 3'
MR1024 reverse	M2	1,024-1,002	5'- AGAAACAAGGTAGTTTTTACTC - 3'
N2F-1 forward	NA	1-20	5' - AGCAAAAGCAGGAGTGAAGA - 3'
N2R-1407 reverse	NA	1,408-1,389	5' - ATATAGGCATGAGATTGATG - 3'
H3HAF6 forward	HA	6-29	5'- AAGCAGGGGATAATTCTATTAACC - 3'
H3HAR1056 reverse	HA	1,056-1,037	5'- GTTCTCTGGTACATTCCGC - 3'

HA: haemagglutinin; NA: neuraminidase.

TABLE 2

Influenza virus detection and typing, northern Greece, influenza seasons between 2004 and 2008

Influenza season	2004-5	2005-6	2006-7	2007-8	Total
Examined	170	140	128	93	531
Influenza A	63(37%)	11(7,9%)	45(35%)	31(33%)	150(28%)
Influenza B	3(1,8%)	42(30%)	6(4,7%)	15(16%)	66(12%)
Negative	104(61%)	87(62%)	77(60%)	47(51%)	315(59%)

Percentage is calculated from the total number of examined samples.

TABLE 3

Influenza A viruses subtyping, northern Greece, influenza seasons between 2004 and 2008

Influenza Season	2004-5	2005-6	2006-7	2007-8	Total
Examined	63	11	45	31	150
H1	36(57%)	0	0	31(100%)	67(45%)
H3	27(43%)	11(100%)	45(100%)	0	83(55%)

Percentage is calculated from the total number of influenza A viruses.

TABLE 4

Analysis of 34 influenza A(H3N2) strains isolated in northern Greece during influenza seasons between 2004 and 2008

Influenza A (H3N2) strains, northern Greece				
Influenza period	Sensitivity/resistance to M2 inhibitors	Known mutations in M2 gene	Phylogenetic groups based on HA analysis	Known mutations in NA gene
2004-5	100% sensitive	Not found	A/Lisbon/3/04-like	Not found
2005-6	13% sensitive	Not found	A/Berlin/2/06-like	Not found
	88% resistant	S31N	A/HongKong/4443/05-like	
2006-7	25% sensitive	Not found	A/Nepal/921/06-like	Not found
	75% resistant	S31N	A/Trieste/25/07-like	

HA: haemagglutinin; NA: neuraminidase.

reverse transcription PCR (RT-PCR) and sequencing were performed. RNA was extracted from HA-positive cell culture supernatants, or allantoic and/or amniotic fluids using the Viral RNA Mini Kit (Qiagen, Germany). Reverse transcription and amplification of the M2, NA and HA genes was done with the Superscript III One-step RT-PCR kit (Invitrogen, UK), using the oligonucleotide primers listed in Table 1. Purified PCR products were sequenced (Lark Technologies, Cogenics Ltd., Essex, UK) using the forward primers for each amplified product (MF8, N2F-1 and H3HAF6). NA and HA sequences from representative strains were added to GenBank under accession numbers EU744874, EU744875, EU744876, EU744877, EU744878, EU744879, EU744880, EU744881, EU744882, EU744883

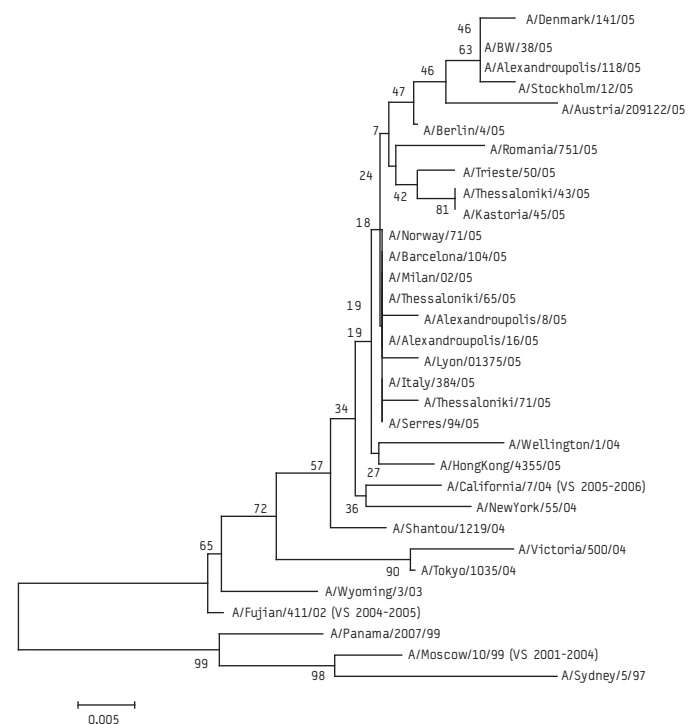
From a total of 83 PCR positive A(H3N2) viruses, 34 were cultured successfully and further studied (41% of the isolates). They covered 14 isolates from the 2004-5 season, eight isolates from the 2005-6 season and 12 isolates from the 2006-7 season were studied. Viral M2 and NA sequences were examined for resistance mutations. Viral HA sequences were also examined and compared with reference strains in order to determine the phylogenetic relationship between the circulating resistant and sensitive strains.

Results

A total number of 83 A(H3N2) viruses were isolated during three out of the four influenza seasons between 2004 and 2008

FIGURE 1

HA sequences of influenza A(H3N2) circulating in northern Greece and Europe in 2004-5, WHO reference and vaccine strains



Phylogenetic tree of HA amino acid sequences, constructed with the Neighbor Joining method and MEGA4.1 software. HA: haemagglutinin; WHO: World Health Organization

in northern Greece. This region represents 38.5% of the Greek population. No A(H3N2) strains were isolated during the last influenza season 2007-8. An overview of the surveillance and typing results is given in Tables 2 and 3.

The results of the sequence analysis are summarised in Table 4 and show that 100% of the A(H3N2) strains that were isolated during the influenza season 2004-5 were sensitive to adamantanes, whereas 88% and 75% of the strains isolated during the 2005-6 and 2006-7 influenza seasons, respectively, contained an amino acid substitution at position 31 (serine to asparagine) known to confer resistance to adamantanes.

No known mutations of the NA gene that confer resistance to NA inhibitors were detected in the A(H3N2) strains that were isolated during the three influenza periods 2004-5, 2005-6 and 2006-7. Nor did we find known mutations in the HA gene that have been implicated in resistance to NA inhibitors. Phenotypic assays are needed to identify resistance to those antiviral drugs due to novel mutations and thus genotypic methods are only used for monitoring of established resistance markers.

Phylogenetic analysis of the HA gene of the isolated strains showed a correlation of phylogenetic clades with the S31N

mutation in the M2 gene that confers resistance to adamantanes. Specifically, during the 2004-5 season, all adamantine-resistant strains belonged to the phylogenetic group represented by the A/Lisbon/3/04 strain, and all were sensitive to adamantanes (Figure 1).

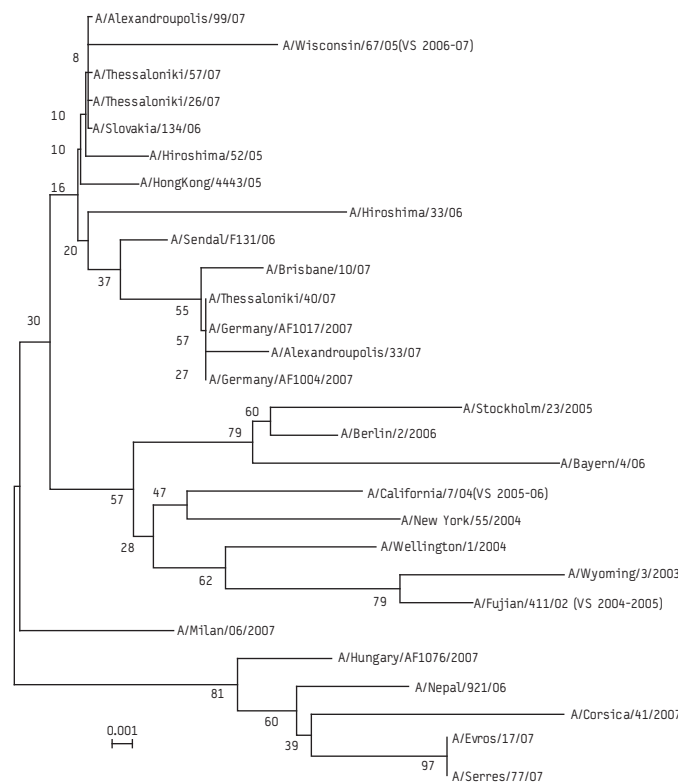
In the season 2005-6, the isolates that carried the S31N resistance mutation were all A/HongKong/4443/05-like, whereas the remaining 12.5% of adamantine-sensitive isolates were A/Berlin/2/06-like and thus belonged to a different phylogenetic group (Figure 2).

In the season 2006-7, 75% of the strains belonged to the phylogenetic group that was represented by A/Trieste/25/07 and were resistant to M2 inhibitors (Figure 3). The remaining strains (25%) belonged to the phylogenetic group represented by A/Nepal/921/06 and were sensitive to adamantanes [7]. In contrast, none of the phylogenetic groups carried mutations in the NA gene that are implicated to confer resistance to NA inhibitors, and thus no phylogenetic relationships could be determined.

Discussion

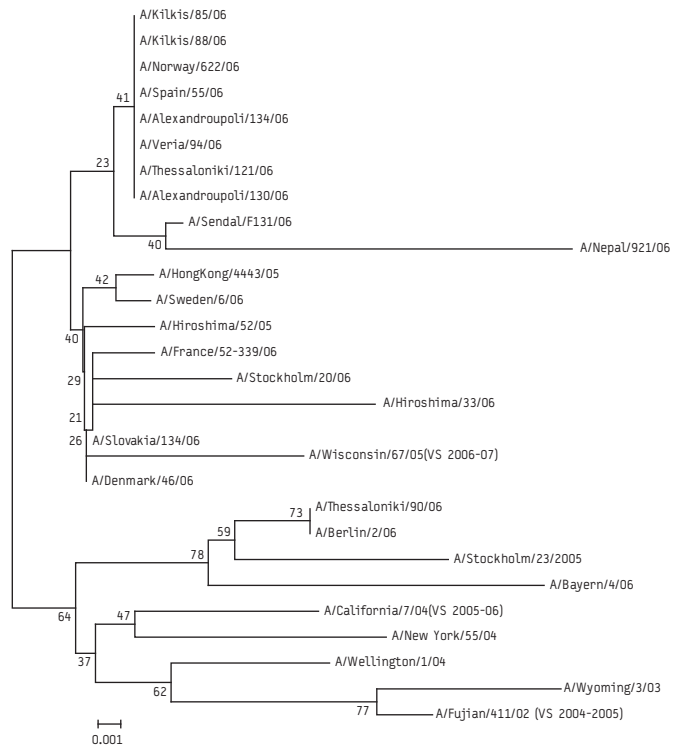
Our results are consistent with the results that have been published in other countries of the European Union, Australia, Asia

FIGURE 2
HA sequences of influenza A(H3N2) circulating in northern Greece and Europe in 2005-6, WHO reference and vaccine strains



Phylogenetic tree of HA amino acid sequences, constructed with the Neighbor Joining method and MEGA4.1 software.
 HA: haemagglutinin; WHO: World Health Organization

FIGURE 3
HA sequences of influenza A(H3N2) circulating in northern Greece and Europe in 2006-7, WHO reference and vaccine strains



Phylogenetic tree of HA amino acid sequences, constructed with the Neighbor Joining method and MEGA4.1 software.
 HA: haemagglutinin; WHO: World Health Organization.

and the US. [2,7-12] Resistance to M2 inhibitors first appeared following extensive drug use in Asia and the US after the SARS epidemic in 2004. The worldwide spread of these resistant strains occurred through replacement of sensitive with resistant viruses, probably because of other selective advantages of the resistant strains connected to other genes than M2.

Resistance of Greek influenza strains has developed despite the absence of selective drug pressure, as these drugs have not been extensively used in Greece [3,8-10,13]. It seems that viruses of different phylogenetic clades are imported to Greece from different parts of the world, not least due to the country's borders with Asia. The fact that our 2007 A/Nepal/921/2006-like isolates possessed M genes that were closely related to A/California/7/2004-like viruses, would seem to support the hypothesis that the sensitivity of viruses isolated more recently could be due to a reassortment event that caused reacquisition of an older, sensitive, M gene, rather than reversion of the resistance mutation. Unfortunately, the available information from southern Europe is limited.

According to the latest available information, the Ministry of Health and Social Solidarity of Greece has stockpiled oseltamivir to cover 5% of the population in case of an influenza pandemic. This corresponds to 500,000 therapeutic courses. A stock of 400,000 courses of amantadine has been created as well [14-16]. However, amantadine is the only drug that is approved for prophylaxis by the National Organisation for Medicines in Greece and can only be used for individuals over the age of five years, while rimantadine is not available at all. Oseltamivir and zanamivir are only approved for therapeutic use, for children over the age of one and 12 years, respectively, and their use can start only after an official announcement by the Ministry of Health that there is an ongoing influenza epidemic or pandemic [14,15,17].

Greece is a known crossroads among three continents (Europe, Asia, Africa) through which resistant strains can spread. Our results emphasise the need for constant monitoring of emerging resistance to the available antiviral drugs, especially after the recent appearance of A(H1N1) strains that are resistant to oseltamivir [18]. Analysis of 65 Greek influenza A(H1N1) strains by WHO found seven of 65 (10.8%) isolates resistant to oseltamivir [17]. In communities in which oseltamivir-resistant viruses are circulating widely, treatment with zanamivir or a combined treatment with adamantanes should be considered [19]. Decision makers in Greece are urged to take into account the results of such studies when they consider alternative stockpiling and usage policies of these drugs both for annual epidemics and for a possible pandemic situation [16].

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USE OF OSELTAMIVIR IN 12 EUROPEAN COUNTRIES BETWEEN 2002 AND 2007 – LACK OF ASSOCIATION WITH THE APPEARANCE OF OSELTAMIVIR-RESISTANT INFLUENZA A(H1N1) VIRUSES

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Variable levels of oseltamivir resistance among seasonal influenza A(H1N1) isolates have been reported in Europe during the 2007-8 northern Hemisphere influenza season. It has been questioned whether oseltamivir use could have driven the emergence and predominance of resistant viruses. This study aimed at describing the levels of use of oseltamivir in 12 European Union (EU) Member States and European Economic Area (EEA)/European Free Trade Area (EFTA) countries. The data were converted into prescription rates and compared with the national proportions of resistant influenza A(H1N1) viruses through regression analysis. Overall use of oseltamivir in European countries between 2002 and 2007 was low compared to e.g. the use in Japan. High variability between the countries and over time was observed. In eight of the 12 countries, there was a peak of prescriptions in 2005, coinciding with concerns about a perceived threat from an influenza pandemic which might have led to personal stockpiling. Ecological comparison between national levels of use of oseltamivir in 2007 and the proportions of A(H1N1) viruses that were resistant to oseltamivir showed no statistical association. In conclusion, our results do not support the hypothesis that the emergence and persistence of these viruses in 2007-8 was related to the levels of use of oseltamivir in Europe. Further investigation is needed to elucidate the reasons for different level of use between the countries.

Introduction

Annual epidemics of human seasonal influenza are associated with a substantial burden of morbidity and mortality, which cumulates in certain groups of the population such as older people and those with chronic medical conditions [1-3]. Annual vaccination remains the mainstay of influenza prevention, and antiviral medications, including the neuraminidase inhibitors (NAIs) oseltamivir and zanamivir, and M2 protein inhibitors (the adamantanes amantadine and rimantadine) play an auxiliary role in the prevention or treatment of influenza infection. They can be especially helpful in controlling outbreaks in nursing homes, in individuals who cannot be immunised or in situations in which vaccine has not been given or in which vaccination is not optimally effective due to a poor match between the vaccine strain and the circulating strains [4-9].

NAIs, especially the oral drug oseltamivir, became increasingly important after a sudden increase in adamantane resistance among seasonal influenza A viruses between 2004 and 2006 [5,10,11]. NAIs have also been preferred in recommendations to amantadine (the most commonly used adamantane) since they show lower levels of adverse neurotoxic reactions [12]. Before the 2007-8 influenza season, resistance to the NAIs among transmitting seasonal influenza A viruses was extremely rare in Europe and elsewhere [13-15] and higher proportions of resistance had been reported only in children: up to 18% of children infected with influenza A(H3N2) and treated with oseltamivir shed virus resistant to oseltamivir [16-17]. However, NAI-resistant viruses detected before 2007-8 showed in most cases a poor ability to transmit from human to human.

This situation changed abruptly during the 2007-8 northern Hemisphere influenza season when influenza A(H1N1) virus isolates highly resistant to oseltamivir were detected as part of surveillance in the Europe through the networks of the European Influenza Surveillance Scheme (EISS)/European Surveillance Network for Vigilance against Viral Resistance (VIRGIL) [13,18]. Laboratory analyses showed that up to 67.4% of all influenza A(H1N1) viruses isolated from specimens collected between November 2007 and April 2008 in Europe either carried the mutation H274Y which is associated with high levels of oseltamivir resistance or tested positively in the IC50 phenotypic examination for oseltamivir resistance (Figure 1) [19]. This was the first indication that influenza A(H1N1) virus resistant to oseltamivir could readily transmit between humans.

The question arises whether current levels of oseltamivir use in European countries could have been associated with the emergence and sustained transmission of resistant influenza A(H1N1) viruses. The aim of the study was thus to describe, using all available data (including data from prescription surveys and databases), oseltamivir usage at population level in several EU Member States and EEA/EFTA countries and to determine if there was any correlation between the level of use and the observed proportions of A(H1N1) viruses that were resistant.

Methods

We used several sources of information on oseltamivir prescriptions as a proxy measure for oseltamivir utilisation in EU Member States and EEA/EFTA countries.

Information on oseltamivir use from a prescription survey

We used data from a continuing survey of a panel of office-based physicians in EU Member States and EEA/EFTA countries from databases maintained by Intercontinental Marketing Services (IMS) Health, an independent commercial company providing information on the use of pharmaceuticals. IMS Health attempts to achieve a high level of representativeness of their panels for the population of all physicians in the involved countries. Participating physicians are being surveyed for two consecutive workdays per quarter of a year and provide information on each patient encounter during this period. The manufacturer of oseltamivir, F.Hoffmann-La Roche Ltd., provided the European Centre for Disease Control and Prevention (ECDC) with the data from IMS Health on the numbers of oseltamivir prescriptions in Austria, Belgium, Finland, France, Germany and Greece for the years 2002 to 2007. We then converted

these data into prescription rates (number of prescriptions per 1,000 inhabitants per year) using Eurostat population data [20]. Four other countries monitored by IMS Health, the Netherlands, Portugal, Switzerland and the United Kingdom (UK), had only negligible prescription levels for oseltamivir.

Information on oseltamivir use from population prescription databases

In Denmark and Norway, data on the number of patients having used oseltamivir at least once each year between 2002 and 2007 and between 2004 and 2007, respectively, were extracted from national, publicly available databases on redeemed prescriptions [21,22]. These numbers of prescriptions were converted into rates of redeemed prescriptions per 1,000 inhabitants per year. In both countries, data included corporate prescriptions, i.e. medicines purchased by business organisations for their employees. The data did not include any supply of antiviral medications to countries for national or corporate stockpiles.

Quarterly prescription information

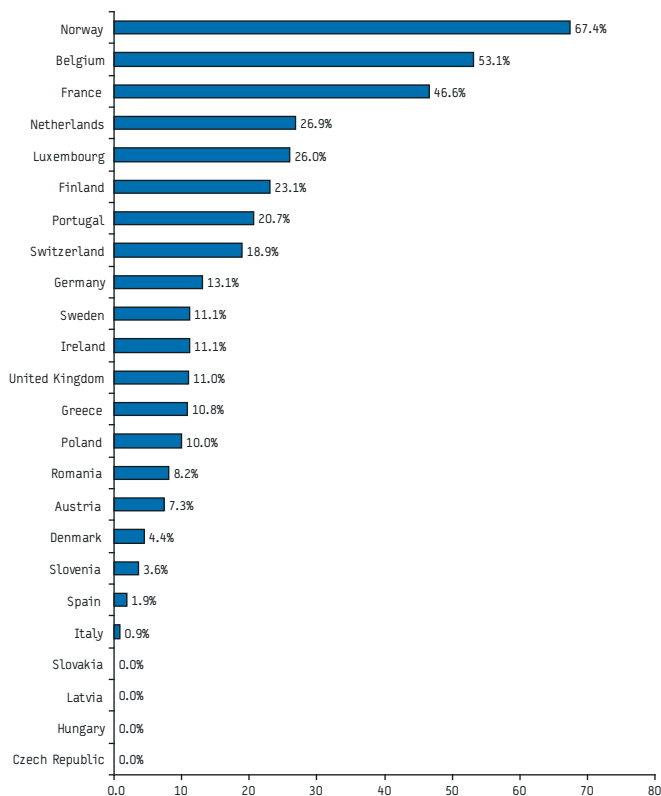
The initial analysis consisted in computing annual figures for oseltamivir prescriptions per 1,000 inhabitants. To examine trends in oseltamivir use over time in more detail, we also obtained quarterly prescription numbers and converted them into prescription rates. Quarterly data were available for eight countries: Austria, Belgium, Finland, Germany, Greece, the Netherlands, Portugal, Switzerland, and the UK.

Investigation of the relationship between oseltamivir use and levels of resistance

Linear regression analysis was performed to determine whether there was any relationship between the use of oseltamivir and the levels of oseltamivir resistance. Proportions of oseltamivir resistance during the 2007-8 influenza season among all A(H1N1) tested strains expressed on the web sites of ECDC, EISS and the World Health Organization (WHO) were regressed on the levels of

FIGURE 1

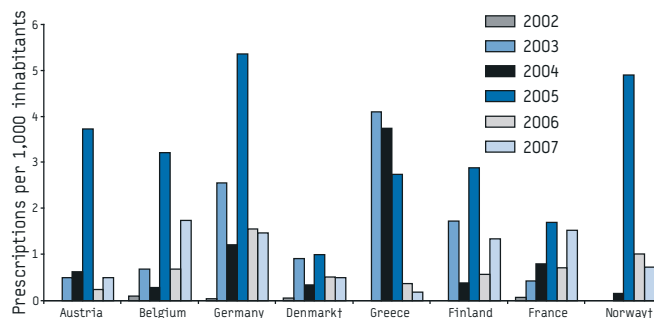
National proportions of antiviral resistance in A(H1N1) influenza viruses for EU/EEA Member States, 2007-8



Data (available as of 6 August 2008) were provided by European Influenza Surveillance Scheme www.eiss.org/index.cgi and the VIRGIL Project www.virgil-net.org. Countries with fewer than 10 test results (Bulgaria, Estonia) are not shown in the graph. EU/EEA/EFTA countries in the EISS network for which no test results were available: Cyprus, Lithuania, Malta. EU: European Union; EEA: European Economic Area EFTA: European Free Trade Area; EISS: European Influenza Surveillance Scheme; VIRGIL: European Surveillance Network for Vigilance against Viral Resistance.

FIGURE 2

Prescriptions of oseltamivir per 1,000 inhabitants in eight European countries*, 2002-2007



* Data only include patient prescriptions. They do not include stockpiles at national/regional level or by hospitals/institutions. Data for Denmark and Norway include corporate prescriptions. Netherlands, Portugal, UK and Switzerland: data not presented due to 'negligible' number of prescriptions. † Denmark and Norway: the data are based on the number of patients, which may slightly underestimate the number of prescriptions. Source: IMS Health data provided by F. Hoffmann - La Roche Ltd., Basel except for: Denmark; data provided by Danish Medicines Agency, and Norway: data provided by Norwegian Institute of Public Health.

oseltamivir use in the countries in 2007. STATA (STATA/SE 10 for Windows, STATA Corporation) was used for statistical analyses.

Results

Annual oseltamivir prescription rates

As shown in Figure 2, the overall prescription rates for oseltamivir remained under six prescriptions/1,000 inhabitants/year in the eight EU Member States for which such data was available. This is low compared to those reported, for example, in Japan where the reported prescription rate in 2005 was 70.9/1,000 inhabitants/year [23].

After a substantial peak in prescriptions in 2005, when three countries exceeded three prescriptions/1,000 inhabitants/year (Austria, Belgium and Norway) and one country exceeded five prescriptions/1,000 inhabitants/year (Germany), the use of oseltamivir decreased to under two prescriptions/1,000 inhabitants/year in 2006 and 2007 in all included countries. However, the trends from 2006 to 2007 differed: an increase occurred in Austria, Belgium, Finland and France, a small decrease in Germany, Greece and Norway, and the rates remained stable in Denmark.

In the most recent year with available data (2007), we observed a substantial variation in oseltamivir prescription rates in EU Member States, with an almost tenfold differences in those countries with any significant use of oseltamivir. The highest rates were seen in Belgium and the lowest in Greece. Countries with negligible use that are not shown in the figure are the Netherlands, Portugal, Switzerland and the UK. Greece exhibited a different prescription pattern with high use in 2003 and 2004.

In summary, our analysis showed low prescription rates of oseltamivir with substantial variation between analysed countries and over time.

Quarterly oseltamivir prescription rates

Figure 3 shows a more detailed comparison of oseltamivir prescription rates in eight countries for which data were available at the level of periods of three months.

It was noticeable that the peak of oseltamivir use observed in 2005 in Austria, Belgium, Finland and Germany (Figure 2) concentrated mostly during the first quarter of that year.

No correlation of prescription data and resistance development

We have analysed oseltamivir resistance in 2007-8 because a sharp increase in resistance was observed during that season. We regressed it against oseltamivir use in 2007 assuming this was a good proxy for oseltamivir use in 2008. However, regression analysis for twelve countries (Figure 4) did not show any statistical association between the levels of oseltamivir resistance during the influenza season 2007-8 and oseltamivir prescriptions in 2007 (R2 = 0.02).

Discussion

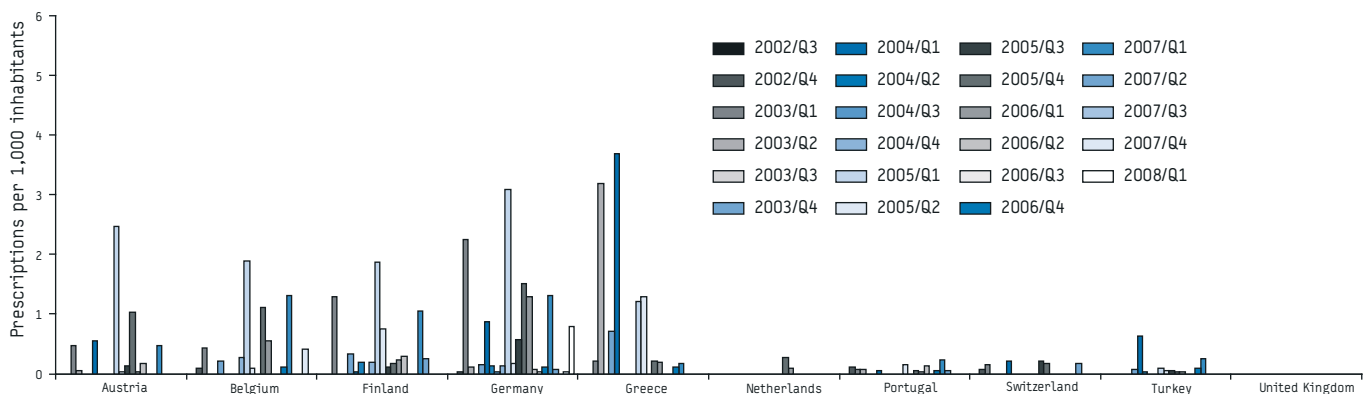
We found overall low levels of oseltamivir use in EU Member States in the period between 2002 and 2007, compared to the use of oseltamivir in Japan, a country with the world's highest per capita use of oseltamivir (70.9/1,000 inhabitants/year), but relatively low levels (3%) of oseltamivir resistance during the 2007-8 season [23,24].

There was a common peak in prescriptions in 2005 in eight countries. One possible explanation for this phenomenon is the concern over 'bird flu' influenza A(H5N1) in 2005 when spread of these viruses from Asia towards Europe received considerable attention in the media. Many of these prescriptions to individuals and families may therefore have gone to form a source of medication for the future ("personal stockpiling"). A similar spike of influenza antiviral medication sales, was observed in October 2005 in New York [25] and, in general, in the autumn and winter of 2005 across the United States [26]. It did not coincide with influenza activity itself, but rather with the beginning media coverage of avian influenza A (H5N1) and the potential for an influenza pandemic [23].

It is more difficult to explain the observation that most of the oseltamivir use in EU Member States in 2005 concentrated in the first quarter of the year. Influenza activity during the season 2004-5

FIGURE 3

Prescriptions of oseltamivir per 1,000 inhabitants in eight European countries*, 2002-2007, by quarter of a year



Source: IMS Health data provided by F. Hoffmann – La Roche Ltd., Basel except for: Denmark; data provided by Danish Medicines Agency, and Norway: data provided by Norwegian Institute of Public Health.

only partially explains this peak. Although the media paid some attention in early 2005 to ongoing outbreaks of avian influenza among poultry in Indonesia, Thailand, and Vietnam and possibly also in Cambodia and Lao People's Democratic Republic, it was the outbreaks of avian influenza in Turkey, Romania, Croatia and the UK in October 2005 which spiked most of the media reports that year [26]. At the time there were public statements in many countries about national antiviral stockpiles being purchased by governments [28,29].

It should be noted that some countries had significant levels of prescribing even before 2005, which could be an indication for therapeutic or prophylactic application by physicians. The contrasting prescription pattern in Greece with high use in 2003 and 2004, may represent the seasonal influenza activity pattern in that country with the highest activity in February-April 2003, and then from December 2003 to the first months of 2004.

We also found a substantial variation in prescription rates between the analysed countries, which is hard to justify on any scientific grounds. Reasons may be differences in national guidelines, clinical practice patterns, marketing strategies or insurance companies' reimbursement [30]. Among the countries with negligible use of anti-influenza drugs, the UK and the Netherlands have medical guidelines on when antiviral medications are indicated that restrict their widespread use [4,12,31], while in Switzerland, most insurance companies do not reimburse the use of antivirals (D. Koch, personal communication). Exploring this phenomenon in more detail would warrant a separate study and would be justified because the wide variations in the use of antivirals for influenza does at present not reflect observed patterns of influenza-like illness/influenza and cannot be seen as having a scientific basis.

Although the analyses had to be restricted to ecological analyses, these preliminary data do not point towards any correlation between a higher prevalence of resistance and higher rates of antiviral use. Hence, it seems very unlikely that oseltamivir use has driven the rise and persistence of 'fit' oseltamivir-resistant influenza viruses

A(H1N1) in Europe in the 2007-8 season. The H274Y point mutation, which confers oseltamivir resistance is most likely a random event, and potential factors influencing its occurrence are not known [32].

Our study had several limitations, apart from being restricted to an ecological level of analysis. Firstly, we obtained information on antiviral medication prescriptions which do not necessarily represent all medications consumed. Indeed, it is possible that some of the purchased medications were not consumed but stored in "private stockpiles". This seems especially likely for the antivirals acquired in the peak year of 2005. Secondly, the IMS Health data are based on a sample of physicians who may not necessarily be representative for all physicians in the analysed countries. Thirdly, data were only available for a limited number of EU Member States and EEA/EFTA countries, and the situation could be quite different in the countries that we could not study. Moreover, for several countries we only had data on oseltamivir resistance for the first quarter of 2008.

Conclusion

While the precise relationship between oseltamivir use and resistance of influenza A(H1N1) to oseltamivir remains uncertain, the available data do not suggest a link between the rapid rise in the proportion of the resistant A(H1N1) and the use of oseltamivir in Europe.

The use of influenza antiviral medication in EU Member States should be closely monitored in the future. More studies are needed to assess how the influenza prescription rates reflect the actual use of the medication by patients, in order to explore the potential causes of the large variation in the number of prescriptions in EU Member States and EEA/EFTA countries. In addition, a scientific discussion is needed about what are the right indicators for use of these drugs. Virological studies are needed to better understand the mechanism behind the development of oseltamivir resistance among A(H1N1) seasonal influenza viruses, and to monitor the possible emergence and spread among other influenza viruses. Epidemiological studies are needed to understand the determinants of resistance development, in order to be able to design targeted interventions and to assess the impact on transmission and clinical outcome.

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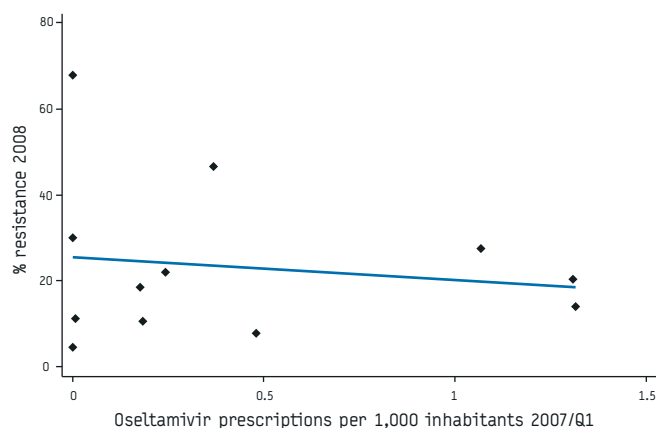
ECDC would like to thank all countries, virologists, clinicians and others for contributing data. Funding for the VIRGIL project comes from the European Union FP6 Research Programme http://ec.europa.eu/research/health/influenza/proj13_en.html and EISS is supported by ECDC. Laboratories in EISS contribute to the Global Influenza Surveillance Network managed by WHO.

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FIGURE 4

Regression of the proportion of resistant strains on the number of prescriptions of oseltamivir per 1,000 inhabitants in European countries



Source as in Figure 3.

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RISK FACTORS FOR SPORADIC CAMPYLOBACTER INFECTION: AN ALL-IRELAND CASE-CONTROL STUDY

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We report the findings of the first case-control study conducted in both the Republic of Ireland and Northern Ireland to determine risk factors for sporadic *Campylobacter* infections. A total of 197 cases and 296 case-nominated controls matched for age, were included. Based on Population Attributable Fraction (PAF), the most important risk factors were consuming chicken [adjusted matched (am) OR 6.8; 95%CI 2.1-21.9], consuming lettuce (amOR 3.3; 95%CI 1.5-7.1) and eating in takeaways (amOR=3.1; 95%CI 1.4-6.6). Contact with sheep (amOR=11; 95%CI 1.6-78), peptic ulcer (amOR=19; 95%CI 3.8-93.7), hiatus hernia (amOR=20.3; 95%CI 2.3-183.3), lower bowel problems (amOR=4.5; 95%CI 1.2-16.8) were also independently associated with infection. Mains water supply showed protective effect (amOR=0.2; 95 CI 0.1-0.9). The findings highlight the continued need for consumer food safety education and further control measures throughout the food chain on the island of Ireland.

Introduction

In line with many western countries, *Campylobacter* is the most common cause of laboratory confirmed bacterial gastrointestinal disease in both the Republic of Ireland (ROI) and Northern Ireland (NI). Between 1999 and 2006 over 20,000 laboratory-confirmed cases were reported in the two jurisdictions, giving a mean incidence rate of 47 per 100,000 population per year and representing about two thirds of all acute reported gastroenteritis [1,2].

Campylobacter infection is of important public health concern as it can cause considerable illness and loss of productivity and may be associated with sequelae, such as reactive arthritis and Guillain Barré syndrome [3-7]. Different risk factors have been reported in various studies conducted in several developed countries, with the most common ones being: consumption and handling of chicken, and in particular undercooked chicken or commercially prepared chicken; unpasteurised milk and dairy products; consumption of untreated water; contact with domestic pets like dogs and cats; contact with farm animals; travel abroad [7-14]. However, differences between risk factors across studies may reflect either different study methodologies or variations in the sources of infection across different countries [6,8]. In addition, epidemiological studies conducted in the United Kingdom (UK)

have suggested that there may be even regional differences in the contributing risk factors for infection [15].

Although the population health burden from *Campylobacter* is considerable, there have not been any analytical studies conducted in Ireland on the epidemiology of the disease in humans. This paper describes the first case-control study that was conducted in both ROI and NI, to identify risk and protective factors for sporadic *Campylobacter* infection on the island of Ireland and estimate the proportion of the risk attributable to the identified factors, in order to guide prevention efforts.

Methods

Study design

A prospective matched case-control study was conducted in all four Health Board areas in NI and the Health Service Executive (HSE) Eastern region in ROI (which includes the greater Dublin area and represents 36% of the ROI population). Data were collected over a 12-month period (from December 2003 to December 2004).

Two controls were nominated by each case matched for age group (0-5, 6-10, 11-20, 21-34, 35-49, 50-64 and 65+ years). Age was chosen as a matching variable because (i) potential high-risk exposures (e.g. food habits, leisure activities) vary considerably among different age groups and (ii) the age profile of campylobacteriosis in Ireland, both ROI and NI, peaks in some age groups, namely 0-4 and 20-34 years.

Cases

A case was defined as a person of any age (living or visiting the study area) whose laboratory confirmed *Campylobacter* spp. infection was reported through the routine surveillance systems in the participating health authorities, during the 12-month study period. Cases were excluded if (i) they were associated with an outbreak reported to the health authority or the national surveillance centre or (ii) at least one matched control could not be identified.

Controls

Cases (or adult respondents, in case of children patients aged less than 16 years) were asked to hand the questionnaires to

two controls matched for age group (such as neighbours, work colleagues, friends or schoolmates, but not household members).

Controls were excluded (i) if they had gastrointestinal symptoms in the 14 days prior to the completion of the questionnaire (ii) if they lived in the same household as the case or (iii) if the completed questionnaire of the matched case was not available.

Sample size

To detect an association with a matched odds ratio (mOR) of 2 at the 5% significance level, with 80% power and a case-control ratio of 1:2, a sample size of 186 case-control sets (i.e. a case with at least one matched control) was required, assuming 70% chicken consumption among controls, as reported in the North/South Ireland Consumption Survey [16]. The calculation was performed by a software written by the Statistics Unit of the Health Protection Agency (HPA) [17].

Study questionnaire

Information on exposures of the cases and their matched nominated controls was collected using a self-administered postal questionnaire. This gathered demographic data (age, gender, employment status, occupation), clinical details of cases (date of onset, duration and symptoms of the disease, if hospitalisation was required), and information on household contacts.

The 86 considered exposures were grouped into five categories:

- Food and drink history, including drinking water (type of water supply, bottled water, other sources), meat (beef, pork, lamb, sausage, ham, salami), fish, chicken, vegetables, fruit, milk and dairy products and eating out (type of restaurant or takeaway);
- Foreign travel (outside the island of Ireland);
- Contact with animals (pets and farm animals);
- Leisure activities (including swimming, gardening, visits to parks or farms, fishing and other sports);
- Medical history and medication (antacids, H2-receptor antagonists, antibiotics).

Dose-responses of the food and drink exposures (frequency of consumption) were investigated. All questions related to exposures in the seven days before the onset of symptoms for cases and seven days before the completion of the questionnaire for controls, except for medication (one month before illness onset/completion of questionnaire) and foreign travel (14 days before). Adult family members were asked to complete the questionnaires on behalf of children under the age of 16. The questionnaire was validated during a pilot study conducted in ROI that involved 20 cases. To increase the response rate, a reminder letter was sent to the cases that had not responded within 14 days.

The study received ethical approval by two Ethics Committees; the Faculty of Public Health Medicine Research Ethics Committee in ROI and the Queen's University of Belfast Research Ethics Committee in NI.

Statistical analysis

Data were entered in a database designed using EpiInfo2003 software (version 3; Centers for Disease Control and Prevention) and were checked for mistakes and inconsistencies (consistency and range checks). Food/drink exposures were treated as dichotomous variables, whereas frequencies of food/drink consumptions were analysed as continuous variables. Age was grouped into the following age bands: 0-5, 6-10, 11-20, 21-34, 35-49, 50-64 and 65+ years. Initial univariate matched analysis was carried out

to calculate age-group adjusted matched OR (mOR) and their 95% confidence intervals (95%CI). Age adjustment was performed to control for the potential residual confounding of age, as matching for age had not been successful in some young cases. Dose-response relationships were also examined between frequencies of food/drink consumptions and the disease.

Multiple conditional logistic regression models were constructed with Stata software (version 8, Stata Corporation, Texas). The initial regression model was developed including age, gender and all other variables for which (i) the p-value (for the OR) was less than 0.05, or (ii) the OR was more than 1.5 or less than 0.67 in the univariate analysis. These cut-off values were considered

TABLE 1

Socio-demographic characteristics of cases and controls included in the final analysis. All-Ireland Campylobacter infection case-control study, 2004

	Cases (n=197) n (%)	Controls (n=296) n (%)
Sex		
Males	91 (46.4)	101 (34.4)
Age group		
0-5 years	26 (13.3)	23* (8.0)
6-10 years	9 (4.6)	9* (3.1)
11-19 years	25 (12.8)	37 (12.9)
20-34 years	46 (23.4)	78 (27.2)
35-49 years	47 (23.9)	61 (21.3)
50-64 years	31 (15.8)	58 (20.2)
65+ years	13 (6.6)	21 (7.3)
Employment status		
Employed	98 (52.7)	175 (61.8)
Unemployed	8 (4.3)	16 (5.7)
Student	34 (18.3)	48 (17)
Retired	19 (10.2)	22 (7.8)
Other (housewife, baby, etc.)	27 (14.5)	22 (7.9)
Food-handler	13 (13.3)†	28 (16.0)‡
Contact with animals (as job)	3 (3.1)	7 (4.0)
Place of residence		
City/town	40 (90.9)	62 (89.9)
Village/rural area	4 (9.1)	7 (10.1)
Type of house		
Apartment	14 (7.7)	18 (6.8)
House	163 (89.6)	238 (90.5)
Farm	5 (2.7)	7 (2.7)
Household contacts		
Mean number of people in household (standard deviation)	3.5 (1.5)	3.8 (2.2)
Child < 5 years in household	44 (22.6)	68 (23.1)
Mean number of children <5 years in household (standard deviation)	0.31 (0.6)	0.31 (0.7)

* Five cases aged 0-5 years and five cases aged 6-10 years nominated controls that were older than 5 and 10 years, respectively

† Among the 98 employed cases

‡ Among the 175 employed controls

important for the specific exposures and the disease. To simplify the model, variables were removed one at a time depending on the significance testing ($p < 0.05$) by the likelihood ratio (LR) test or the alteration of OR. Because of several missing values, frequency of food/drink consumption variables were not included in the models and food/drink items as dichotomous variables were included instead. Potential interactions among all variables in the final model, age and country (ROI vs NI) were also examined. The population-attributable fractions (PAF) for all risk factors in the final model were calculated, using the following formula for matched case-control studies: $PAF = [P' * (amOR - 1) / amOR]$, where P' is

the proportion of cases exposed and amOR is the adjusted matched OR which was derived from the final conditional regression model.

Results

Response rate

A total of 978 persons fulfilling the case definition were contacted and 402 (41.1%) (215; 37.7% in ROI and 187; 45.7% in NI) returned a completed questionnaire. Of these, 197 (49%; 52.5% in ROI and 44.9% in NI) had at least one control that responded. The final analysis was made up of 197 cases (113 in ROI and 84 in NI) and 296 controls (172 in ROI and 124 in NI). Of the 197

TABLE 2

Univariate analysis of risk and protective factors (travel, eating out, poultry, meat and fish, vegetables and fruit consumption) for campylobacteriosis. All-Ireland Campylobacter infection case-control study, 2004.

Exposure	Cases (n=197) † n (%)	Controls (n=296) † n (%)	Crude OR * (95% CI)	P-value
Male	91 (46.4)	101 (34.4)	2.0 (1.2-3.2)	0.005
Foreign travel				
Foreign travel (outside the island of Ireland)	41 (20.8)	22 (7.5)	3.5 (1.7-7.3)	<0.001
Travel to United Kingdom	7 (3.6)	8 (2.7)	1.8 (0.6-5.7)	0.34
Travel to Europe	29 (14.7)	20 (6.8)	2.8 (1.4-5.8)	0.005
Travel to places outside Europe	12 (6.1)	2 (0.7)	17.8 (2.2-143)	0.007
Poultry and poultry products				
Chicken	181 (92.3)	251 (85.1)	3.0 (1.5-6.1)	0.002
Undercooked chicken	13 (6.7)	2 (0.7)	9.5 (2.1-43.5)	0.004
Duck	10 (5.1)	12 (4.1)	2.0 (0.7-5.6)	0.18
Turkey	11 (5.6)	44 (14.9)	0.3 (0.1-0.6)	0.002
Handle raw chicken bought from butchers	9 (4.6)	34 (11.5)	0.4 (0.2-0.9)	0.02
Meat and fish				
Any meat and fish	189 (95.9)	290 (98)	0.4 (0.1-1.5)	0.15
Beef (including mince)	143 (73)	244 (82.7)	0.6 (0.4-1)	0.03
Sausages	114 (58.5)	196 (60.2)	0.6 (0.4-1)	0.03
Pate	12 (6.1)	13 (4.4)	1.9 (0.7-4.8)	0.25
Salami	17 (8.7)	33 (11.2)	0.6 (0.3-1.2)	0.14
Fresh fish	40 (20.5)	84 (28.5)	0.5 (0.3-0.9)	0.02
Frozen fish	42 (21.5)	89 (30.2)	0.6 (0.4-1)	0.07
Any meat cooked rare	17 (8.8)	14 (4.7)	1.6 (0.7-3.6)	0.26
Handle raw meat	73 (37.4)	144 (49.5)	0.6 (0.4-1)	0.05
Vegetables and fruit	178 (90.4)	281 (94.9)	0.5 (0.2-1.1)	0.07
Lettuce	124 (63.6)	181 (61.4)	1.6 (1.0-2.6)	0.06
Prepared salad - other than lettuce, e.g. coleslaw	71 (36.4)	147 (50.1)	0.6 (0.4-0.9)	0.01
Milk and dairy products	187 (94.9)	283 (95.9)	0.9 (0.4-2.3)	0.88
Cold milk	87 (44.4)	171 (58.2)	0.5 (0.3-0.8)	0.00
Ice-cream	86 (44.3)	166 (56.5)	0.6 (0.4-0.9)	0.02
Yoghurt	99 (50.8)	184 (62.8)	0.6 (0.4-0.9)	0.01
Eating out	143 (73)	204 (69.2)	1.4 (0.9-2.2)	0.13
Fish and Chip shop	24 (12.2)	64 (21.7)	0.5 (0.3-0.9)	0.03
Indian Restaurant/ /takeaway	12 (6.1)	14 (4.7)	1.3 (0.5-3.1)	0.61
Chinese Restaurant/ /takeaway	61 (31.1)	89 (30.2)	1.1 (0.7-1.8)	0.55
Other Restaurant/takeaway	54 (27.6)	46 (15.6)	2.6 (1.5-4.4)	<0.001
Plane	19 (9.7)	5 (1.7)	7.8 (2.2-27.1)	0.001

* Matched odds ratio adjusted for age † For several variables answers were not available from all participants (denominators in percentages vary).

case-control sets, 101 cases were matched to one control each, 93 cases to two controls each, and three cases to three controls each (as these three cases handed questionnaires to three controls instead of two).

The participant (n=197) and non-participant cases did not differ significantly in terms of gender (p=0.24) and age group (p=0.13). In addition, the response rate of cases was similar in each season, suggesting that the number of participant cases by season reflected

the seasonal distribution of reported campylobacteriosis cases (data not shown).

Cases and controls

The main socio-demographic characteristics of cases and controls are shown in Table 1. The median age (32 years; range 0-76) of cases did not differ significantly from the median age (33 years; range 0-81) of controls (p=0.253). Regarding gender, 46.4% of cases and 34.4% of controls were male.

TABLE 3

Univariate analysis of risk and protective factors (alcohol and drinking water, contact with animals, leisure activities, medical history) for campylobacteriosis. All-Ireland Campylobacter infection case-control study, 2004.

Exposure	Cases (n=197) † n (%)	Controls (n=296) † n (%)	Crude OR* (95% CI)	P-value
Alcohol	98 (50.5)	174 (59.4)	0.7 (0.4-1.2)	0.15
Alcohol during meals	59 (30.3)	73 (24.9)	1.8 (1-3.2)	0.04
Drinking water				
Mains water supply	184 (93.4)	268 (96.3)	0.4 (0.2-1.0)	0.06
Well	4 (2.0)	3 (1.0)	2.4 (0.5-11.0)	0.27
Group water scheme	2 (1.0)	2 (0.7)	1.5 (0.2-12.0)	0.70
Tap water	151 (77.0)	245 (83.6)	0.8 (0.5-1.3)	0.37
Own pet(s)				
Own any pet	5 (2.5)	5 (1.7)	1.8 (0.5-7.1)	0.38
Dog (as pet)	16 (8.1)	36 (12.2)	0.5 (0.2-1.1)	0.08
Cat (as pet)	4 (2.0)	11 (3.7)	0.6 (0.2-2.1)	0.46
Fish (as pet)	4 (2.0)	10 (3.4)	0.3 (0.1-1.4)	0.12
Pet ill with diarrhoea	57 (28.9)	110 (37.4)	0.7 (0.4-1.1)	0.09
Contact with animals (other than pets)				
Other dogs	39 (19.3)	93 (31.6)	0.5 (0.3-0.8)	0.01
Horses	4 (2.0)	7 (2.4)	0.5 (0.1-2.6)	0.39
Sheep or lambs	8 (4.1)	5 (1.7)	3.8 (0.7-19)	0.11
Outdoor and leisure activities				
Swimming or water sports in the sea	10 (5.1)	8 (2.7)	2.4 (0.7-8.6)	0.18
Running/Jogging/Athletics	18 (9.2)	38 (12.9)	0.6 (0.3-1.1)	0.12
Play in a garden or park	42 (21.4)	90 (30.6)	0.4 (0.2-0.7)	0.00
Own a garden	175 (89.7)	262 (89.4)	1.4 (0.7-3.1)	0.35
Using manure in the garden	6 (3.1)	7 (2.4)	1.4 (0.4-4.6)	0.55
Health and medical history				
Any of the following health problems	73 (37.1)	63 (21.3)	2.3 (1.42-3.6)	0.00
Stomach ulcer	23 (11.7)	8 (2.7)	5.4 (2-14.7)	<0.001
Gall stones or liver disease	5 (2.6)	2 (0.7)	4.3 (0.8-24)	0.10
Hiatus hernia	18 (9.2)	4 (1.4)	11.9 (2.7-52)	0.00
Lower bowel problems	23 (11.7)	14 (4.8)	4.2 (1.8-9.6)	<0.001
Lower bowel problems	8(4.1)	6 (2)	2.7 (0.8-9.2)	0.11
Lower bowel problems	12 (6.2)	5 (1.7)	3.8 (1.2-11.3)	0.03
Any of the following drugs in the month before	47 (24)	52 (17.7)	1.4 (0.8-2.3)	0.21
Antacid medicines	21 (10.8)	34 (11.6)	0.9 (0.5-1.7)	0.70
Ulcer medicines	21 (10.8)	4 (1.4)	10.3 (3-35.7)	<0.001
Steroid tablets	4 (2.1)	4 (1.4)	1.7 (0.4-7.2)	0.47
Antibiotics	17 (8.7)	15 (5.1)	1.4 (0.7-3)	0.39

* Matched odds ratio adjusted for age † For several variables answers were not available from all participants (denominators in percentages vary).

Information on place of residence (city/village/rural area) was only available for 38.3% of the respondents. Of those, the majority (90.9% of cases and 89.9% of controls) reported living in a town or city.

There were no significant differences in the employment status between cases and controls (Pearson $\chi^2 = 8.1712$; $p = 0.086$). Among employed cases and controls, 13.3% and 16.0% respectively reported handling food as part of their occupation.

The median number of people living in the same household as the cases was three (range 1-8) compared to four (range 1-9) people living in the same household as controls ($p = 0.35$). Of the cases, 22.6%, and of the controls, 23.1% had at least one child under five years of age in their household ($p = 0.47$).

Clinical features of cases

The main symptoms reported by the 197 cases were diarrhoea (99.1%) (including bloody diarrhoea reported by 39.1%), abdominal pain (89.3%) and fever (63.8%). Almost a fifth of cases were admitted to hospital, with the median duration of hospitalisation being four days (range 1-14 days).

Univariate and multivariate analysis of risk factors

The results of the univariate analysis for selected risk and protective factors are shown in Table 2 and Table 3. Cases were more likely than controls to have consumed chicken, and undercooked chicken in particular, duck, lettuce, to have eaten in a takeaway restaurant, to have eaten in a plane, to have travelled outside Ireland, to have drunk from a well, to have had contact with a sheep or lamb and to have swum in the sea. Cases were also more likely than controls to suffer from peptic ulcer, hiatus hernia or lower bowel problems.

TABLE 4

Final conditional logistic regression model. All-Ireland *Campylobacter* infection case control study, 2004.

Risk and protective factor	Crude OR* (n=457)	Adjusted OR* (n=457)	PAF †
Chicken	2.8 (1.4-5.7)	5.6 (1.8-15.6)	83.2
Lettuce	1.5 (0.9-2.5)	2.6 (1.3-5.2)	58.5
Other restaurant/takeaway	2.6 (1.5-4.4)	3.1 (1.4-6.6)	24.7
Hiatus hernia	11.9 (2.7-52.4)	20.3 (2.3-183.3)	21.6
Peptic ulcer	5.4 (2-14.7)	19.0 (3.8-93.7)	32.7
Lower bowel problems	4.2 (1.8-9.6)	4.5 (1.2-16.8)	14.4
Contact with sheep	3.8 (0.7-19.1)	11.0 (1.6-78)	14.5
Turkey	0.7 (0.5-1.1)	0.2 (0.1-0.5)	--
Beef	0.6 (0.4-1)	0.4 (0.2-0.8)	--
Salad (other than lettuce)	0.6 (0.4-0.9)	0.4 (0.2-0.8)	--
Mains water supply	0.4 (0.2-1)	0.2 (0.1-0.9)	--
Contact with a dog other than one's own	0.5 (0.3-0.8)	0.4 (0.2-0.8)	--
Playing in a park	0.4 (0.2-0.7)	0.3 (0.1-0.7)	--
Male	2.0 (1.2-3.2)	2.4 (1.2-4.7)	--

* Crude and adjusted matched odds ratios (ORs) are calculated based on 457 subjects, due to missing values in some variables
 † Population-attributable fraction

In the final conditional logistic regression model, seven variables remained significant independent risk factors for *Campylobacter* infection (consuming chicken, consuming lettuce, eating in a takeaway, contact with sheep, suffering from peptic ulcer, suffering from hiatus hernia and suffering from lower bowel problems) and six were protective factors (consuming turkey, consuming beef, consuming salad other than lettuce, having mains water supply at home, contact with dog other than one's own and playing in a park) (Table 4).

Eating chicken was the only risk factor that showed a dose-response relationship, as more frequent consumption of chicken increased the risk of infection by 20% per time of consumption (amOR 1.2 ; 95%CI: 1-1.4). When we stratified the results by country (ROI versus NI), country-specific ORs were not found significantly different. However, the numbers in the strata were not large enough to allow safe conclusions and the corresponding 95% CIs were wide (data not shown).

Discussion

Risk factors for transmission of infection

This study identified some independent risk factors for *Campylobacter* infection that could account for the majority of sporadic cases on the island of Ireland. The most important, based on PAF, was eating chicken, consuming lettuce and eating from a restaurant takeaway (other than Chinese or Indian) in the seven days before onset of illness. The other risk factors identified were: contact with sheep, suffering from stomach ulcer, peptic ulcer or gastritis, suffering from hiatus hernia, suffering from lower bowel problems.

Our study showed an increased risk associated with chicken consumption in general, and an even (three times) higher risk associated with undercooked chicken consumption. This finding, which was also supported by an observed dose-response relationship, was not unexpected as chicken and, in particular, undercooked chicken has been the most consistent finding in studies of risk factors for campylobacteriosis [4,5,7,8,11,12,15,18,19,20]. However, the PAF suggests that the consumption of chicken may account for an unusually high proportion (i.e. the majority) of sporadic cases that occur in Ireland, both ROI and NI. Given that chicken consumption exceeds 70% among the Irish population, this finding receives more importance [16]. Recent microbiological studies of raw poultry conducted in NI and ROI have shown that 50%-70% of raw chickens tested at retail level, were contaminated with *Campylobacter* [21-26]. Those contamination rates were consistently the highest among all food items examined. Furthermore, the genotypic characterisation by Pulsed Field Gel Electrophoresis (PFGE) of both clinical and food isolates and comparative cluster analysis during a recent all-Ireland study, has revealed that a high proportion of indistinguishable *Campylobacter* isolates found in poultry products were also found in human cases [21], re-emphasising the significant role that chicken plays in the epidemiology of human *Campylobacter* infection in Ireland.

Although, in line with several other studies, our study has found negative associations with salad vegetables, lettuce was identified as an important risk factor for infection. Lettuce consumption has previously been described in association with outbreaks [27-29], but, to our knowledge, it has never been identified as a risk factor for sporadic cases. The consumption of lettuce was implicated as the likely vehicle of infection in a recent large *Salmonella* Newport outbreak in NI [30]. Lettuce could be contaminated

with *Campylobacter* before or after the point of sale. Contamination at source could occur through the presence of contaminated soil, the use of contaminated water during harvesting or even flies [31]. During food preparation, fresh lettuce may also be cross-contaminated by kitchen tools or surfaces already contaminated from previous contact with other food [32]. Cross-contamination was the most frequent contributory factor identified in a review of foodborne outbreaks in England and Wales (including five due to *Campylobacter*) linked with lettuce and other salad vegetables and fruit [28,29]. A recent study in ROI, in common with several other studies, has demonstrated that *Campylobacter* can easily spread from fresh food, mainly chicken, to domestic kitchen surfaces and tools and subsequently to lettuce and other salad vegetables [33]. This suggests that cross-contamination of fresh products is very likely to happen in kitchens, particularly through poor handling or storage practices.

Consumption of food from takeaways (other than Chinese or Indian) was an important risk factor, based on PAF (PAF=25). This association suggests that food-hygiene preparation practices in these settings may be poor. Several other studies have implicated exposure to food (most often poultry) prepared outside the home to be associated with campylobacteriosis [7,18,34]. However, it was not possible in this study to identify specific food items associated with infection, bought from takeaways. Further attention to sources of food and food-handling practices in these restaurants in Ireland are needed. Cross-contamination of ready-to eat foods may be an important source of infection, given evidence from experimental studies suggesting that *Campylobacter* is frequently present in a variety of foods and has a low infectious dose (ranging from 500-10,000 cells) [22,35].

A small proportion of cases could be explained by contact with sheep. This association, though apparently uncommon, is entirely plausible. Sheep are known to be carriers [36], excrete *Campylobacter* and therefore may transmit infection to humans. Previously, occupational contact with animal faeces, living on a farm and contact with cattle have all been described as risk factors for *Campylobacter* infection [15].

The association between campylobacteriosis and suffering from peptic ulcer or hiatus hernia yielded the highest aOR among all risk factors identified, although this factor explained a small percentage of cases, overall. Many of the patients who suffer from these gastrointestinal diseases may be receiving long-term treatment with acid suppressants, such as proton pump inhibitors (omeprazole) and H₂ antagonists. These drugs have been previously reported to increase risk for *Campylobacter* infection probably by increasing gastric PH and therefore making the stomach a much less hostile environment for bacteria [37].

To our knowledge, the independent association between lower bowel problems and campylobacteriosis has not been previously reported. The biological plausibility of this finding is unclear, particularly, as data were not collected on specific diseases of the lower bowel. It is possible that some conditions or their treatment may lead to a prolonged gastrointestinal transit time and slow clearance of the organism. Alternatively, this finding may be due to a bias, as the case ascertainment for campylobacteriosis may have been higher for patients with pre-morbid bowel problems who may be more likely to submit a faecal stool sample. This bias may also apply to the previously mentioned diseases, i.e. peptic ulcer or hiatus hernia. However, more research is needed to clarify this apparent effect and the mechanisms behind it.

Protective factors

The role of mains water supply as a protective factor is interesting. It has been previously reported that inadequately treated water may cause *Campylobacter* infection in humans and this pathogen was implicated in several waterborne outbreaks in some countries [38-42]. In addition, a recent ecological study in Sweden indicated that water might be an important route of transmission for *Campylobacter* infection [43]. Water can be contaminated through animal faeces [44] and sewage and some *Campylobacter* strains can survive for long in untreated water sources [45]. In this study, cases were twice more likely to drink water from a source other than the mains water supply (e.g. wells or other water schemes). This association, however, was not statistically significant probably because this exposure was uncommon (reported only by 13 cases and 7 controls). It is possible that the protective effect of the public water supply reflects the association of infection with sources of untreated water.

Many of the other protective factors (mainly food items such as beef, turkey and salad vegetables other than lettuce) might indirectly confirm the association with chicken and lettuce as our data suggest that controls, who ate chicken or lettuce less frequently than cases, were more likely to replace those food items with another kind of meat (including poultry) or salad vegetable respectively.

The protective effect of playing in the park and of having contact with a dog other than one's own is less clear. It is possible that these individuals may have a healthier life-style and therefore be less prone to infections or may have engaged in some practices, not evaluated in the study, which protect them from *Campylobacter* infection.

Limitations of the study

The study only involved campylobacteriosis cases reported through the routine surveillance system, that constitute a subset of all cases occurring in the community. Epidemiological studies in the UK have shown that only one in eight cases of *Campylobacter* infections occurring in the community is reported through routine surveillance [15]. In addition, due to the relatively low response rate and the matched design of the study, cases that were included in the analysis may have not been representative of all reported cases. However, the available demographic data (age and gender), suggest that there were no statistically significant differences between participant and non-participant campylobacteriosis cases reported to the health authorities.

All *Campylobacter* species were included in the case-definition and no information on speciation was collected. It is possible that risk factors vary according to campylobacter species. Approximately 90-95% human infections are due to *C. jejuni* in the developed world [38], and sporadically available typing data suggest that Ireland has a similar distribution [1].

Controls were not randomly selected from the source population, which would have assured their representativeness in terms of the exposures, but were case-nominated. Choosing controls among friends, work colleagues and neighbours might lead to over-matching, as cases and controls may be similarly exposed to common risk factors (especially food). In addition, some of the controls may have come from the same household as the cases. This could have increased the risk of over-matching even more. This effect, however, may have only reduced the strength of

any association. We excluded those cases and controls from the analysis, whenever this was evident.

As in all case-control studies, cases as a result of their illness may be more able to recall exposures than controls, which may result in recall bias. However, cases that received their questionnaire long after their onset of symptoms in this study (median delay 16 days), may have reported a list of food preferences rather than their definite food exposures. This may have been less of a problem for controls, as exposure period referred to the week prior to their completing the questionnaire, i.e. a more recent period.

Information on place of residence was limited in this study, as only 38.3% of the respondents answered the relevant question. Surveillance data have shown increased incidence of *Campylobacter* infection in rural areas compared to urban areas and studies have suggested that exposure to risk factors may be different for people living in rural compared to those in urban areas [46]. Accurate knowledge of the geographical distribution of cases and controls would have been of added value, and it could have potentially resulted in different guidelines for rural and urban areas.

Conclusion and recommendations

The study suggests that consumption of chicken, lettuce and food from takeaways accounts for the majority of *Campylobacter* infections in the island of Ireland, both ROI and NI. All these factors could be prevented using basic food-hygiene measures. The findings of this study therefore, highlight the need for an improved and more efficient approach to basic food-hygiene measures to prevent campylobacteriosis and other infectious gastrointestinal illnesses in the community. Further measures are needed throughout the food chain from production, transport, retail and catering to reduce the risk of contamination and cross-contamination. Improved catering practice, whether in the domestic or commercial setting, is an important last line of defense in reducing exposure to potentially *Campylobacter*-contaminated products. In addition, it is essential to raise awareness in the population of the importance of good basic food-hygiene practices, using means of communication easily and readily accessible. Further efforts are needed to identify the defective points in the food chain and enable appropriate measures to reduce the overall burden of this infection in the Irish community. Linkage of epidemiological data with *Campylobacter* speciation and the development of new molecular diagnostic tests will also provide a greater understanding of *Campylobacter* infection.

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HEPATITIS A SEROPREVALENCE IN TUSCANY, ITALY

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Information regarding the current seroprevalence of hepatitis A virus (HAV) is useful for the control of HAV infections. The objective of our study was to evaluate the prevalence of anti-HAV antibodies among children (1-5 years old) and young adults (15-20 years old) in Tuscany, in central Italy. A total of 565 sera were collected in three years 1992, 1998 and 2004, equally distributed between the two age groups. The overall proportion of those that tested positive for anti-HAV antibodies was 8.3%. The proportion of immune children (1-5 years old) statistically significantly increased over the years. The percentage of immune subjects among 15-20-year-old young adults varied over the years, not showing a significant statistical trend, nevertheless our findings indicate that in a low endemicity area, adolescents and young adults are becoming increasingly susceptible to HAV infection. On-going monitoring of immunity to HAV is necessary for detecting trends over time.

Introduction

Hepatitis A is generally an acute, self-limiting liver infection transmitted through the faecal-oral route by a picornavirus, hepatitis A virus (HAV) that occurs worldwide and causes about 1.5 million cases of clinical hepatitis each year [1]. The degree of endemicity is closely related to hygienic and sanitary conditions, the socio-economic level and other development indicators [2]. In recent decades Italy has experienced a declining trend of HAV prevalence [3], probably related to improved health and sanitary conditions which have been responsible for the progressive decline in the infection rate among children under 14 years of age and a major shift towards the highest incidence in susceptible teenagers and young adults [4]. Nevertheless, Italy is considered to be an area with low/intermediate endemicity of hepatitis A. Data from the national surveillance system for acute viral hepatitis infection (Sistema Epidemiologico Integrato dell'Epatite Virale Acuta, SEIEVA), suggest a steady decrease in the incidence of reported cases of HAV infection over the past few years (from 10 per 100,000 inhabitants in 1985 to 3.6 per 100,000 in 2004) [5].

However, the epidemiological situation varies from region to region within Italy [4]. The practice of consuming contaminated raw seafood still causes outbreaks, especially in southern Italy. In particular, Puglia and Campania, two regions in the south, experienced a large outbreak during 1996-7 with approximately 11,000 cases of HAV infection reported, accounting for an annual incidence rate of approximately 130 per 100,000 population [6,7]. Moreover, in 2004 another outbreak, involving 882 cases, was described in Campania [8].

The availability of a safe, highly immunogenic vaccine that provides long-term protection against HAV has been proven to be useful in the containment of hepatitis A in endemic areas [9]. However, high costs and the limited availability of the HAV vaccine have raised some concerns regarding mass vaccination [4,10]. In Italy the current National Vaccination Plan (Piano Nazionale Vaccini PNV 2005-2008), recommends vaccination against HAV only for specific population groups (travellers to endemic areas, drug users, men who have sex with men (MSM), soldiers, sewage workers, patients presenting with liver disease, recipients of liver transplants and HAV-negative haemophiliacs) [11,12]. Since 1998, after a large epidemic of hepatitis A, the Puglia region (south-eastern Italy) has introduced a free-of-charge mass vaccination program (the first ever in Italy since safe and highly effective hepatitis A vaccines became available in 1995) for newborns (15-18 months of age) and adolescents (12 years of age), as part of the routine immunisation schedule, in order to reduce transmission [9]. For this reason, since 2001, when the Italian National Health System was decentralised, the regional health authorities have implemented vaccination strategies according to their own judgment. However, the region of Tuscany does not include hepatitis A vaccination in the regional infant and adolescent immunisation calendar. Preventive hepatitis A vaccination, however, is considered, in Tuscany and all other Italian regions, for close contacts of clinical cases as control measure in case of an epidemic.

Moreover, although hepatitis A is usually a self-limited disease, the likelihood and severity of symptomatic illness are age-related. In a low endemicity area the highest frequency of HAV infection is observed in adults, who are more likely to have clinical symptoms since the infection causes significant morbidity, along with absenteeism, hospitalisations and occasional mortality, while infants and young children are usually asymptomatic [13,14]. In the last decade, in Italy, a progressive reduction of the prevalence of the infection in children, teenagers and young adults has been described. However, the symptomatic/asymptomatic ratio and the percentage of patients with a more severe clinical presentation have progressively increased [15].

The objective of the present study was to determine the prevalence of anti-HAV antibodies in children and young adults in Tuscany, in central Italy, and to present epidemiological data on HAV infection in this area.

Methods

Serum samples

Serum samples from individuals aged 1-5 and 15-20 and collected during three different years: 1992, 1998 and 2004, in the region of Tuscany, were tested. Serum specimens were obtained by using leftover serum from specimens taken for diagnostic purposes and submitted to the Laboratory from individuals who received care in the University Hospital of Siena. The sera collected from the two ranges of age were randomly selected from the laboratory stock. We excluded samples from individuals known to be HIV-seropositive. Samples were collected anonymously and only the age of the subject and the date of the sample were recorded.

A total of 565 sera (283 for the age group 1-5 years and 282 for the age group 15-20 years, approximately 100 samples for each year) were collected and stored at -20°C. Of the 565 serum samples collected all were tested for the anti-HAV-IgG antibody: 47 (8.3%, 95% CI: 6.03-10.57) were positive, 509 (90.1%, 95% CI: 87.64-92.56) were negative and 9 (1.6 % CI: 0.57-2.63) gave ambiguous results. Thus, 556 samples were included in the statistical analysis, as the nine giving ambiguous test results were excluded (Table).

Serological test

Serological testing was performed at the Department of Physiopathology, Experimental Medicine and Public Health of the University of Siena. All serum samples were tested for detection of anti-HAV-IgG antibodies. The antibody index (calculated as sample OD/cut-off serum mean OD x 10), was determined by a commercial enzyme immunoassay test (Enzywell HAV Antibody, DIESSE) following the manufacturer's instructions. According to the manufacturer, the test has a sensitivity and specificity of 100%.

Samples with an index of < 0.8 were considered positive, samples with an index > 1.2 were considered negative, and samples with an index ranging from 0.8 to 1.2 were considered equivocal. Equivocal sera were retested for confirmation.

A positive result for total anti-HAV IgG antibodies is not able to distinguish recent infection from a previous one, thus a test for determining IgM antibodies is necessary for correct detection of current infection. However, the test for determining total anti-HAV IgG antibodies is important since this indicator allows for the identification of cohorts of subjects that are still at risk of infection.

TABLE

Samples tested for the prevalence of antibodies against hepatitis A virus, Tuscany, Italy, by patients' age group, gender and the year of obtaining the specimen

Year of obtaining the sample	Age group (gender: male - female)	
	1-5 years n (m - f)	15-20 years n (m - f)
1992	73 (42-31)	80 (21-59)
1998	97 (44-53)	71 (22-49)
2004	108 (54-54)	127 (65-62)
Total	278 (140-138)	278 (108-170)

There also is no way of determining whether the positive test results are due to past infection or due to vaccination. Detection of hepatitis A IgG antibodies indicates either past infection or vaccination.

Statistical analysis

Age-specific (1-5 years and 15-20 years) seroprevalence rates were calculated, along with the corresponding 95% confidence intervals (CI). The statistical analysis was performed by Epi Info version 6 program using the chi-squared (χ^2) test, as well as χ^2 test for trend to evaluate possible tendencies. Differences were regarded as significant when $p < 0.05$.

Results

Of the 565 serum samples collected, all were tested for the anti-HAV-IgG antibody: 47 (8.3%, 95% CI: 6.03-10.57) were positive, 509 (90.1%, 95% CI: 87.64-92.56) were negative and 9 (1.6 % CI: 0.57-2.63) gave ambiguous results.

The nine specimens with equivocal results were eliminated from the statistical analysis. Among these, five were taken from 1-5 years old children (four males, one female) and four were from patients aged 15-20 years (two males, two females). Thus, 556 samples were suitable for calculating the results.

Of the 278 serum samples from 1-5-year-old children 26 were positive (9.4%, 95% CI: 6.20-13.40) and of the 278 serum samples from the age group 15-20 years 21 were positive (7.6%, 95% CI: 4.73-11.31).

The percentage of immune children (1-5 years) increased from 2.7% (95% CI: 0.33-9.5) in 1992, to 6.2% (95% CI: 2.30-13.0) in 1998, and to 16.7% (95% CI: 10.19-25.05) in 2004 (Figure). The annual trend was statistically significant ($\chi^2 = 8.9$, p value = 0.0027).

The percentage of immune young adults, aged 15-20 years, varied from 7.5% (95% CI: 2.80-15.61) in 1992, to 11.3% (95% CI: 5.0-21.0) in 1998, and to 5.5% (95% CI: 2.24-11.03) in 2004 (Figure). In this case, the yearly trend was not statistically significant.

Discussion and conclusion

The results of this study showed an increasing trend of the seroprotection rate in children aged 1-5 years with a particularly high antibodies titre in 2004 (χ^2 for linear trend=10.7; $p=0.0011$). The high anti-HAV antibodies rate among children in 2004 is likely to have been related to extensive vaccination of this age group in consequence of a small epidemics that occurred in communities of children in central Italy during that period [16]: children attending primary schools were contaminated by infected schoolmates who had contracted hepatitis A by eating raw seafood in endemic areas (Campania and Puglia) during Christmas holidays.

One case-control study conducted during an outbreak of hepatitis A which occurred in 2004 in southern Italy and affected different municipalities of Campania region, in the municipality with the highest attack rate showed that raw seafood consumption, particularly when it was illegally stored in water, was strongly associated with HAV infection [8]. The major role played by shellfish consumption in HAV transmission in Italy is supported by data from the surveillance system for type-specific acute viral hepatitis (SEIEVA) [5].

On the other hand, the prevalence of anti-HAV antibodies in young adults (15-20 years old) was higher during 1992 and 1998 (7.5% and 11.3%), followed by a decrease to 5.4% in 2004 (χ^2 for linear trend=0.40; $p=0.53$). However, this is not enough to support the presumption that there is an increasing trend in the risk of infection in young adults.

These results must be considered cautiously because the samples studied cover only three years (1992, 1998 and 2004) during a period of eight years (from 1992 to 2004). Nevertheless, these findings confirm the shift of the seroprevalence of hepatitis A virus infection in younger age groups, as observed in the urban population of India [17], in an area with low endemicity where adolescents and young adults are becoming increasingly susceptible to HAV infection.

In the past few years similar studies have been conducted in Italy but only two described the situation in the whole country. However, direct comparisons with these studies are difficult due to the differences in the age groups considered. One, conducted in 1990, showed an anti-HAV immune prevalence of 2.3% among 3 to 5 year-old children, and of 16.3% in teenagers aged 17-19 [18]. The other study performed on sera collected in 1997-1998 showed a prevalence of 34.9%, 12.9% and 14.6% in age groups of 0-1, 2-5 and 12-19 years respectively [19].

Other seroepidemiological investigations have only been conducted in specific areas or among certain risk groups. In 1994 the seroprevalence of HAV antibodies was tested in north-east Italy: the prevalence obtained was 0.7% among the group aged 10-19 years old and 6.0% in the group of over 19 years. Anti-HAV antibodies prevalence in army recruits was 66% in 1981, 30% in 1990 and 5% in 2003 (ranging from 2% in the north to 8% in the south) [20]. Furthermore, a specific report on the prevalence of hepatitis A virus (HAV) in a group of drug users in Italy showed an overall seroprevalence of 28.7% [21].

In conclusion, information regarding the current status of hepatitis A immunity, including the seroepidemiological survey described here, is crucial for providing new and timely parameters useful for mathematical modelling, such as the one proposed and investigated for temporal trends in southern Italy [22]. A

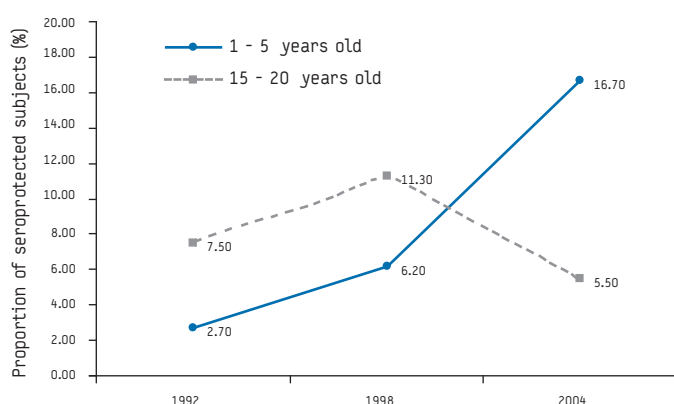
vaccination program for risk groups, travellers and food handlers would be suitable for the control of this virus at present, however, the current epidemiological situation in Italy does not suggest the need for mass vaccination of newborns and adolescents outside the endemic areas, such as Puglia and Campania. The same is true in other southern European countries, such as Spain, where universal immunisation seems economically ineffective and sufficient evidence for targeted vaccination has not yet been gathered [4].

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FIGURE

Seroprevalence of antibodies against hepatitis A virus, in age groups of 1-5 and 15-20 years, Tuscany, Italy, 1992, 1998, 2004



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USE OF SEVEN-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV7) IN EUROPE, 2001-2007

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The first pneumococcal vaccine targeting the youngest age groups, a seven-valent conjugate vaccine (PCV7), was licensed in Europe in 2001. Since then several European countries have introduced PCV7 in their childhood vaccination schedules. Still, information on vaccination schemes, vaccine uptake and impact of vaccine introduction is scarce in Europe. The following article summarises the characteristics of national pneumococcal vaccination programmes for children in 32 European countries and provides an estimate of vaccine use based on sales data for 22 countries between 2001 and 2007. There were wide variations in the recommended PCV7 vaccination schemes and in PCV7 use. High vaccine uptake was not always related to the presence of a national vaccination programme.

Introduction

Pneumococcal infection is an important cause of otitis media, pneumonia, septicaemia and meningitis leading to significant morbidity and mortality, particularly in young children and elderly people. The first vaccine targeting children, a seven-valent pneumococcal conjugate vaccine (PCV7), was first licensed in the United States in 2000 [1] and vaccination coverage has since increased from 89% (≥ 1 dose PCV7) and 68% (≥ 3 doses PCV7) among children born in 2001 to 95% and 84%, respectively, among children born in 2005 [2].

Following the European Union (EU)'s authorisation in 2001 for PCV7 use in children aged between the age of two months and five years [3], European countries have gradually introduced PCV7 in their vaccination schedules. In contrast to the situation in United States, there is little data on PCV7 vaccination coverage in European countries. This article provides an overview of the current national pneumococcal vaccination programmes in children and uses country-specific sales data to provide an estimate of PCV7 use in European countries.

Material and methods

Information about current national pneumococcal vaccination programmes for children in 32 European countries, including all 27 EU countries plus Croatia, Iceland, Norway, Switzerland and Turkey, was submitted by the national public health or surveillance institutions to the European surveillance network for vaccine-preventable diseases (EUVAC.NET) hub (see Acknowledgments). Data were collected between March 2008 and March 2009.

The only PCV7 licensed so far in the EU is a vaccine covering *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, conjugated to the CRM197 carrier protein and adsorbed on 0.5 mg of aluminium phosphate (Prevenar™, Wyeth). Annual PCV7 sales data in 22 European countries for this period were provided by Wyeth, the marketing authorisation holder and manufacturer of the vaccine. For each country, population data by age group were obtained from the online database of Eurostat, the statistical office of the European Communities [4]. PCV7 use was estimated by calculating (a) the yearly rate of PCV7 doses sold per 100 live births between 2001 and 2007, and (b) the cumulative number of completed vaccination courses (based on either three or four doses, according to the national schedules) per 100 live births for the period from 2005 to 2007. The yearly number of live births (birth cohort) was used as the denominator for all countries, including those that recommend vaccinating risk groups, as data on the size of the different risk groups was not available.

Results

National PCV7 vaccination programmes

By January 2009, 24 (75%) of the 32 participating European countries had introduced or decided to introduce vaccination against pneumococcal disease in their childhood vaccination schedule (see Table). Seven (29%) of these schedules offer PCV7 to risk groups only. In Italy, either risk-based or universal vaccination programmes are used, depending on the region. Twenty (83%) of 24 countries with a vaccination programme against pneumococcal disease started the programme in 2005 or later. Twelve (50%) countries recommend a 3+1 dose vaccination regimen and 11 countries recommend a 2+1 regimen. Switzerland uses a 3+1 regimen for risk groups and a 2+1 regimen for other children.

There is some variation regarding reimbursement of the vaccine. However, most of the countries (92%, $n=22$) with an established programme offer the vaccine free of charge or at least offer cost sharing for the respective target group. In Italy, the reimbursement policy (full reimbursement versus cost-sharing) varies depending on the region. Among countries with universal vaccination programmes, 11 have implemented catch-up programmes with different schemes.

PCV7 use

In almost all countries, and especially in the countries that have already introduced PCV7 in their childhood vaccination schedule,

TABLE

Characteristics of national pneumococcal vaccination programmes for children in 32 European countries

Country	Extent of PCV7 vaccination programme	Date of implementation	Vaccination regimen	Catch-up programme	Reimbursement	Comments
Austria	Universal	September 2004	3+1	No	No	Free of charge for children under the age of two years in risk groups.
Belgium	Universal	January 2005	2+1	Yes ^b	Total	Free of charge for children under the age of two years since January 2007
Bulgaria	None	-	-	-	-	Inclusion of PCV7 as a recommended vaccine on an individual voluntary basis is being considered based on a decision of the expert committee on national immunisations (24 July 2008).
Croatia	Risk-based	November 2006	3+1	n/a	Total	Since August 2008 free of charge for children at the ages of two, four and six months, with a booster dose at the age of 12-15 months (3+1). In addition, a catch-up programme is implemented for children up to the age of 59 months.
Cyprus	Universal	August 2008	3+1	Yes	Total	Free of charge for children under the age of five years since January 2007.
Czech Republic	Risk-based	January 2007	3+1	n/a	Total	Free of charge for children under the age of five years since January 2007.
Denmark	Universal	October 2007	2+1	Yes ^c	Total	
Estonia	None	-	-	-	-	
Finland	Risk-based	January 2009	2+1	n/a	Total	Since January 2009, free of charge for children under the age of five years in risk groups. In addition, one dose of pneumococcal polysaccharide vaccine is given to children over the age of two years in risk groups.
France	Universal	June 2006	2+1	Yes ^d	Cost sharing/total	In October 2008, the vaccination regimen changed from 3+1 to 2+1. 65% of the price of PCV7 is reimbursed by social security. The rest is reimbursed by private insurance (for the 80% of the population that have one). The vaccine is free of charge in mother and child care services.
Germany	Universal	July 2006	3+1	Yes ^e	Total	Since January 2008, reimbursement of all recommended vaccinations has been regulated on a national level.
Greece	Universal	March 2006	3+1	Yes ^b	Total	Fully reimbursed since March 2008
Hungary	Universal	October 2008	3+1	Yes ^b	Total	Since October 2008, PCV7 has been given on a voluntary basis and free of charge to children under the age of two years with the 3+1 regimen. As of April 2009, PCV7 will be given free of charge to children at the age of two and four months, with a booster dose at the age of 15 months (2+1 regimen).
Iceland	Risk-based	December 2006	2+1	n/a	No	Free of charge for all children.
Ireland	Universal	September 2008	2+1	n/a	Total	
Italy	Universal/ risk-based	May 2005	2+1	No	Cost sharing/total (regional variation)	In 15 of 20 regions, PCV7 is offered to all children either free of charge or with cost sharing. In five regions, PCV7 is recommended to children at risk only and is free of charge.

Latvia	None	-	-	-	-	-	Voluntary vaccination of children in risk-groups is planned for 2009.
Lithuania	None	-	-	-	-	-	
Luxembourg	Universal	October 2004	3+1	Yes ^d	Total		
Malta	Risk-based	January 2007	3+1	n/a	Total		
The Netherlands	Universal	June 2006	3+1	-	Total		
Norway	Universal	July 2006	2+1	Yes	Total		PCV7 was introduced in the national childhood vaccination programme on 1 July 2006, with a catch-up programme for children born after 1 January 2006.
Poland	None	-	-	-	-		
Portugal	None	-	-	-	-		The Portuguese National Vaccination Committee is in the process of discussing the implementation of PCV7 into the national vaccination programme.
Romania	None	-	-	-	-		
Slovakia	Universal	April 2008	2+1	n/a	Cost sharing		Universal: recommended to children under the age of two years as complementary (optional) vaccination for optimal individual protection. 96% of the costs are reimbursed by the national health insurance.
Slovenia	Risk-based	January 2006	2+1		Total		Free of charge to children under the age of two years belonging to risk groups.
Spain	Risk-based	September 2005	3+1	n/a	Total		Fully reimbursed since September 2005.
Sweden	Risk-based	June 2001	3+1	n/a	Total		Free of charge for children under the age of five years since June 2001.
Switzerland	Universal	January 2009	2+1	n/a	Total		Since January 2009, PCV7 has been part of the national childhood vaccination programme and is recommended to all children born from October 2008 onwards.
Turkey	Universal	November 2005	2+1	Yes ^d	Total		Universal: recommended as complementary (optional) vaccination for optimal individual protection; fully reimbursed since August 2006.
United Kingdom	Risk-based	July 2001	3+1				Risk-based: fully reimbursed since July 2001.
	None	-	-	-	-		
	Universal	September 2006	2+1	Yes ^b	Total		Free to all children

n/a = not applicable

a Number of PCV7 doses given during first year + number of booster doses

b Until 23 months of age for all children

c Until 18 months of age for all children

d Until 23 months of age for all children and until 59 months of age for children with particular co-morbidities

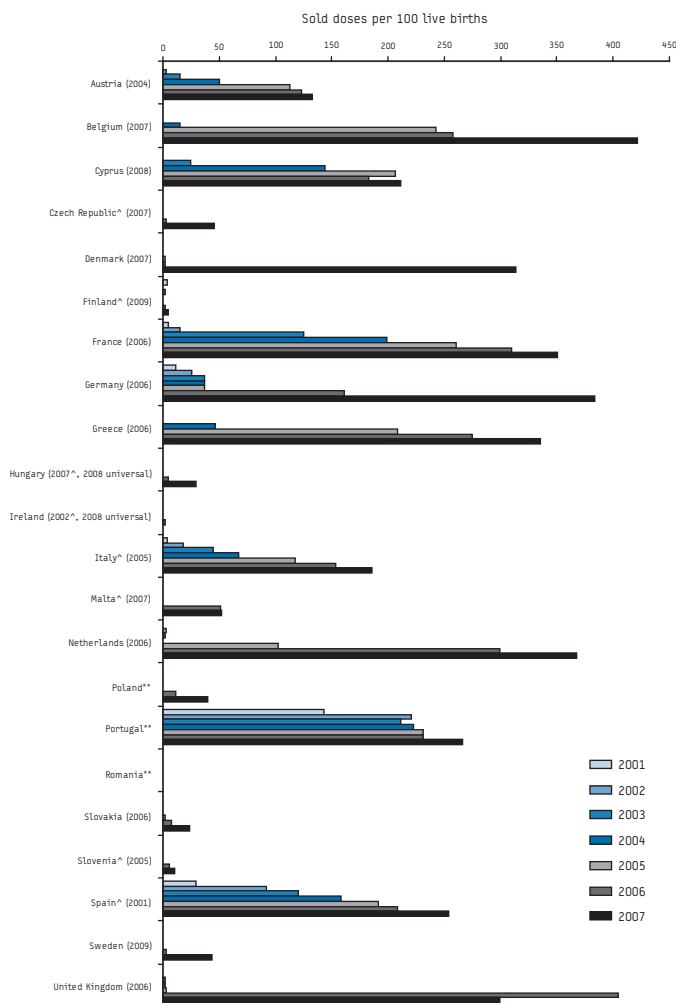
e Until 59 months of age for children with particular co-morbidities

Source: EUVAC.NET

PCV7 sales increased in the period from 2001 to 2007 (Figure 1). An increasing trend in PCV7 sales could be observed in nearly all countries and the increase in PCV7 sales was especially marked in the year the childhood pneumococcal vaccination programme started or shortly thereafter.

The highest PCV7 use was registered in Belgium in 2007 (422 doses per 100 live births), followed by the United Kingdom in 2006 (405 doses per 100 live births). In both cases, the peak coincided with the introduction of PCV7 in the childhood vaccination schedule. An estimate of the cumulative number of complete PCV7 courses per 100 live births for each country in 2005-2007 is presented in Figure 2.

FIGURE 1
Sold PCV7 doses per 100 live births in 22 EU countries, 2001-2007

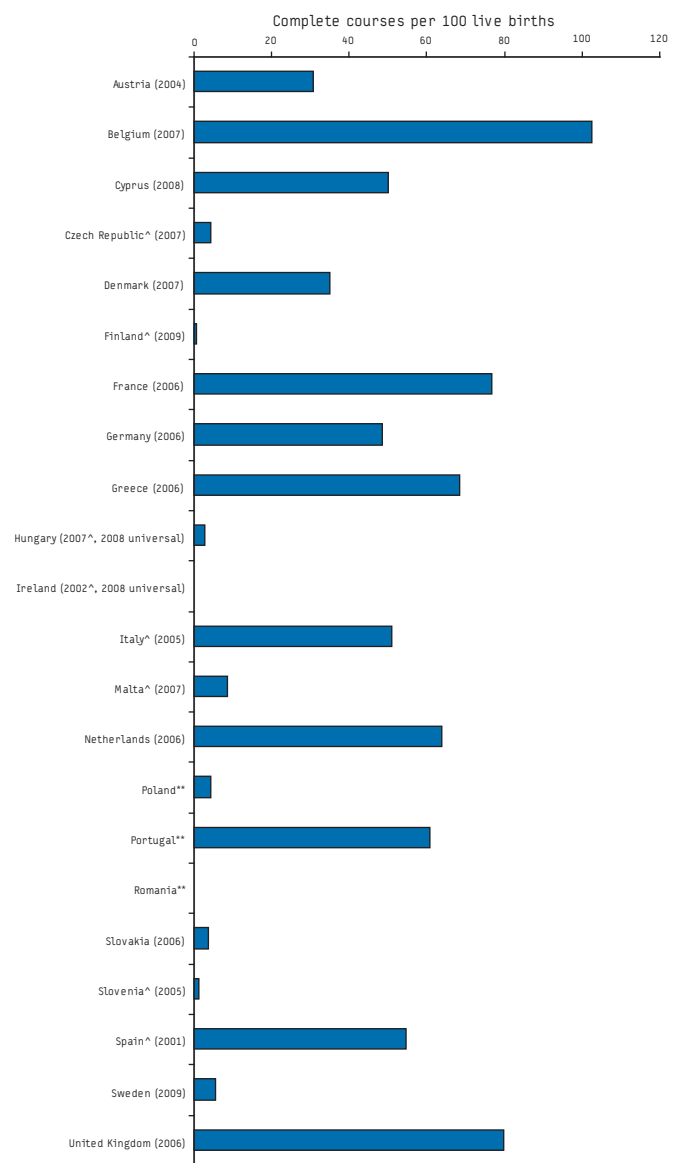


The data are shown as yearly sold doses per 100 live births in the respective year for 22 EU countries, for which sales data were available. For each country, the year of PCV7 introduction into the childhood vaccination schedule is shown in parenthesis.
 ^ Country with risk group programme only. Italy has a mix of universal and risk group programmes depending on the region.
 ** Country without childhood programme for vaccination against pneumococcal disease.

Discussion

This study presents the latest information on current national pneumococcal vaccination programmes in children in European countries. It also presents information on PCV7 use in the years from 2001 to 2007, based on sales data provided by the only PCV7 manufacturer in Europe during that time period. At the time of a previous review of PCV7 vaccination programmes in 2006, 19 European countries had recommendations for pneumococcal

FIGURE 2
Estimated number of complete PCV7 courses per 100 live births in 22 EU countries, 2005-2007



The cumulative number of complete PCV7 courses was estimated based on either three or four doses, according to the national schedules. For each country, the year of PCV7 introduction into the childhood vaccination schedule is shown in parenthesis.
 ^ Country with risk group programme only. Italy has a mix of universal and risk group programmes depending on the region.
 ** Country without childhood programme for vaccination against pneumococcal disease.

vaccination in children [5]. Among these, 10 had started a universal childhood pneumococcal vaccination programme. Three years later, seven additional European countries have introduced a universal pneumococcal vaccination programme for children. Although progress has been made to introduce PCV7 globally, only few countries outside Europe have introduced this vaccine into their national immunisation programmes for all children, and these are primarily high-income countries, i.e. the United States, Canada, Australia and New Zealand [6].

In each European country, the decision to introduce a new vaccine in the vaccination schedule is the result of careful discussions. In the case of PCV7, budget constraints have often been the principal driver in the decision-making process, especially in lower income European countries. PCV7 is an expensive vaccine to be proposed for childhood vaccination. Although the vaccine has been shown to decrease the incidence of invasive pneumococcal disease and pneumococcal pneumonia in children [7-11], different methods have been used to evaluate its cost-effectiveness and uncertainty remains as to whether a universal PCV7 vaccination programme in children would be cost-effective [12]. In the absence of adequate surveillance data, there have been concerns that the available vaccine may not cover all circulating pneumococcal strains. There have also been concerns about possible replacement of serotypes used in PCV7 by serotypes not covered by the vaccine [13,14]. As a consequence, the recommendations for PCV7 vaccination in children vary even between countries of similar income levels.

Publicly available data on PCV7 vaccination coverage in European countries is scarce [14-16]. Data on the number of sold PCV7 doses that were actually used, as well as the number of doses used for each child were not available, and PCV7 vaccination coverage could therefore not be calculated in this study. Two different rates were calculated to estimate PCV7 uptake and differences in use between European countries. Firstly, we calculated the number of sold PCV7 doses per 100 live births for the 22 countries for which sales data were available. From the sales data, we also estimated the number of - theoretically possible - complete PCV7 courses per 100 live births for the three most recent years for which data on sales and births were available (Figure 2). We assumed that all PCV7 doses sold in a specific year were given only to children born in that same year, that PCV7 doses were offered according to the vaccination schemes (3+1 or 2+1) recommended in each country at the time, and that the vaccination scheme was completed in the same year. We are aware that this rather simplistic approach is likely to have overestimated the real number of completed vaccination courses. However, it made it easier to benchmark the PCV7 use in the countries.

In Belgium, Denmark and the United Kingdom, the estimates of complete PCV7 courses, based on the respective PCV7 vaccination schemes in use, were above 100% just after the start of the vaccination programme. This could be an indication of increased efforts at the beginning of the programme to include every child in the target group definition.

PCV7 sales were high in countries with a national programme for universal childhood vaccination for pneumococcal disease. They were also remarkably high in Portugal and Spain, countries that do not have such a universal programme. Spain has had a risk-based PCV7 vaccination programme since 2001 and a single universal programme in the Madrid region since 2006 [17]. A study performed in northern Portugal in 2002 aimed at estimating the use of meningococcal and pneumococcal vaccine, which were

both not part of the Portuguese childhood vaccination schedule at the time. That study showed that one third of the 1,877 children born in northern Portugal in 1999 were vaccinated against pneumococcal disease and that most of these children had been vaccinated at an age over 23 months, i.e. later than during the age range recommended in most other countries [18]. The application of both vaccines – the one for meningococcal and the one for pneumococcal disease - was highly correlated. The high vaccine use in the absence of a programme or reimbursement policies was attributed by the authors, at least partly, to high media coverage during a peak of meningitis cases in the region. This single study, however, cannot explain the regular high annual sales of PCV7 in this country.

In conclusion, our study showed large variations in the recommended PCV7 vaccination schemes and in PCV7 use across Europe. While it has to be said that higher vaccine uptake is not always related to the presence of a national vaccination programme, this observation highlights the need for harmonisation of the decision making process in the EU in order to improve access of all European citizens to preventive services such as vaccination. As for other vaccine-preventable diseases, epidemiological surveillance is paramount to provide decision makers with solid data on burden of disease and impact of vaccination. Detailed data on pneumococcal strains circulating in children are currently lacking in many European countries. New conjugated pneumococcal vaccines with broader serotype coverage are under licensure review and more are under development. In this context, establishing surveillance of pneumococcal disease, collection of information on circulating strains and whether these strains are covered by PCV7, as well as surveillance of upcoming conjugated pneumococcal vaccines, is a priority for Europe.

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Research articles

COMPLIANCE WITH BOIL WATER ADVICE FOLLOWING A WATER CONTAMINATION INCIDENT IN THE NETHERLANDS IN 2007

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In May 2007, *Escherichia coli* was detected in tap water supplied by a company in North Holland. The company issued advice through mass media to boil tap water before consumption; this advice was lifted six days later. A cross-sectional study was implemented to investigate compliance among residents in this area. Based on postcode, a total of 300 households, chosen randomly from a database of a private company performing internet-based surveys for different marketing purposes, were sent a self-administered questionnaire for this study. The questionnaire contained questions on demographic information, source of information regarding the advice, response to it and personal opinions on the company's reaction and the advice. Ninety-nine (66%) households of the affected area and 90 (60%) households from non-affected areas served by the same company replied to the survey. All respondents knew about the advice. 81.8% of the respondents in the affected area and 5.6% of the non-affected areas reported complying with the advisory. Most respondents from the affected area still used unboiled water to brush teeth, wash salads and fruits. There was no difference in compliance between men and women. Using the mass media was proved to be efficient to inform the public and could be used in the future in similar settings. However, more detailed wording of boiling advices should be considered in the future.

Introduction

Consumption of drinking water may cause waterborne disease which can be prevented by protection of the source water, efficient treatment processes and reliable distribution systems. The European Union Drinking Water Directive [1] demands monitoring of tap water for different parameters, such as *Escherichia coli*, to indicate possible faecal contamination from humans and animals.

System failure or human error may cause an increase in the level of pathogens in the water posing a risk of waterborne disease. For example, in 2001, a large outbreak of gastroenteritis occurred due to accidental introduction of partially treated water to the drinking water supply system in the Netherlands, resulting in 921 households being exposed to contaminated water [2].

In the event that faecal contamination is detected the drinking water company may issue an advice to boil tap water before using it for domestic purposes. On 15 May 2007, *E. coli* was detected in samples collected the day before of the finished tap water delivered by a company in the province Noord-Holland (North-Holland) in the Netherlands. For preventive reasons, on the same day the company

issued an advice for consumers to "boil tap water for two minutes before consumption but that this was not necessary for taking a shower or washing". This information was broadcasted through mass-media including the national and regional television channel, radio and newspapers. In addition, a public website used during emergency situations (www.crisis.nl) and a toll-free telephone number were made available for the public to provide information to households in the affected area.

The boil water advice had an impact on approximately 180,000 households in the affected area comprising 13 municipalities. The advice was lifted a week later, on 22 May 2007, as risk for public health was no longer present. In September 2007, the water company published a press release informing that the cause of the water contamination was due to run-off of rainwater contaminated with faeces of breeding gulls on the roof that had seeped into one of the six storage rooms [3].

Elevated levels of microorganisms in drinking water may represent a public health risk. For this reason, we investigated compliance with boil water advice issued by the private water company following the 2007 incident.

Methods

A cross-sectional study was implemented to investigate factors that may have affected water consumption habits of the residents in the area supplied by the water company. For this purpose, on the company's behalf, a self-administered questionnaire was sent to 300 households in June 2007. Households were selected on the basis of their residence postcodes; half in the area where the advice was valid and half in areas served by the same company but where the advice did not apply. These participants were derived from a database of a private company that conducts online consumer surveys for marketing purposes.

The questionnaire contained questions on demographic information, level of urbanisation, source and time of receiving the information regarding the advice, initial and secondary response to the advice and personal opinions on the company's response and the advice itself. The data were sent back to the drinking water company and the National Institute for Public Health and the Environment, where they were analysed. The statistical analysis was done with STATA v10.

Results

Ninety-nine households (66%) from the area affected by water contamination and 90 households (60%) from control areas supplied with water by the same company replied to the survey. Women more often than men responded to the questionnaire in both the affected and the non-affected areas (57.7% of all responders). The respondents represented 189 households with a total population of 505 people, 176 (34.9%) of whom were below the age of 18 years. There was no statistically significant difference in the number of children per household between the affected and the non-affected areas ($p=0.112$). Descriptive results for the two different areas are presented in Table 1.

All 189 respondents (100%) in both areas answered that they had been informed about the advice. Ninety-five (50.3%) of them said they had first heard about it through the television. Other sources were radio (24.3%), friends, relatives or neighbours (22.8%), newspapers (19.6%) and the internet (7.4%).

Persons living in the affected area were more frequently disappointed (14.1%) about the choice of the company to use mass media for the advice than people residing in the non-affected area (2.2%). In the affected area, seven (9.3%) of the respondents had first reacted with fear to the information on the possible contamination of water, 34 (45.3%) responded with self-control and 34 (45.3%) with the intention to take measures. The corresponding percentages for the non affected area were 15.7%, 72.9% and 11.4%. About half (48.5%) of the respondents from the affected area said they had looked for more information when they had heard about the advice, while the corresponding proportion of respondents from the non-affected area was only 8.9% ($p<0.001$). The most common source of active search for more information was the website of the water supply company.

Eighty-one (81.8%) of all respondents in the affected area said they had complied with the advice. This was done by buying bottled water (43.4% of all respondents in affected area) or boiling tap water for two minutes before consuming it (70.7%). None of the respondents in the area stopped consuming tap water completely. Five (5.6%) of the respondents in the non-affected area were buying bottled water and three of them (3.3%) were boiling tap water during the advice. These numbers were considerably lower than the corresponding ones in the affected area, but showed that compliance exceeded beyond the affected area.

Even though it had not been advised to boil water for activities such as washing and showering, 26 (26.3%) of the respondents in the affected area stated that they had not been aware of that.

Concerning the image of the drinking water company, 177 respondents (93.7%) thought that the company had done well informing the consumers about the water contamination and its response to it. This prevailing opinion was not different between respondents from the affected area and those from the non affected area.

The respondents' compliance with the advice was independent of sex, age and the presence of children in the household. However, the respondents were 138.6 times more likely to follow the advice if a second person in the household was following it as well ($p<0.001$).

Reasons for non-compliance with the advice are given in Table 2.

Some of the respondents replied that they had been using boiled water for uses other than drinking, too. These results are shown in Table 3.

The majority of the respondents stated that their image of the company had not changed after the incident and the six-day advice (78.8% in the affected area and 88.9% in the non-affected area).

Factors affecting compliance

The type of mass media from which people in the affected area found out about the advice played no significant role in the subsequent compliance of the respondents. The highest compliance rates occurred among those in the affected area who heard about the advice from the internet (90%) or from friends (89.5%). Respondents informed by more than one source were more likely

TABLE 1

Survey on boil water advice in the North Holland province in the Netherlands, 2007, demographic characteristics of the respondents

	Affected area (n=99)	Non-affected area (n=90)	Total (n=189)	p-value
Respondent's age (years)	47.7	48.4	48.0	0.7549
Number of people living in the household	2.62	2.82	2.72	0.2526
Number of children living in the household	0.78	1.11	0.93	0.0510

TABLE 2

Reasons for non-compliance with boil water advice in the affected area in the North Holland province, the Netherlands, 2007 (n=11)

Reason given	N	%
I have enough immunity	1	9.1
The risk was small	1	9.1
I was not worried	3	27.3
It was too much inconvenience	2	18.2
I forgot about it	2	18.2
I had only just found out	2	18.2
Total	11	100.0

TABLE 3

Use of boiled water for uses other than drinking in the affected area in the North Holland province, the Netherlands, 2007 (n=99)

Domestic use	N	%
To brush teeth	30	28.1
To wash salads	48	35.6
To wash fruits	51	48.4
To make coffee	56	54.7
To make ice cubes	89	87.2
To give to pets	73	69.4

to have complied with the advice (90.9% against 79.2%) but this difference was not statistically significant. The source of information did not depend on the age ($p=0.6532$). Compliance with the advice did not differ between households with children and those without children ($p=0.536$).

Respondents who undertook active search for more information may have been more likely to follow the advice than those who did not proceed to further active search for more information (89.4% vs. 74.5%, $p=0.058$).

Since all respondents knew about the advice, it was not possible to estimate unwitting compliance rates.

Conclusions

Since excess of standard levels of certain microorganisms such as *E. coli* indicate faecal contamination and the possible presence of pathogens in tap water, the time between the water sampling, water analysis and the boil water notice is essential. During this period, consumers may be exposed to tap water of unacceptable quality. The choice of mass media for broadcasting the advice is therefore believed to be an effective measure to prevent panic and to protect public health.

From this study, it can be concluded that participating consumers not only thought that they had been informed about the advice in a timely manner, but that also the response of the company to ensure the advice would reach the public had been satisfactory as well as the choice of communication channels. Thus, the incident did not lead to customers' dissatisfaction or a degradation of the company's image.

The sample in our study derived from a database of people who subscribed to be included in different research surveys. This could raise questions regarding the representativeness of the study population. We agree that there is a need for similar studies with samples deriving randomly from the whole population and not from potentially biased data sources. For example, 100% of the participants stated that they had been informed about the boiling water advice; however, subscribers to online databases for marketing purposes may be more likely to regularly follow the news than the general population.

In the Netherlands, boil water notices are not harmonised but are determined by the drinking water company itself. This results in different advice with respect to, for instance, boiling time. Internationally recognised guidelines, such as the World Health Organization (WHO) Guidelines for Drinking Water Quality [4], could be taken into consideration in case of similar "crises" in the future. According to data from the water company involved in our study, about thirty boil water advices are issued per year in their responsibility area (ca. 700,000 households); involving on average 100 households per time. So, the chance to receive a boil water advice is small but existing.

The inclusion of recommendations including use of water for brushing teeth, washing fruits and vegetables may also prove helpful in future advice, since it is not only consumption of water through drinking that may pose a risk to the consumer. Bathing and showering may also need to be addressed separately, as a possible link between this kind of exposure to contaminated water and itching has been described elsewhere [2]. Also, although this

conclusion does not directly follow from our results, vulnerable groups should be targeted separately in the advice; elderly people and children may easily miss information disseminated through the means of mass media [5,6].

Few studies have been published on boil water notices and their results seldom reach the public. Further research would also be useful to incorporate findings from compliance studies to model health effects of drinking contaminated water during similar events.

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INTERNATIONAL TRAVELS AND FEVER SCREENING DURING EPIDEMICS: A LITERATURE REVIEW ON THE EFFECTIVENESS AND POTENTIAL USE OF NON-CONTACT INFRARED THERMOMETERS

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Several countries plan to introduce non-contact infrared thermometers (NCIT) at international airports in order to detect febrile passengers, thus to delay the introduction of a novel influenza strain. We reviewed the existing studies on fever screening by NCIT to estimate their efficacy under the hypothesis of pandemic influenza. Three Severe Acute Respiratory Syndrome (SARS) or dengue fever interventions in airports were excluded because of insufficient information. Six fever screening studies in other gathering areas, mainly hospitals, were included (N= 176 to 72,327 persons; fever prevalence= 1.2% to 16.9%). Sensitivity varied from 4.0% to 89.6%, specificity from 75.4% to 99.6%, positive predictive value (PPV) from 0.9% to 76.0% and negative predictive value (NPV) from 86.1% to 99.7%. When we fixed fever prevalence at 1% in all studies to allow comparisons, the derived PPV varied from 3.5% to 65.4% and NPV was $\geq 99\%$. The low PPV suggests limited efficacy of NCIT to detect symptomatic passengers at the early stages of a pandemic influenza, when fever prevalence among passengers would be $\leq 1\%$. External factors can also impair the screening strategy: passengers can hide their symptoms or cross borders before symptoms occur. These limits should be considered when setting up border control measures to delay the pandemic progression.

Introduction

The emergence of Severe Acute Respiratory Syndrome (SARS) in 2003 underlined the role of international travels in the rapid spread of infectious diseases and prompted countries to set up border control strategies, in order to reduce the risk of introduction of an infection. Traditional measures such as information for travellers, self-completion of health cards or visual inspection of passengers were implemented by most countries. In addition, non-contact infrared thermometers (NCIT) were introduced in some international airports or other gathering areas such as bus or railway stations. The principle of NCIT is that heat emitted by any organic body can be detected in the infrared radiation spectrum through a remote sensor and transformed into colour images on a monitor. The clinical applications of non-contact infrared thermography include the diagnostic of inflammatory disorders or cancers, or the surveillance of body temperature in neonatology wards through the monitoring of changes in the skin perfusion over time [1,2]. Yet, the extension of these non-invasive diagnostic tools to a public health application,

for instance mass screening of breast cancers or fever, has not been thoroughly assessed. Early reports from the SARS experience suggested a low efficacy of NCIT at international airports [3-5] and some authors stressed that NCIT were not currently manufactured for a fever screening purpose [6]. Nevertheless, given the increasing threat of pandemic influenza, some countries envisage to introduce thermal screening at their borders [7]. Their objective is to delay the introduction of the infection through the early detection and isolation of the first infected cases, thus allowing for more time to organise the response.

In this review we summarise the available information on the sensitivity, specificity and predictive values of NCIT used with the objective of fever screening, in airports or other gathering areas. We discuss their potential benefits under the hypothesis of pandemic influenza.

Materials and methods

We performed a systematic MEDLINE search on the literature from 1975 to August 2008. We used the following key words: fever; screening; non-contact, infrared thermography or thermometers; thermal imagers or scanners or pyrometers; thermal screening. The apparent redundancy for some words was necessary because there did not seem to be a standardised vocabulary for the subject. Among the abstracts identified through these key words, we selected the publications which provided the sensitivity and specificity values of NCIT used in a fever screening objective, whatever the cause of the fever.

For international airports, we found partial data from three mass screening interventions using NCIT: two aimed at detecting SARS among international passengers in Canada [4] and in Singapore [8] and one aimed at detecting dengue fever in Taiwan [9]. The numbers of passengers screened and those subsequently confirmed as SARS or dengue cases were provided in these publications but the numbers of passengers who presented with fever due to another cause, i.e. the total numbers of true positive cases, were not available. We therefore discarded these publications which did not allow to derive the sensitivity, specificity and predictive values of NCIT to screen a fever of any origin.

Our search also focused on fever screening interventions in other settings than airports. We selected those which were carried out under conditions considered to be close to a mass screening at international airports, for instance studies implemented in gathering areas, with no preliminary selection or preparation of the tested subjects. We found six studies, performed mainly in hospitals, in which all subjects who were present and accepted to participate were tested. These selected studies summarised in Table 1, included: one in Singapore [10], two in Hong Kong [11,12], two in Taiwan [13,14] and finally one in France [15]. In all, temperatures measured by NCIT were compared to reference values measured by tympanic thermometers i.e. contact thermometers. The authors considered that tympanic thermometers reflected the actual core body temperature with enough confidence, were easy to use because they were routinely used in many hospitals and were more acceptable for the tested subjects than rectal thermometers. The positive and negative predictive values (PPV and NPV) were reported in three of the selected studies. For the others, we derived these values from the available sensitivity, specificity and prevalence data. Finally, because the prevalence of fever in the study populations varied and in order to allow comparisons, we assumed a fixed fever prevalence of 1% in all studies and derived predictive values based upon the sensitivity and specificity as reported in each study. We considered that 1% prevalence was a plausible assumption of the proportion of febrile subjects among international passengers, based on findings from a review of interventions to control SARS [3].

Through our search, we also identified other studies on NCIT with sensitivity and specificity values but these were discarded because they were carried out under strict surrounding conditions which did not fit with our specific objective which was to assess the performances of NCIT under mass screening conditions, in crowded/gathering areas. For instance, participating subjects were asked to refrain from drinking caffeine-based beverages or from exercising the day before. Elsewhere, the device was scanned

across the forehead in order to identify specific skin areas where the physiological variations of the skin temperature were reduced. Finally, we also excluded a large number of reports identified through Internet searches, other than Medline, in which information was too scarce.

Results

The study populations ranged from 176 to 72,327 persons (Table 1). They were composed of either hospitalised patients, or persons presenting for emergency or for outpatient consultations, or supposedly healthy persons selected among hospital visitors or sports clubs. Information on age or gender was mostly unavailable. The fever thresholds varied between 37.5°C and 38°C (these were mainly based on the thresholds which were used in the respective countries during the SARS outbreak). The body areas targeted by NCIT systematically included the forehead; the inner eye corner or the external auricular meatus were other skin areas occasionally targeted by the devices. Different types of devices were tested. In four studies, hand-held thermometers were assessed. This implied a shorter distance between the device and tested subjects (≤ 50 cm) than in the two other studies which used remote sensors linked to a monitor (> 50 cm). The devices were calibrated according to the respective producers' recommendations. Two studies were carried out in stable external environments consisting of a single dedicated room with stable ambient temperature and ventilation system [12,14].

The prevalence of fever measured by reference contact tympanic thermometers varied from 1.2% to 20.7% in the respective samples, with variable fever thresholds (Table 2). This prevalence was either based on the entire study population or was estimated from a sub-sample. The sensitivity, specificity and predictive values of NCIT targeting the forehead area largely differed between studies. The sensitivity varied from 4.0% to 89.6%, the specificity from 75.4% to 99.6%, the PPV from 0.9% to 76.0% and the NPV from 86.1% to 99.7%. The lowest PPV was found in the study by Chiu

TABLE 1

Summary of studies on fever screening by non-contact infrared thermometers, 2004-2008

First author, reference	Country, area	Study population (N)	Settings	Sample size *	Temperature threshold	Target area(s)	Device	Environmental conditions
Ng E [10] <i>Microvasc Res</i> 2004	Singapore	502	Hospital	310	37.7° C	Forehead	Flir® S60 Hand held	na
				310		Inner eye corner		
Liu CC [14] <i>Infect Control Hosp Epidemiol</i> 2004	Taiwan	500	Outpatient consultation	500	37.5° C	Forehead	Thermofocus® Hand held	Stable
				500		Auricular meatus		
Chan LS [11] <i>Travel Med</i> 2004	Hong Kong	176	Hospital, consultations and sports club	188	37.5°C & 38°C	Forehead	Flir® -3 models Remote sensors	na
				116		Auricular meatus		
Ng DK [12] <i>Ann Trop Paed</i> 2005	Hong Kong	500	Inpatients (Age:1 month-18 years)	500	38° C	Forehead	Standard ST® Hand held	Stable
Chiu WT [13] <i>Asia Pac J Public Health</i> 2005	Taiwan	993	Hospital visitors	993	37.5° C	Forehead	Telesis® Remote sensors	na
		72.327	Patients + visitors	72.327	37.5° C	Forehead		na
Hausfater P [15] <i>Emerg Inf Dis</i> 2008	France	2026	Emergency department (Age 6 – 103 years)	2.026	38° C	Forehead	Raynger® ** Hand held	Dedicated nurse

* Number of measurements done in each population
na: Information not available

et al. [13] in their second series of measures conducted among 72,327 patients and hospital visitors, in which fever prevalence was not given.

Receiver operating characteristic (ROC) curves were assessed by three teams; the values of the area under the curve reached 0.96, 0.92 and 0.86 in the studies of E. Ng et al. [10], Hausfater et al. [15] and D. Ng et al. [12], respectively. The correlation coefficient between the forehead and reference tympanic temperatures varied from 0.25 to 0.51 in the two studies where it was quantified [11,14] and was 0.71 when we derived it from the available data in E. Ng [10].

The external auricular meatus area was tested in two studies. This target area yielded higher sensitivity results than the forehead: 82.7% vs. 17.3% [14] and 67.0% vs. 4.0% [11], respectively. Specificity remained high: 98.7% and 96.0%, respectively.

When we fixed the fever prevalence at 1% in all studies and used the sensitivity and specificity values as reported by the respective authors, the derived PPV for the forehead area varied from 3.5% to 65.4% and the derived NPV was =>99% (Table 3).

Discussion

Interpretation and comparison of findings were made difficult by the limited number of selected studies and their wide heterogeneity in terms of methods, study design and environmental conditions. Also, the level of available details in the published papers varied regarding the different study populations which included either healthy or sick persons, and the different types of tested NCIT which included hand-held or remote sensors. The relevance of tympanic (contact) thermometers as reference measurements might also be discussed, but the authors selected feasible and acceptable methods. Another important bias resides in the devices themselves: under operational conditions, the detection of fever by NCIT can be affected by three types of factors [10]. Individual factors such as

the consumption of hot beverages or alcohol, pregnancy, menstrual period or hormonal treatments can increase the external skin temperature. Inversely, intense perspiration or heavy face make-up can have a cooling effect on the cutaneous temperature without a parallel decrease of the actual body temperature. The targeted body area scanned by the detector also plays a role, because of physiological differences in vascularisation and consequently in heat distribution. The forehead is subject to important physiological variations but is preferred in screening programmes for feasibility reasons. Inversely, the inner eye corner or the auricular area are less subject to variations but are less accessible: targeting the external auricular area yields better results but travellers would have to be asked to remove their scarves, etc. from around the ear, generating a longer preparation time. Finally, environmental factors can also affect the measurements [2,10], such as the subject-sensor distance, the ambient temperature or humidity and the surrounding ventilation systems, as well as the fact that the person tested should remain immobile for a few seconds in front of the detector.

Despite these constraints, there are several advantages in using NCIT to screen fever at international airports. NCIT save time (temperature is displayed within a few seconds) and reduce close contacts with infected individuals. But, although NCIT appear suitable for entry screening because of high specificity and NPV, the low sensitivity values reported in the studies suggest that the risk of missing febrile individuals (1-sensitivity) would reach 83 to 85% [11,14]. In addition, given the low PPV, hostile reactions may arise among a high proportion of passengers mistakenly classified as febrile by the sensors and subsequently referred for medical examination. Because of these limitations, most authors were extremely cautious in their respective conclusions, stating for instance that NCIT may serve as a proxy tool [11] or that surveillance and contact tracing would be more beneficial [14].

TABLE 2

Fever screening by non-contact infrared thermometers, 2004-2008: sensitivity, specificity and predictive values according to the body area targeted

First author, country, publication year	Sample size	Target area(s)	Temperature threshold	Fever prevalence %	Sensitivity %	Specificity %	PPV %	NPV %
Ng E Singapore 2004	310	Forehead	37.7°C	16.9	89.6	94.3	76.0*	97.8*
	310	Inner eye corner	37.7°C	16.9	85.4	95	77.7*	97.0*
Liu CC Taiwan 2004	500	Forehead	37.5°C	-	17.3	98.2		
	500	Auricular meatus	37.5°C	-	82.7	98.7		
Chan LS Hong Kong 2004	188	Forehead	38°C	14.3	4	99	40.1*	86.1*
	-	Forehead	37.5°C	Na	15	98		
	116	Auricular meatus	38°C	20.7	67	96	81.4*	91.8*
Ng DK Hong Kong 2005	500	Forehead	37.5°C †	12.3 †	89.4	75.4	33.7	98.1
Chiu W Taiwan 2005	993	Forehead	37.5°C	1.2	75	99.6	69.9*	99.7*
	72.327	Forehead	37.5°C	-	-	-	0.9*	
Hausfater P France 2008	2.026	Forehead	38.0°C	3.0	82	77	10	99

* Values derived from the available information are in **bold italic**

† The 37.5°C cut-off corresponds to the optimal sensitivity and specificity values reported by the authors whereas the prevalence (12.3%) is based on a 38°C threshold.

PPV: Positive predictive values; NPV: Negative predictive values

Under a pandemic influenza scenario, one could expect a higher PPV, because of a higher prevalence of fever (>1%). But it is in the very early stages of the pandemic that NCITs would be considered as a way to delay the introduction of infection in a given area. In these early stages, the number of infected cases would be very low and the overall prevalence of fever among international passengers would remain below the 1% rate which we set in our analysis.

Finally, even if better-performing devices were manufactured and implementation costs were affordable for national authorities, the overall efficiency of the screening intervention would still need to be examined. As stated by an international experts committee [16], the overall sensitivity of border control is likely to be limited. Modelling works show that border control strategies aimed at reducing the risk of introduction of SARS or influenza in a country have poor sensitivity [17] and limited impact [18-21]. The epidemiological characteristics of the infection play a major role, as illustrated by the differences between SARS and influenza. For SARS, infectiousness peaks after the onset of symptoms, therefore early detection of patients may indeed contribute to their early isolation and thus reduce transmission. For pandemic influenza, because it is assumed that infectiousness starts a few hours before the onset of symptoms, some cases would be missed and would generate secondary cases after their entry in the country. Sociological factors can also affect the efficacy of border control measures. Knowing that thermal screening is organised in international airports may motivate some symptomatic passengers to delay their travel, but inversely, others may try to hide their symptoms or by-pass border control [22;23]. The psychological reassuring effect on the public can influence the decision to implement such screening, as was the case in Singapore and Canada [24-26], but these countries also recognised that the public may lose confidence in this measure if an undetected case had entered the country and generated secondary cases. Because public perceptions are important, policy makers may feel some pressure to use NCIT but the decision making process should not ignore the poor scientific evidence on NCIT's efficacy to delay the introduction of a novel influenza strain. For transparency reasons, the surrounding sociological, demographic, epidemiological and environmental factors which can influence the screening strategy must also be taken into consideration.

TABLE 3
Fever screening by non-contact infrared thermometers (NCIT), 2004-2008: positive and negative predictive values of NCIT for forehead temperature screening, assuming a fever prevalence of 1%

First author, country	Sample	Fever threshold	Sensitivity %	Specificity %	PPV %	NPV %
Ng E. Singapore	310	37.7°C	89.6	94.3	13.7	99.9
Liu CC Taiwan	500	37.5°C	17.3	98.2	8.8	99.2
Chan LS Hong Kong	188	38°C	4	99	3.9	99.0
	188	37.5°C	15	98	7.0	99.1
Ng DK Hong Kong	1.000	37.5°C	89.4	75.4	3.5	99.9
Chiu W Taiwan	993	37.5°C	75	99.6	65.4	99.7
Hausfater P France	2.026	38°C	82	77	9.9	99.3

PPV: Positive predictive values; NPV: Negative predictive values

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FIRST IDENTIFICATION OF METALLO-BETA-LACTAMASE-PRODUCING *PSEUDOMONAS AERUGINOSA* IN THE CZECH REPUBLIC

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Since 2005, invasive isolates of *Pseudomonas aeruginosa* have been collected in the Czech Republic as part of the European Antibiotic Resistance Surveillance System (EARSS). Forty-eight microbiology laboratories throughout the country including approximately 81% of the population provide consecutive isolates from blood and cerebrospinal fluid. Surprisingly, no metallo-beta-lactamase (MBL) was found in 1,259 invasive isolates tested over the past three years until the detection of two MBL-producing strains in mid-2008. Both strains were isolated from patients hospitalised in one regional hospital. The MBL was identified as IMP-7, which had been seen previously in Canada, Japan, Malaysia and Slovakia.

Metallo-beta-lactamases (MBL) belong to the group of clinically important carbapenemases produced by Gram-negative bacteria. They have been found mostly in *Pseudomonas* spp., *Acinetobacter* spp. and *Enterobacteriaceae* [1]. Due to increasing diversity, the rapid spread of these enzymes, and the fact that they are often encoded on mobile genetic elements (integrons, transposons, plasmids) together with other resistance genes, MBL-producers belong to the group of clinically relevant multidrug-resistant bacteria [1]. Treating infections caused by such strains is a therapeutic challenge because of the limitations posed by high-level resistance to various groups of antibiotics [1].

Since 2005, invasive isolates of *Pseudomonas aeruginosa* have been collected in the Czech Republic by the National Reference Laboratory (NRL) for Antibiotics of the National Institute of Health in Prague (NIPH) within the European Antibiotic Resistance Surveillance System (EARSS). Forty-eight microbiology laboratories provide consecutive isolates from blood and cerebrospinal fluid (CSF). These laboratories are geographically spread throughout the country. As of 2007, the catchment population of the cooperating laboratories totalled approximately 81% of the Czech population [2].

The minimum inhibitory concentrations (MIC) of 12 antibiotics are determined by broth microdilution method, in accordance with recommendations by the Clinical and Laboratory Standards Institute (CLSI) [3]. MBL production in strains showing resistance to meropenem is verified by the standard synergy test with ethylenediaminetetraacetic acid (EDTA) and meropenem, imipenem and ceftazidime [1,4].

Case description

In 2008, the NRL for antibiotics identified for the first time two MBL-producing strains of *P. aeruginosa* in isolates from blood. Both isolates had been collected from patients hospitalised in the same hospital unit in Hospital A in the North Bohemian region.

The first strain was isolated in July 2008 from a patient with obstructive hydrocephalus complicated with shunt meningitis. The patient, a 25 year-old man whose first cerebral shunt had been implanted in 1994 had been admitted to the department of neurosurgery three times since April 2008 to have his shunt repositioned. During his third hospitalisation in July, the patient developed meningitis complicated with sepsis caused by MBL-producing *P. aeruginosa*. After recovery, the patient was transferred to another hospital in August 2008.

The second strain was identified in November 2008 in a 71 year-old woman with a diagnosis of neurological ependymoma. In July 2008, the patient had been admitted to the department of neurosurgery in Hospital A during the same time period as the patient described above. After surgery, the patient had developed obstructive hydrocephalus treated by use of a shunt. She was sent first to a different hospital and subsequently to a nursing home. In November, she was again transferred to the department of infectious diseases of Hospital A for treatment of a brain abscess. During this hospitalisation, she developed sepsis and an MBL-producing strain of *P. aeruginosa* was isolated from her blood. The patient ultimately died as a result of organ failure.

Because the patient was hospitalised in many different health-care settings, it is unfortunately not possible to describe complete data about antibiotic therapy.

Laboratory analysis

MBL production in the isolates from both cases was verified by the spectrophotometric method described by Lauretti *et al.* [5]. Preliminary identification of MBL was based on multiplex PCR according to Ellington *et al.* [6]. Subsequently, the entire coding regions of the class 1 integron were amplified (as proposed by Fiett *et al.* [7]) and sequenced. Sequencing revealed the MBL enzyme to be IMP-7, encoded on the class 1 integron in both isolates. The *bla*IMP-7 gene cassette was found together with other resistance

genes (*aac(6')-Ib*). As only a partial sequence of the integron is available, it is not known at this stage whether the two strains encode further resistance genes.

These are the first two documented appearances of IMP-7 in the Czech Republic.

Pulsed-field gel electrophoresis (PFGE) was used to type the IMP-7 producers, as described by Struelens *et al.* [8], using the restriction enzyme *Xba*I; banding patterns were interpreted according to Tenover *et al.* [9]. The PFGE patterns in both isolates were indistinguishable.

Conclusions

The only hospital unit in which both patients had been simultaneously hospitalised was the department of neurosurgery of hospital A. However, as no epidemiological data or isolates are available from that unit, it was not possible to identify a reservoir or source of the MBL producers. The second patient may have become infected when returning to Hospital A in November 2008, but may also have been colonised already since her stay in that hospital in July 2008. We can therefore not be certain whether the *Pseudomonas* strain was present in Hospital A between July and November or only in July. Control epidemiological screening was proposed in the department of neurosurgery and the department of infectious diseases in Hospital A. At the time of publication of this report, we have no data from this screening.

Because both patients had been hospitalised in several health-care institutions in the North-Bohemian region, the potential risks of spreading MBL-producing bacteria in this geographical area are high.

MBL-producing strains have been described in all countries bordering the Czech Republic. Surprisingly, no MBL had been responsible for resistance to carbapenems in the 1,259 invasive isolates referred to the National Reference Laboratory for Antibiotics in the past three years.

IMP-7 MBL was first described in Canada in strains of *P. aeruginosa* that had caused nosocomial outbreaks in two rehabilitation wards in 1995 and 1996 [10]. Later, this enzyme was found in Malaysia [11], and most recently in Japan [12] and Slovakia [13]. Integron typing has been presented only in the first publication by Gibb *et al.* [10]. It is not clear if this enzyme is alone responsible for the resistance to carbapenems, because when cloned into an *E. coli* laboratory strain, it provided only slight protection against meropenem and no protection against imipenem [10].

With the exception of Italy, IMP-type-producing bacteria seem to be less common in Europe than VIM-type producers [14]. Where and how the IMP-7 MBL entered the Czech Republic therefore remains unclear.

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TREATMENT FAILURE IN CASE OF TYPHOID FEVER IMPORTED FROM INDIA TO CZECH REPUBLIC, DECEMBER 2008 - JANUARY 2009

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In this report we describe a case of typhoid fever in a Czech patient with history of travel to India and discuss antibiotic treatment failure which led to the relapse of fever.

Case report

Travel history

A previously healthy 31-year-old man from the Czech Republic visited India from 2 October to 28 November 2008. Before leaving the Czech Republic he had received neither vaccination (travellers to India are advised to get vaccinated at least against viral hepatitis A and typhoid fever) nor antimalaric chemoprophylaxis. He climbed the Himalayas, and in the last week of his stay he visited Varanasi at the Ganga River. There, he drunk a soft drink from a cup washed in water of unsure origin at the market place. His travelling companion had the same food without this soft drink, and had no problems afterwards.

A week before returning home the man experienced fever (temperature 40°C), fatigue and vomiting without diarrhoea. While still in India he took ciprofloxacin bought at the chemist's. He returned home on 28 November 2008. On 1 December the patient was examined by his general practitioner and sent to the Department of infectious diseases in Ostrava because of malaria suspicion.

First hospitalisation

After admission malaria was excluded, and hepatosplenomegaly was proved by ultrasonography. Laboratory analyses showed increased C-reactive protein (109 mg/l), and alanine aminotransferase (ALT) was elevated (100.2 U/l). Widal test was repeatedly negative during hospitalisation. On 3 December *Salmonella* sp. was found in blood culture and in stool, and on the next day *Salmonella typhi* (*S. typhi*) was identified.

The patient was first treated by cefotaxime in a dose of 6 g per day. As fever continued, after five days of cefotaxime, ciprofloxacin of 800 mg per day was added. Although fever gradually dropped, the temperature stayed at 38.5°C for 10 days and at 37.5°C for next five days. Laboratory results were subsequently improving (a decline of C-reactive protein and ALT). Cefotaxime was administered for a total of 19 days, ciprofloxacin for a total of 15 days. The patient was discharged on 22 December 2008 after 21 days of hospitalisation and after seven days without fever.

Second hospitalisation

At home the patient was feeling weak but his condition was gradually improving. On 31 December (nine days after leaving hospital), the patient had a new episode of fever (temperature 38.5°C) and on 1 January 2009 he was hospitalised again with the temperature of 39.5°C, fatigue and sweat. Malaria was excluded again. Ciprofloxacin was used in the therapy. As treatment showed no effect on the third day ciprofloxacin was replaced by meropenem, however, despite therapy change the patient's temperature continued to peak daily at 39.5°C. Laboratory analyses showed increasing C-reactive protein (from 42 mg/l to 96 mg/l) and decreasing platelet count (from 195 to 83 x 10⁹/l). Hepatosplenomegaly was proved by ultrasonography again. When *S. typhi* was detected in blood culture again on 5 January 2009 the patient was administered intravenous chloramphenicol in dose of 6 g per day for 17 days. Finally his temperature dropped within 36 hours and the patient started to feel better without further complications. Laboratory results were gradually improving. He was discharged home after 22 days.

Discussion

In endemic areas typhoid fever occurs as asymptomatic or mild illness. According to the World Health Organization the case-fatality rates were 10-20% during the pre-antibiotic era, and can be reduced to less than 1%, with prompt and appropriate antibiotics therapy [1]. Fluoroquinolones had previously been very effective in the treatment of typhoid fever but in the past decade, progressive increase in the minimum inhibitory concentration (MIC) of ciprofloxacin and high incidence of clinical failures to quinolones have been described [2]. The third generation cephalosporins are now being increasingly used but they are associated with a long time of defervescence and high rates of relapses [2].

The annual incidence of typhoid fever in India is 493.5 per 100,000 inhabitants, and quinolones treatment failure is common there [3]. In India there have also been sporadic reports of high-level resistance to ceftriaxone in *S. typhi* and return of sensitivity to chloramphenicol [1,3]. Multi-drug resistance was seen in 32% of strains [4]. There are reports of the emergence of fluoroquinolone-resistant isolates in various part of Asia and descriptions of resistance to third-generation cephalosporins in the same region. However, many of these reports are coupled with evidence of re-

emergence of sensitive isolates in the same region [1]. In South America incidence of fluoroquinolone-resistant strains is low [5].

In the case described in this paper, sensitivity tests performed during the first hospitalisation showed that *S. typhi* had MIC of cefotaxime equal to 0.016 mg/l, of ciprofloxacin 0.250 mg/l and of meropenem 0.016 mg/l. *S. typhi* was sensitive to chloramphenicol, but MIC was not assessed. During the second hospitalisation *S. typhi* had MIC of cefotaxime 0.016 mg/l, of meropenem 0.016 mg/l, of ciprofloxacin 1.000 mg/l, of ampicilin >128.0 mg/l and of cotrimoxazol > 64.0 mg/l. MIC of 0.250 mg/l has been described as resistance to ciprofloxacin [6,7].

In our patient typhoid fever therapy with ciprofloxacin plus cefotaxime showed to be ineffective, despite of adequate dose, duration of therapy and susceptibility to cefotaxime in vitro. Even though the results of blood tests were improving, the temperature declined very slowly and a relapse of typhoid fever appeared two weeks after stopping the treatment. In spite of good sensitivity to meropenem, this agent was also ineffective. Only traditional chloramphenicol actually showed to be effective.

In typhoid fever diagnostics Widal test is very commonly used but has very variable sensitivity and specificity and problems in interpretation [2]. In our patient Widal test was repeatedly negative.

Conclusion

The 2003 World Health Organization guidelines recommend treatment with fluoroquinolones for both complicated and uncomplicated cases of typhoid fever. However, sensitivity profiles of *S. typhi* vary geographically, so the initial antibiotic choice for typhoid fever treatment should be based on the sensitivity data of the area in which the infection was acquired [5]. In a patient returning from India *S. typhi* resistance to quinolones has to be presumed. The third generation cephalosporins represent treatment alternative, although resistance to these drugs is gradually increasing [3]. Chloramphenicol can be an option of antibiotic choice for typhoid fever treatment when another therapy fails.

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Rapid communications

NATIONWIDE OUTBREAK OF STEC O157 INFECTION IN THE NETHERLANDS, DECEMBER 2008-JANUARY 2009: CONTINUOUS RISK OF CONSUMING RAW BEEF PRODUCTS

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The Netherlands experienced a nationwide outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157 with onset of symptoms from the end of December 2008 until the end of January 2009. A total of 20 laboratory-confirmed cases were linked to the outbreak strain, serotype O157: H-, stx1, stx2, eae and e-hly positive. The investigation into the source of this outbreak is still ongoing, but evidence so far suggests that infection occurred as a result of consuming contaminated raw meat (steak tartare).

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) can cause bloody diarrhoea which progresses to a life-threatening condition known as the haemolytic uraemic syndrome (HUS) in 2-15% of the cases, particularly children [1,2]. STEC O157 is the serogroup most commonly identified in outbreaks.

STEC is found in the guts of many animals, but ruminants and cattle in particular are believed to be the main reservoir of human pathogenic STEC. Although infection can occur through direct contact with animals, most human infections are probably foodborne; water or food products (undercooked beef, raw milk, vegetables such as lettuce and alfalfa) contaminated with manure have often been linked to common source outbreaks. Person-to-person transmission can also occur [3,4].

An enhanced laboratory-based surveillance for STEC O157 was implemented in the Netherlands in 1999 and STEC non-O157 serogroups were added to this surveillance in 2007 [5]. Since 1999, STEC has been notifiable by law. All STEC-positive isolates identified in laboratories across the country are sent to the National Institute of Public Health and the Environment (RIVM). Since the start of the surveillance the number of STEC O157 infections reported annually ranged from 35 to 57 between 1999 and 2006, increased to 83 cases in 2007 as a result of a national outbreak [6], and dropped to 46 sporadic cases in 2008.

In week 4 of 2009, an increase to nearly half the predicted annual number of STEC O157 cases (n=20) was noted thanks

to the intensive surveillance system. This prompted a further epidemiological and microbiological investigation, the results of which are presented here.

Methods

An outbreak investigation was initiated on 29 January 2009 in response to laboratory confirmation of a nationwide outbreak of STEC O157. An outbreak case was defined as a person diagnosed with a laboratory-confirmed STEC O157 infection since 10 December 2008 and a pulsed-field gel electrophoresis (PFGE) profile belonging to the outbreak cluster. Municipal Health Authorities in the Netherlands routinely follow up laboratory-confirmed STEC cases using a standardised questionnaire to collect information on clinical symptoms and exposure to possible risk factors in the week preceding onset of illness. Due to the dispersed distribution of cases within the Netherlands suspicion was raised that the cause could be a common food source or supplier; Municipal Health Authorities were requested to pay particular attention to the completeness of responses to questions pertaining to food history and location of purchase in their follow up of laboratory-confirmed cases.

All STEC positive isolates sent in to the RIVM are tested for genes encoding Shiga toxin type 1 and type 2 (*stx*₁ and *stx*₂), the *E. coli* attaching-and-effacing gene (*eae*) and the enterohaemolysin encoding EHEC-*hly* gene (*e-hly*). DNA fingerprints are generated by PFGE, using *Xba*I as the restriction enzyme. The fingerprints are processed with BioNumerics® (Applied Maths, Kortrijk, Belgium; Dendrogram type=UPGMA, Similarity coefficient=Dice) [4].

Statistical analysis

Analysis of food exposures was conducted using a case-case study design to compare laboratory-confirmed STEC O157 outbreak cases with sporadic cases of STEC O157 reported in the enhanced surveillance in 2008. Food items that were reported to have been definitely or possibly consumed were compared with items that were reported to have not been consumed. An attack rate and odds ratio for each food item was calculated using STATA 10. Individuals

who did not provide information on a food item were excluded from analysis of this particular food. Three possible secondary cases, defined as members of the same family as a case and with onset of symptoms later than that of the first family member, were also excluded from the analysis.

Food tracing

A trace back of suspected food items was initiated. The Food and Consumer Products Safety Authority (VWA) collected samples of any available left-over meat products from patients' homes for testing for STEC O157. The VWA also investigated the supermarkets and producers of various meat products mentioned by the cases.

International cooperation

The Netherlands is member of the European food and waterborne diseases and zoonoses surveillance network (formerly ENTERNET) administered by the European Centre for Disease Prevention and Control, which covers, amongst others, STEC infections. Using this network, in week 6 a request was made to all member countries to provide details of any occurrences of STEC O157 with a similar PFGE pattern.

Results

Between 27 December 2008 and 22 January 2009, 20 cases of STEC O157 (including three secondary cases) were attributed to the outbreak strain in the Netherlands (Figure 1). One additional STEC O157 case, with symptom onset on 13 December 2008, was possibly associated with the outbreak strain in accordance with the PFGE case definition and three isolates are pending PFGE typing to determine whether they are related to the outbreak.

Cases were spread throughout the Netherlands and were aged between 6 and 76 years of age (median age 41), with an equal number of males and females.

The outbreak strain was characterised as serotype O157:H-, and stx1, stx2, eae and e-hly positive and, all isolates, with the exception of one, were sorbitol-nonfermenting (Figure 2). This exact PFGE pattern has been seen on only two occasions in the Netherlands, both in 2005.

Sixteen of the 20 outbreak-related cases (80%) completed the questionnaire, three of which were secondary cases. Seven cases were hospitalised and none developed HUS. The questionnaire was

also returned by 36 non-outbreak cases of STEC O157 with onset of symptoms in 2008. These cases represented 78% of the total

FIGURE 2
Pulsed-field gel electrophoresis (PFGE) pattern of the outbreak strain (middle three lanes) in the national outbreak of Shiga toxin-producing Escherichia coli (STEC) O157 in the Netherlands, December 2008 - January 2009

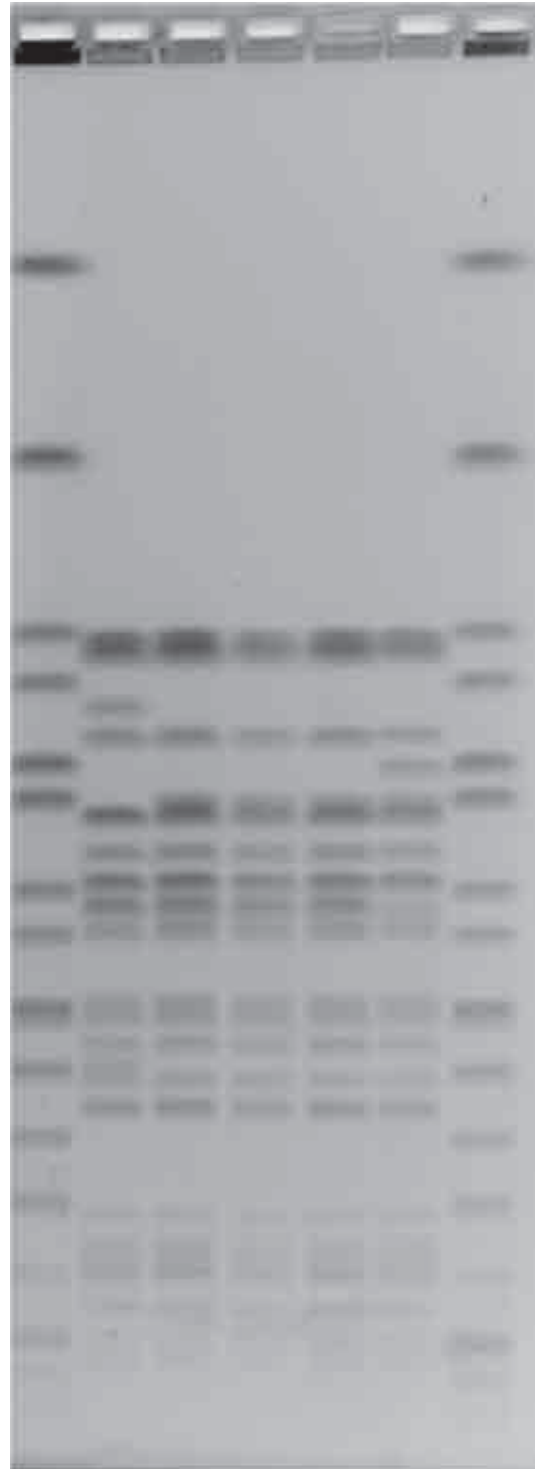
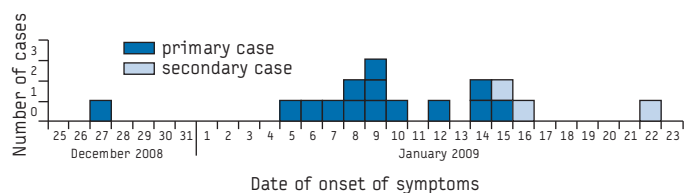


FIGURE 1
Distribution of confirmed cases of the outbreak strain of Shiga toxin-producing Escherichia coli (STEC) O157 in the Netherlands, December 2008 - January 2009, by date of onset of symptoms (n=17)*



* Date of onset was unknown for three of the twenty outbreak-related cases

number of STEC O157 cases reported in 2008, ranged in age from 1 to 65 years and 67% were women. Two food items were frequently reported to have been consumed by outbreak cases: minced meat and steak tartare. There appears to be a link between consumption of steak tartare and STEC O157 infection: 83% of the 13 primary outbreak cases who provided the relevant information reported eating steak tartare in the week before illness compared to 18% of the sporadic 2008 cases (OR 16.3; $p < 0.001$, Table).

Many of the cases purchased food at more than one supermarket or store, and the precise location where individual items were purchased was not recorded. The supermarket or butcher where the implicated steak tartare was bought is therefore unknown.

Results of food tracing

All left over food samples collected by the VWA tested negative for STEC O157. However, the food trace-back investigation is still ongoing and it is possible that the STEC cases pending PFGE results may be linked to the outbreak strain. Even if the contaminated batch of meat has been long-since consumed it is still worthwhile to further investigate the production flow in supermarkets, producers of steak tartare and possibly also slaughterhouses to try and gain information to support the epidemiological link.

International response

Nine countries responded to the information request (Belgium, Germany, Finland, Ireland, Norway, England and Wales, Scotland, United States and Denmark), none of which reported current STEC O157 cases with PFGE profiles related to the outbreak strain. Finland reported two strains isolated in 1998 and 2004 with identical PFGE patterns, both patients had reported travel to Turkey.

Conclusions

This is not the first time that a nationwide outbreak of STEC O157 has occurred in the Netherlands. In 2005, an outbreak was linked to steak tartare [3] and in 2007 an outbreak was associated with lettuce [4]. Based on the case-case study it seems probable that the outbreak reported here was also caused by steak tartare; steak tartare consumption was strongly and significantly associated with being an outbreak case and could explain the majority of the cases. The age distribution of cases seems also consistent with these findings; 28% of the non-outbreak cases of STEC O157 infection in 2008 were five years old or younger but the youngest outbreak-related patient was six years old which is not surprising as very young children are less likely to eat raw meat products.

TABLE

Risk factors for outbreak of Shiga toxin-producing Escherichia coli (STEC) O157 in the Netherlands, December 2008 – January 2009

	Outbreak case (n=13)	Non-outbreak case (n=36)	Univariate odds ratio	
Food item	n (%)	n (%)	OR (95%CI)	p-value
Minced meat	10 (77)	21 (60)	2.2 (0.4-14.5)	0.23
Steak tartare	10 (83)	6 (18)	16.3 (3.2-238.6)	<0.001

Note: Percentages were calculated taking as denominator the number of persons who provided the relevant answer

The incubation period of STEC O157 is generally considered to be 1-10 days. Thirteen of the 14 primary cases with known date of symptom onset became ill within 11 days of each other. Raw meat can become contaminated during slaughter, and by cutting and mixing the meat a point source contamination can result in the contamination of a large batch of meat. Hygienic slaughter processes are imperative but contamination of carcasses cannot be completely avoided. This outbreak is another sign that despite control measures and legislation, raw meat products continue to pose a risk for the health of the general population. Raising consumer awareness in relation to consumption of raw meat is still needed [1,7,8].

Tracing of meat products continues to be a difficult task because insufficient detail is collected in the routine questionnaires about the precise type of meat, such as whether it is beef or veal, pre-packed or fresh from a butcher. Although questionnaire data is very useful, in our investigation several cases mentioned shopping at more than one supermarket chain without distinguishing which items were purchased where. This made it difficult to trace back the place of production and purchase of the implicated steak tartare. Obtaining supermarket receipts from cases could assist with the trace back, particularly in investigations in which one supermarket chain is frequented or when it is unclear what products were purchased where. This could improve the efficiency of the food trace back, but it is also time-consuming when more than one supermarket is involved and there is no protocol in place. It would also be useful to collect detailed information about steak tartare in the routine questionnaire. It is also apparent that we still do not have a good insight into the production chain of steak tartare, despite two large outbreaks in recent years.

Even though the current outbreak was confined to the Netherlands, international trade in meat and vegetable products makes it important to raise the alert internationally and rapidly and accurately trace suspected food items. However, effective prevention of future outbreaks caused by consumption of steak tartare may be very difficult.

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SALMONELLOSIS CASES CAUSED BY A RARE SALMONELLA ENTERITIDIS PT6C ASSOCIATED WITH TRAVEL TO BULGARIA, JUNE-JULY 2008

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In June 2008 an outbreak of gastroenteritis was registered in Sunny Beach resort situated on the Black Sea coast in Bulgaria, affecting 14 employees of a hotel, five of whom tested positive for *Salmonella* Enteritidis. During June-July 2008 four sporadic *S. Enteritidis* cases were also reported and two of them were foreign tourists. In the same period *S. Enteritidis* cases connected with travel to Bulgaria were reported to the European Centre for Disease Prevention and Control (ECDC) from Finland, United Kingdom, Sweden, Germany and Norway. We describe a study performed to find out relatedness between Bulgarian and Finnish *S. Enteritidis* isolates using phage typing (PT) and pulse-field gel electrophoresis (PFGE). Fifteen *S. Enteritidis* isolates from Bulgaria and 195 from Finland (including 28 from travellers to Bulgaria) were phage typed. Within Bulgarian isolates four different PTs were found and PT6c with eight strains was predominant. Nineteen out of 28 strains isolated from the Finns visiting Bulgaria belonged also to PT6c. PFGE typing (with one enzyme) of all *S. Enteritidis* PT6c strains (8 Bulgarian and 19 Finnish isolates) showed indistinguishable PFGE profile. The typing results thus demonstrated a link between Bulgarian and Finnish *S. Enteritidis* isolates. We conclude that *S. Enteritidis* PT6c was the cause of a salmonellosis outbreak in Sunny Beach and was exported to Finland, and likely to the United Kingdom, Norway, Sweden and Germany.

Introduction

Background

Salmonella has long been recognised as an important food-borne pathogen which can cause symptoms in humans ranging from self-limited enteric infections to enteric fever. In the European Union (EU), serovars *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most frequent causes of gastroenteritis in humans. In 2006, more than 160,000 cases of salmonellosis were reported in the EU resulting in an annual incidence of 34.6 cases per 100,000 population [1]. In 2001-2007, the annual number of salmonellosis cases in Bulgaria has varied between 800-1000 (incidence 9.3-15.4/100,000). Most of the Bulgarian cases have been sporadic. However, a few outbreaks have also emerged every year due to consumption of contaminated eggs and/or dairy products. Approximately 70% of the strains isolated in Bulgaria are of serovar *S. Enteritidis*.

Cases in Bulgaria

In the beginning of June 2008, a salmonellosis outbreak caused by *S. Enteritidis* occurred among personnel of a hotel in Sunny Beach resort situated on the Black Sea coast, in Nessebar municipality, Burgas region, Bulgaria. In all, 14 persons with symptoms of fever ($\leq 39.5^{\circ}\text{C}$), vomiting, abdominal pain and diarrhoea were reported of whom seven sought medical care in the local hospital. At the same time, during June and July 2008, four sporadic *S. Enteritidis* cases were also reported in the Burgas region two of whom were foreign tourists including an eight-year-old Finnish girl.

Cases in other countries

Finland: In June - July 2008, the Gastrointestinal Infection Unit of the National Institute for Health and Welfare (THL) in Helsinki identified 195 *S. Enteritidis* cases: 28 of them were connected to a trip to Bulgaria, including 19 that were of the phage type PT6c.

United Kingdom: On 8 July 2008 the Health Protection Agency reported an increased number of *S. Enteritidis* cases with an unusual phage type PT6c to the European Centre for Disease Prevention and Control (ECDC). Twelve patients were followed up and it turned out that all had been on holiday in Bulgaria preceding their illness.

Sweden: On 10 July 2008 Sweden reported to ECDC 29 *Salmonella* cases among travellers returning from Bulgaria during June and July. In Sweden, *Salmonella* strains related to travels abroad are not routinely serotyped. Nevertheless, 10 strains that were serotyped were all *S. Enteritidis*, and six of these cases were traced back to hotels in Nessebar and Sunny Beach. The 10 *S. Enteritidis* strains were also phage typed but the strains were not PT6c.

Germany reported to ECDC two *S. Enteritidis* cases linked to Bulgaria. One of these travellers had stayed in hotel at Nessebar from 23 to 30 May 2008 and had symptoms of salmonellosis starting from 28 May. More information about the other case was not available.

Norway reported a total of 76 salmonellosis cases in 2008 linked with travelling to Bulgaria, of which 48 isolates had been identified as *S. Enteritidis*. Of these isolates, eight were phage typed and four of them were of PT6c.

Aims of the study

Effective epidemiological surveillance of salmonellosis requires accurate subtyping of the strains in order to trace the potential sources of infection and the geographical distribution of different *Salmonella* serovars. A number of different phenotypic and genotypic methods have been used in microbiology laboratories for subtyping. Phage typing (PT) and pulsed-field gel electrophoresis (PFGE) are currently the only internationally standardised typing methods for *S. Enteritidis*. In order to find out relatedness between Bulgarian and Finnish *S. Enteritidis* isolates potentially associated with an outbreak occurring in Bulgaria, we initiated an investigation of those strains by phage typing and PFGE.

Methods

Surveillance

Salmonellosis is one of the notifiable communicable diseases in Bulgaria. The surveillance of salmonellosis in the country is laboratory-based. The primary diagnostics is performed by the regional clinical microbiology laboratories that are legally required to record and report all cases discovered in their regions. They send all outbreak-associated and some sporadic *Salmonella* strains to the National Reference Laboratory for Enteric Pathogens for confirmation, serotyping and antimicrobial susceptibility testing.

Outbreak investigation

Following notification of the outbreak in a hotel in Sunny Beach resort, field epidemiological investigation was performed including interviews with cases and contact persons, and active case-finding among hotel personnel. Stool samples were taken from 14 symptomatic employees of the hotel and 100 asymptomatic

contacts identified among personnel and families of cases, and were cultured by standard methods for *Salmonella*.

Collection and laboratory investigation of food samples

Food samples taken from five dishes prepared in the hotel restaurant and suspected based on the interviews (scrambled eggs with chopped peppers and tomatoes, chicken soup, chicken goulash, fish fried in egg and bread-crumbs, chicken giblets with rice) were tested for salmonellosis. Additionally, mash potatoes and two kinds of eggs, disinfected and not disinfected, were also examined in the microbiology laboratory at the Regional Inspectorate of Public Health Protection and Control in Burgas. ISO standard 6579 was used for the investigation of those food samples.

Pheno- and genotyping of the isolates

Subtyping of the *S. Enteritidis* strains was conducted at the National Reference Centre for *Salmonella* in Finland. *S. Enteritidis* isolates from Burgas region available at the National Reference Laboratory of Bulgaria (n=15) and all *S. Enteritidis* strains isolated in Finland during June and July 2008 (n=195) were examined. Isolates were phage typed using the method described in Ward et al. [2]. In addition, the 15 Bulgarian strains and the 19 Finnish PT6c isolates were analysed for genetic relatedness by PFGE using XbaI according to the Centers for Disease Control and Prevention (CDC) PulseNet protocol [3]. The PFGE patterns were named using international standardised nomenclature of PulseNet Europe.

Results

In the outbreak investigation, five of the 14 symptomatic persons among the hotel personnel and eight of the 100 contacts tested positive for *S. Enteritidis*. The food samples were all negative for *S. Enteritidis*. Nevertheless, scrambled eggs with chopped peppers and tomatoes were suspected as the most likely source of the

TABLE

Phage types and PFGE types of *S. Enteritidis* strains isolated in Bulgaria from stool specimens of 16 patients, June – July 2008

Date of receipt in NCIPD	Patient's nationality	National region	Occupation (link to a Sunny Beach hotel)	Phage type	PFGE type
14/05/2008	Bulgarian	Burgas	other (sporadic)	1	SENTXB.0001
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	6c	SENTXB.0010
20/06/2008	Bulgarian	Burgas	other (sporadic)	4	SENTXB.0001
20/06/2008	Bulgarian	Burgas	other (sporadic)	4	SENTXB.0001
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
24/06/2008	Bulgarian	Burgas	personnel	6c	SENTXB.0010
02/07/2008	Bulgarian	Burgas	other (sporadic)	1	SENTXB.0001
02/07/2008	Bulgarian	Burgas	other (sporadic)	21	SENTXB.0001
02/07/2008	Bulgarian	Burgas	other (sporadic)	4	SENTXB.0001
02/07/2008	Bulgarian	Burgas	other (sporadic)	1	SENTXB.0001
25/07/2008	Finnish		tourist	6c	SENTXB.0010

Abbreviations: NCIPD: National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria, PFGE: pulse-field gel electrophoresis

outbreak, since all persons affected reported having eaten this dish before onset of symptoms.

As a result of subtyping performed at the National Reference Centre in Finland, within the 15 Bulgarian *S. Enteritidis* strains, four different PTs were found: PT1, PT4, PT6c and PT21 (Table). The predominant PT was PT6c: eight out of 15 strains belonged to this phage type.

Among the 195 *S. Enteritidis* strains isolated in Finland, 15 PTs were found, the most common being PT21 with 54 strains. The cases were associated with trips to 25 countries, most commonly to Greece (34 cases), Bulgaria (28 cases), Turkey (27 cases) and Estonia (18 cases). The 28 strains isolated from Finns who visited Bulgaria in June or July were identified as PT6c (19 cases), PT6 (1 case), PT13a (2 cases), PT14b (2 cases) and PT22 (4 cases). PT6c strains were found only in samples of patients returning from Bulgaria.

PFGE analysis showed that the 15 *S. Enteritidis* strains from Bulgaria and the 19 strains from Finland could be assigned to only two characteristic PFGE patterns (SENTXB.0001 and SENTXB.0010) which were obtained after XbaI digestion (Figure). The *S. Enteritidis* PT6c strains (8 Bulgarian and 19 Finnish isolates) were indistinguishable from each other by this analysis and were classified as SENTXB.0010.

Discussion

Numerous reports of salmonellosis associated with foreign travel and caused by different *Salmonella* serovars have been published [4,5,6,7,8]. In this study, we report multinational cases of salmonellosis caused by *S. Enteritidis* PT6c associated with travel to Bulgaria.

S. Enteritidis PT6c is a rare phage type. None of the *S. Enteritidis* strains isolated from 978 patients and typed at the National Reference Centre in Finland in 2007 and 2008 (by June) belonged to this PT (A. Siitonen, unpublished data). Also, to our knowledge, there are no previously published reports on outbreaks caused by this phage type. Several European countries, namely

Austria, Norway, Hungary, Ireland, Finland, United Kingdom, Germany and Sweden reported to ECDC sporadic *S. Enteritidis* cases in 2008 among tourists returning from Bulgaria. United Kingdom, Finland and Norway found *S. Enteritidis* PT6c among *Salmonella* strains isolated from the samples taken from their citizens returning from Bulgaria.

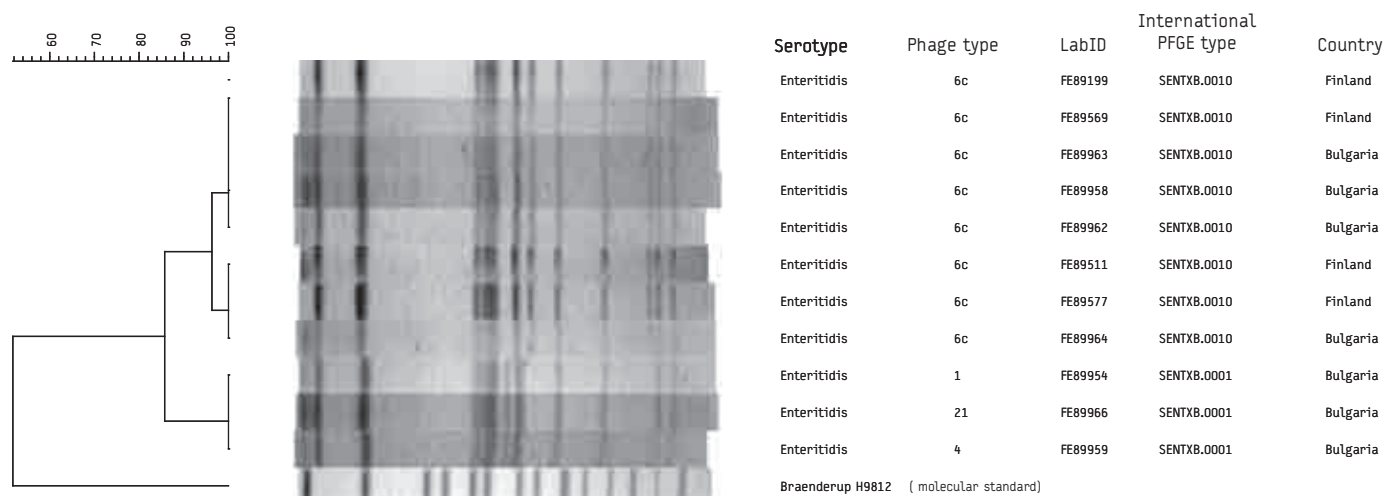
In our study, 27 strains (8 Bulgarian and 19 Finnish) proved to be *S. Enteritidis* PT6c. The facts that i) the Bulgarian and Finnish isolates were of the same phage type and ii) among the Finnish *S. Enteritidis* strains, phage type PT6c was only found in isolates from patients who returned from Bulgaria, indicate an epidemiological link between them. Among Bulgarian *S. Enteritidis* PT6c isolates, four strains were taken from cases associated with *S. Enteritidis* outbreak in a hotel in Sunny Beach resort. *S. Enteritidis* PT6c was also isolated from an eight-year-old Finnish girl who had stayed in another hotel in Sunny Beach resort (Table). In the hotels of this resort, many Finns and people of other nationalities spent their summer holidays in 2008. After July, PT6c was still found in Finnish *S. Enteritidis* cases returning from Bulgaria in August (n=4), September (n=9) and October (n=1) but no cases were detected in November.

The finding that all tested Bulgarian and Finnish *S. Enteritidis* PT6c isolates had the same PFGE profile (SENTXB.0010) also suggests that these strains could have the same origin and be epidemiologically linked. However, this PFGE profile can be found in *S. Enteritidis* strains of several PTs, including common phage types PT4 [9], PT1 and PT21 (A. Siitonen, unpublished). This emphasises the importance and applicability of phage typing over genotyping in epidemiological surveillance of salmonellosis.

Tourism is the fastest growing industry worldwide. The globalisation leads to faster spreading of infectious diseases including salmonellosis and requires us to consider them from a global perspective. International networks worldwide and collaboration of the health authorities are essential for an effective control of salmonellosis.

FIGURE

Cluster analysis based on the PFGE profiles of *S. Enteritidis* isolates originating from Bulgaria and Finland, June-July 2008



In conclusion, the alert system administered by ECDC, the effective collaboration between EU countries and the use of internationally standardised subtyping methods such as phage typing and PFGE, enabled us to establish an international clustered record of *Salmonella* infections caused by a rare *S. Enteritidis* PT6c and its association with travelling to Bulgaria. We believe that *S. Enteritidis* PT6c was the cause of outbreaks of salmonellosis in resorts situated at the Bulgarian Black Sea coast and was exported to Finland and most likely to the United Kingdom, Norway, Sweden and Germany. In this study, the importance of a multinational approach for the determination of potential sources of salmonellosis and its geographical distribution was demonstrated.

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HUMAN CASE OF SWINE INFLUENZA A (H1N1), ARAGON, SPAIN, NOVEMBER 2008

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A human case of swine influenza A (H1N1) in a 50-year-old woman from a village near Teruel (Aragón, in the north-east of Spain), with a population of about 200 inhabitants, has been reported in November 2008.

On 8 November 2008, a 50-year old woman developed fever, cough, extreme tiredness, myalgia, irritation of the nasal/oral mucosae and shivers of sudden onset. During a medical visit on 12 November 2008, the general practitioner (GP) who treated the case and is a member of the sentinel influenza surveillance system, took a throat swab sample and sent it to the Microbiology Laboratory of the Miguel Servet University Hospital in Zaragoza, Aragón in the context of the Spanish Influenza Surveillance System. The patient, with no history of recent travel, did not need specific treatment or hospitalisation and recovered fully.

Epidemiological investigation

The case worked on a family swine farm and had direct and close exposure to pigs. No other family members or co-workers reported flu-like symptoms before or after this case and no symptoms were observed in the pigs. However, the GP who took the throat swab sample reported influenza-like illness (ILI) after visiting the patient. No samples from the GP were taken at that time.

A low level influenza activity, with no activity for the geographical spread indicator, was reported in Spain and specifically in the province of Teruel during week 46/2009 when the case was notified. The GP did not report any other influenza case for the whole season up to week 53.

After the initial report of a possible case of A(H1N1) of swine origin from the National Influenza Reference Laboratory on 13 January, the following actions were taken: an active surveillance was implemented on site, including collection of blood samples for serological investigations from the case, the treating physician and the four household contacts of the case on January 20. Informed consent was required from all of them and a specifically designed questionnaire was used to interview the six mentioned people. So far, no more cases related to the farm have been detected.

Following the requirements of the International Health Regulations (IHR, 2005), this event was notified to the World Health Organization (WHO) as a human case of influenza caused by an influenza virus different from those circulating in humans.

Laboratory investigation

Respiratory secretions were first inoculated in cell cultures (MDCK) at the Microbiology Laboratory of the Miguel Servet University Hospital. The cell cultures were positive for influenza A virus, but the assays routinely used in this laboratory (immunofluorescence with monoclonal antibodies and PCR assay) failed to subtype the virus. After consulting the National Influenza Reference Laboratory (National Influenza Centre-Madrid, Instituto de Salud Carlos III, Spain) the specimen and influenza isolate were sent to this laboratory for further characterisation. Different PCR approaches allowed to partially sequence and identify the haemagglutinin gene. On 13 January, 2009, the Reference Laboratory reported an influenza A subtype H1 phylogenetically close to the human isolate A/Switzerland/8808/2002 of swine origin [1] indicating a sporadic human infection of possible swine origin.

Other genes (NA, M, NP and NS) were also sequenced and analysed, which confirmed that the influenza A virus was phylogenetically related to swine H1N1 viruses. Partial sequences of the five genes have been submitted to the GenBank database (accession numbers from FJ713784 to FJ713788)PPB. Avian-like H1N1 swine influenza viruses are enzootic in the swine population of Western Europe. In order to undertake a serological survey and further virological studies the virus is being propagated in embryoned hen eggs.

Discussion

The epidemiological and virological information points towards a human infection with an influenza virus of swine origin in a person with professional exposure to pigs. No further cases have been identified amongst family members or fellow workers. Sporadic human infections due to influenza viruses of swine origin have been described previously, mostly in young persons (<25 years) in contact with pigs [2-4]. Transmission to humans for unknown reasons seems to be inefficient. Although it is expected that similar

cases could appear in the future this event could not be considered unexpected. All these considerations have led us to investigate this case in order to contribute to a better knowledge of the interaction between swine and human influenza.

The treating physician reported mild influenza-like symptoms after contact with the patient. Based on the available information, human to human transmission could not be confirmed. Ongoing serological studies may be of help to determine whether further transmission of the swine virus has taken place. Human to human transmission has been reported before; however in these cases transmission was limited to one generation [5].

To conclude, this event cannot be considered unexpected and does not pose a public health risk which would require specific public health measures.

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Rapid communications

INCREASE IN HEPATITIS A CASES IN THE CZECH REPUBLIC IN 2008 – AN UPDATE

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In 2008, 1,616 cases of hepatitis A were reported in the Czech Republic, more than a 10-fold increase compared with the annual number of cases registered in 2003-2007. The infection was initially associated with injecting drug users, most probably by person-to-person contact or parenteral transmission, and in the second half of the year continued to spread among the general population with increased susceptibility.

Introduction

Since the end of May 2008, an increase in reported cases of hepatitis A virus (HAV) infection has been observed in the Czech Republic. From 1 January to 31 December 2008, a total of 1,616 laboratory-confirmed cases of were reported. The objective of this paper is to provide basic information on hepatitis A morbidity and outbreak control measures taken by the Public Health Protection Authorities (PHPA) in the Czech Republic.

Methods

In the Czech Republic, hepatitis A is a reportable disease. The attending physician (most often the general practitioner) recommends quarantine of the patient with confirmed or suspected hepatitis A and reports the case to the respective PHPA without delay. The hospital infectious disease departments report admission of each patient with the indication of diagnosis at admission to the respective PHPA. Any patient quarantined or placed under medical

supervision with suspected hepatitis A is examined clinically, biochemically and by laboratory tests for the detection of diagnostic markers of HAV. In the Czech Republic, the laboratory tests include the screening of sera for the presence of specific antibodies against HAV (anti-HAV IgM).

Hepatitis A prevention in the Czech Republic is specified in the Guidelines of the Ministry of Health [1]. A confirmed case of hepatitis A is defined as a person who meets the clinical and laboratory criteria in accordance with the European Union (EU) case definition [2].

The confirmed cases of hepatitis A are reported by the respective PHPA to the national reporting system for infectious diseases EPIDAT. Identification data and standardised results of the epidemiological investigation and laboratory analyses are entered into the EPIDAT system.

Results

From 1 January to 31 December 2008, 1,616 laboratory-confirmed cases of hepatitis A were reported in the Czech Republic, i.e. 15.7 cases per 100,000 population. This is a 10.6 fold rise in comparison with the annual average number of cases reported in 2003 to 2007 (mean 153 cases, range 70 – 322 cases) (Figure 1).

FIGURE 1

Annual incidence rates of viral hepatitis A per 100,000 population, Czech Republic, 2003-2008

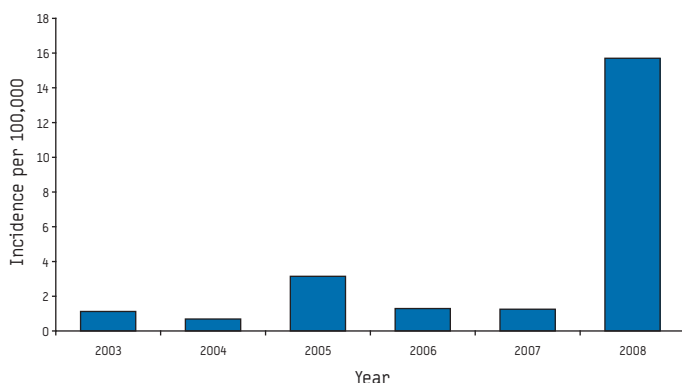
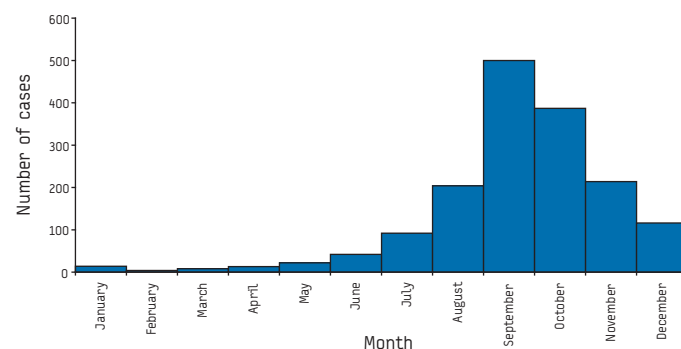


FIGURE 2

Cases of viral hepatitis A reported in the Czech Republic, in 2008, by month of onset (n=1,616)



A marked increase in hepatitis A cases had been observed since the end of May 2008 [3], with a total of 61 cases reported in the first five months of the year, compared to 1,555 cases in the period June to December 2008 (Figure 2).

Two cases were fatal. One was a 33-year-old non-vaccinated drug addict co-infected with hepatitis A, B and C and the other was a 75-year-old man vaccinated as a family contact one day prior to the onset of disease. The latter patient was hospitalised because of relapsed hepatitis. In accordance with the International Classification of Diseases (ICD 10) [4], the final diagnosis was B15.9: hepatitis A without hepatic coma.

The majority of cases were reported in the following three of the 14 administrative regions: Prague region (878 cases, i.e. 54.3% of the reported total), Central Bohemian region (206, i.e. 12.7%) and Olomouc region (147, i.e. 9.1%). In the remaining regions, sporadic cases and small, mostly family outbreaks were reported. The family outbreaks included 382 hepatitis A cases (23.6 % of the total). The absolute numbers of cases are shown in Figure 3.

FIGURE 3
Cases of viral hepatitis A in the Czech Republic, in 2008, by region (n=1,616)

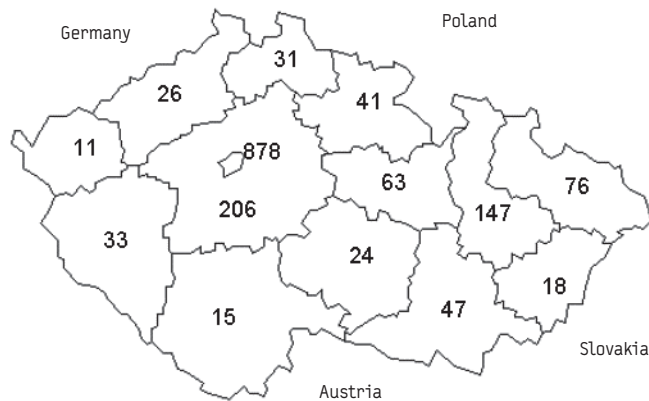
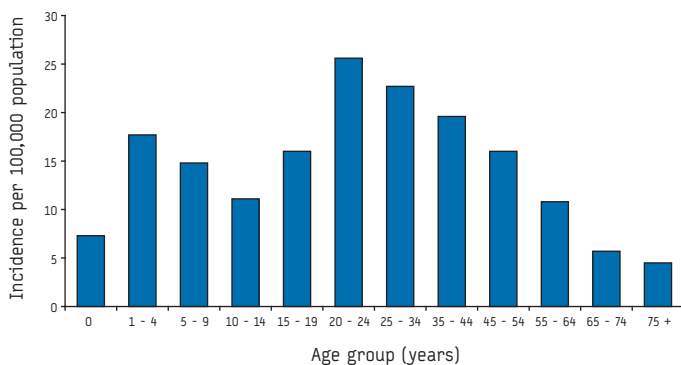


FIGURE 4
Cases of viral hepatitis A reported in the Czech Republic, in 2008, incidence rate per 100,000 population, by age (n=1,616)



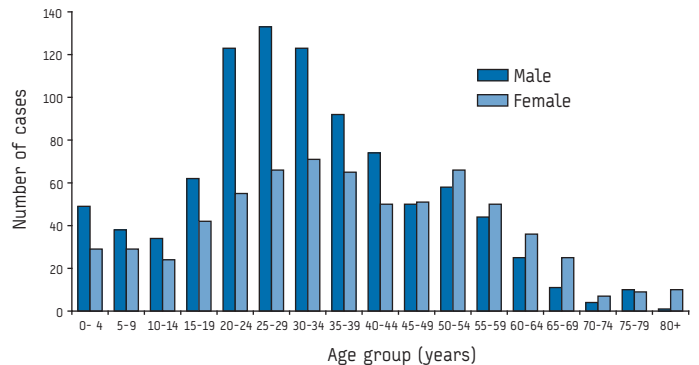
As for age distribution, most hepatitis A cases (82.7 %) were reported in patients aged 15 to 64 years. The most affected age group was that of 25-34 years (393 cases). The highest age-specific incidence rate was reported in the age group of 20-24 years (25.7 cases per 100,000 population). In children aged 0-14 years, 203 cases (12.3% of the total) were diagnosed (Figure 4). As expected, increase in hepatitis A cases in children was observed in September and October 2008 with the start of the new school year and the return of children to school and preschool communities.

Of the total of 1,616 cases of hepatitis A, 931 (57.6%) were reported in males and 685 (42.4%) in females. The greatest difference in sex distribution of cases was found in young adults, with up to 2.5 times more affected males than females (Figure 5).

At the very beginning, the increase in hepatitis A cases was significantly associated with injecting drug users (IDUs), with the highest contribution of the age group of 25-34 years, particularly in the administrative regions of Prague and Central Bohemia where epidemic outbreaks were reported. In the first weeks, IDUs accounted for 2/3 of the cases. HAV transmission in high-risk groups was due to sub-standard hygiene. In the second half of 2008, hepatitis A spread significantly among the adult general population and the proportion of cases in IDUs considerably decreased. In 2008, 226 hepatitis A cases (i.e. 14.0% of the total) were reported in IDUs. Altogether 421 (26.1%) cases were reported in persons considered to be at a higher risk of infection (homeless individuals, prisoners, drug users, alcoholics and persons engaging in high-risk sexual behaviour).

The number of imported cases of hepatitis A in 2008 was 68, about twice as high as reported annually during the last decade, but as a proportion of the total number of cases it was as low as 4.2%. The largest number of imported cases from a single country came from Egypt (20 cases), followed by Slovakia (9 cases), Greece and Croatia (5 cases per country), Tunisia (4 cases), Spain (3 cases), Ukraine, Turkey, France, Italy and Canary Islands (2 cases per country) and 10 other countries (single cases). None of the imported cases came from Latvia where a large outbreak has been ongoing [5,6].

FIGURE 5
Cases of viral hepatitis A reported in the Czech Republic, in 2008, by age group and sex (n=1,616)



Measures and recommendations

Standard outbreak control measures coordinated by the Ministry of Health continue to be taken. They include particularly isolation of patients, medical supervision of close contacts. Medical supervision that consisted in clinical and laboratory follow-up of contacts throughout the maximum incubation period was provided to more than 7,000 persons. Close contacts involved in epidemiologically significant activities (e.g. in food industry) have been instructed to stop such activities and to remain under enhanced surveillance for 50 days after the last contact with the hepatitis A patient. Other measures are disinfection and targeted vaccination in the focus of infection. Post-exposure prophylaxis with vaccine was provided to 7,519 known or probable contacts. The vaccination was fully covered by the state through the Ministry of Health. As many as 100 of the vaccinated contacts developed hepatitis A. These cases are currently analysed in detail from the point of view of the used vaccine, number of administered doses and interval between vaccination and onset of disease.

Vaccination was also offered to IDUs and homeless persons in Prague and Central Bohemia; 2,002 were vaccinated of whom four developed hepatitis A. This vaccination can be characterised as combined pre- and post-exposure prophylaxis. The costs were covered by the respective PHPA. In addition, 7,900 children from the first classes of elementary schools in the Central Bohemian region were vaccinated, with no case of hepatitis A reported in this population. The expenses were covered by the Regional Authority of Central Bohemia.

In addition, PHPA issued information on hepatitis A for school facilities and general practitioners (GPs). Information for the general public has been available primarily at the websites of the National Institute of Public Health and Ministry of Health of the Czech Republic, regional PHPA and in the mass media. Active surveillance of viral hepatitis in the Czech Republic continues.

Conclusion

The European Centre for Disease Prevention and Control (ECDC) organised a technical meeting on hepatitis A held in Riga on 11 November 2008 with the participation of representatives from Latvia, Slovakia, Estonia, Germany, Italy, the Netherlands, United Kingdom and the Czech Republic. The conclusions drawn at the meeting are also relevant to the Czech Republic which is true particularly of the statement that hepatitis A outbreaks are associated with increase in the susceptible population with improved standard of hygiene as documented by higher numbers of hepatitis A cases not only in children and youth but also in adults. Other contributing factors are increase in imported cases coming from endemic countries and higher incidence of hepatitis A in IDUs and other individuals with high-risk behaviour. It was suggested that ECDC should recommend general immunisation against hepatitis A across the EU. The significance of post-exposure prophylaxis with vaccine included in the Guidelines of the Ministry of Health of the Czech Republic was discussed [1]. In a longer perspective, the implementation of serological surveys is considered important to determine susceptibility of the EU population to HAV infection. Results of serological surveys would provide background data for the development of the vaccination strategy guidelines.

HAV RNA sequencing and phylogenetic analysis of HAV isolates from outbreaks would be of relevance. In the Czech Republic, serum and stool samples are being collected in the most affected

areas. The kind offer of the National Institute of Public Health and Environment in Bilthoven, the Netherlands (RIVM) to analyse a part of samples and to provide the guidance for the completion of analyses in the National Reference Laboratory for Viral Hepatitis in the Czech Republic will be accepted.

Based on the available data, it is possible to exclude water- or food-borne and sexual transmission of HAV in the Czech Republic in 2008. The spread of hepatitis A in 2008 started among IDUs, most probably by person-to-person contact or parenteral transmission, and continued among other high-risk groups (homeless persons) in conditions of sub-standard hygiene. Subsequently, the infection spread among the general population with increased susceptibility. Higher susceptibility to HAV is likely to result from long-term low prevalence of hepatitis A.

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Rapid communications

COMMUNITY-WIDE OUTBREAK OF HEPATITIS A IN LATVIA IN 2008 – AN UPDATE

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An outbreak of hepatitis A has been ongoing in Latvia with 2,817 confirmed cases reported between 20 November 2007 and 31 December 2008. Initially the spread of infection was due to transmission among drug users and other high-risk groups, as well as several outbreaks in Rīga (affecting a school and a restaurant), but in the second half of the year led to a community-wide increase in the number of cases. Molecular analysis of 100 strains showed that 95 belonged to genotype IA, of which 89 were identical and six were single nucleotide variants of the same sequence.

Introduction

The Latvian Public Health Agency (PHA) updates through this article the information on epidemiological situation of hepatitis A in 2008 in Latvia. An increase in number of cases of hepatitis A has been observed since November 2007. A total of 2,817 confirmed cases of hepatitis A were notified between 20 November 2007 and 31 December 2008, and 419 suspected cases were still under investigation on 31 December 2008. The highest number of cases (678) was notified in October 2008. The distribution of confirmed and suspected cases of hepatitis A by month of onset is shown in Figure 1.

Methods

Hepatitis A is a disease under mandatory notification in Latvia. Clinicians should notify suspected and confirmed cases and

laboratories are required to report positive hepatitis A virus (HAV) results according to the European Union (EU) case definitions [1].

A probable case is defined as a person with a clinical picture compatible with hepatitis (discrete onset of symptoms and jaundice or elevated serum aminotransferase levels) and with an epidemiological link. A confirmed case is defined as any person meeting the clinical criteria and with serum IgM antibodies against hepatitis A virus (IgM anti-HAV) [1].

Upon receiving notification reports from clinicians or laboratories, all cases of hepatitis A are investigated by epidemiologists from the Public Health Agency (PHA) local branch.

To characterise the HAV circulating in the outbreak, 100 serum samples from the Latvian State Agency "Infectology Center of Latvia" were sent to the Dutch National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) for genotyping.

Results of epidemiological investigations

The age of the cases ranged from five months to 86 years, with a median age of 31.7 years. The age and sex distribution of confirmed cases of hepatitis A is shown in Figure 2.

FIGURE 1

Number of reported cases of hepatitis A, by month of onset, Latvia, November 2007 - December 2008 (n=3,236)

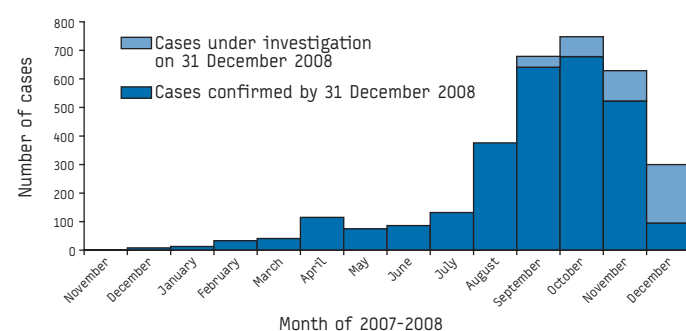


FIGURE 2

Age and sex distribution of confirmed cases of hepatitis A reported in Latvia from November 2007 to December 2008 (n=2,817)

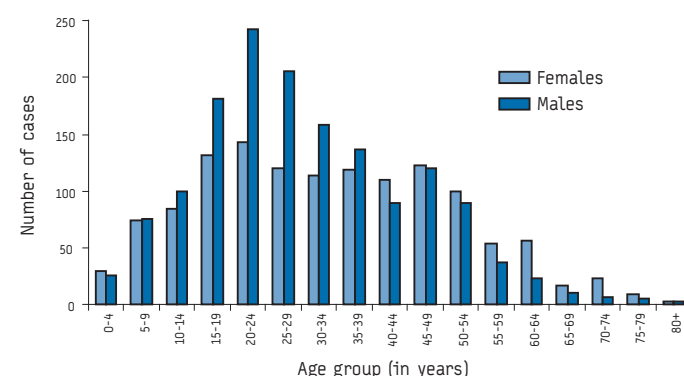


FIGURE 3

Number of cases of hepatitis A per 100,000 population, by age and sex, Latvia, November 2007 – December 2008

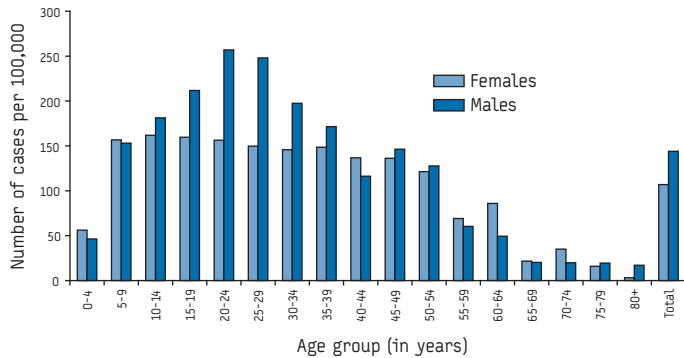


FIGURE 4

Cases of hepatitis A among drug users and the proportion of drug users among all hepatitis A cases, Latvia, November 2007 – December 2008 (n=191)

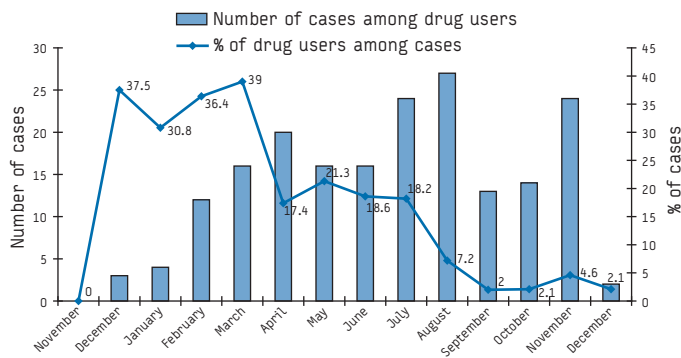


FIGURE 6

Number of death cases of hepatitis A, by age and sex, Latvia, November 2007 – December 2008 (n=17)

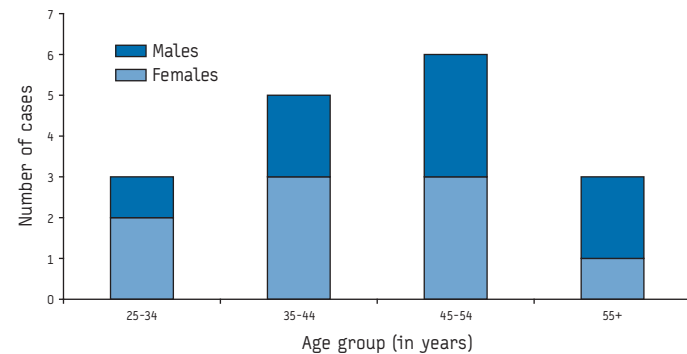


FIGURE 5

Geographical distribution of reported cases of hepatitis A in Latvia, November 2007 – December 2008

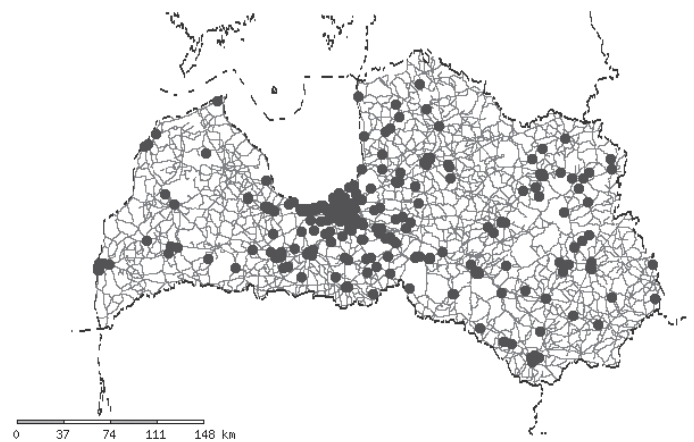
a) Cases reported between 20 November 2007 and 30 April 2008 (n=211)



b) Cases reported between 1 May and 31 August 2008 (n=669)



c) Cases reported between 1 September 2008 and 31 December 2008 (n=1,937)



Significant proportion of cases among adults could be explained by low hepatitis A infection activity in Latvia in recent years and absence of naturally acquired immunity in their childhood. The last biggest outbreak of hepatitis A occurred in 1988-1990 with almost 20,000 cases registered during three years. Since then the number of cases steadily declined and during the last 10 years was very low – an average of 100 cases per year. The lowest number of cases of hepatitis A (n=22) was registered in 2007, the year before the current epidemic.

The proportion of males amongst hepatitis A patients was 72% (range 66 to 73%) in the first six months of the epidemic (November 2007 - April 2008), and 52% in the following period (May - December 2008).

The overall male to female ratio was 1.15 to 1, with the highest rate of 1.55 to 1 in the age group 15 – 34 years.

The overall incidence rate per 100,000 population was 124. The incidence rate amongst males was about 1.35 times higher than amongst females (Figure 3)

The difference in infection risk between the sexes could be partly explained by significant number of cases among male drug users (DUs).

Hepatitis A in drug users

During the observation period, 191 drug users (of whom up to 90% were injecting drug users, IDUs) were notified as hepatitis A patients. The highest numbers were reported in July and August (23 and 27, respectively), but the proportion of drug users amongst all cases was highest in the beginning of the epidemic – up to 39%. The estimated number of problem drug users in Riga is 4,757. As the number of hepatitis A cases among DUs in Riga was 153, the incidence rate in this group could be as high as 3,216 cases per 100,000 in 2008.

Since September, the number of cases among DUs and, in particular, the proportion of DUs among all cases had declined. The reason for this is still unclear although one of the explanations may be that the epidemic in this group started earlier and therefore peaked and declined earlier compared to the outbreak in the general population.

Geographical distribution

The majority of cases of hepatitis A (2,132, 76%) occurred in inhabitants of Riga, 199 in the population of the wider Riga region, 88 cases in Jūrmala and the remaining cases were distributed

among other six cities and 23 districts in Latvia which reported between one and 66 cases each.

Clinical outcome

Of the 2,817 confirmed cases, there were 17 deaths (0.6%). 91% of cases of hepatitis A were treated in hospitals.

All death cases were registered in patients with underlying diseases and / or other risk factors (alcohol, drugs). An increase in mortality has been observed during the epidemic, ranging from 0 in the period of time November 2007- March 2008 to 0.77% in October – December 2008 (see Table 1).

There was no difference in mortality rates among cases of hepatitis A by sex.

Genotyping results

One hundred serum samples from Latvian patients were tested for the presence of HAV RNA. All samples were positive and were further processed for genotype analysis by sequencing of 460 nucleotides of the VP1/P2A region. Sequences were compared to each other and to sequences available in public databases. One of the 100 sequences was of genotype IB, with a maximum match of 99% with three sequences originating from North and West Africa. Four of the 100 sequences were of genotype IIIA. The four sequences were identical and have as nearest neighbors 20 sequences in the database that match at 99%. These sequences mostly have their origin in Pakistan.

By far the largest group of sequences from Latvian patients belonged to genotype IA. Of these 89 were identical, and six were single nucleotide variants of this sequence type. These 95 sequences represent the outbreak strain of Latvia 2008. In the databases only two strains were found with sequences matching at 99% or greater. Both were isolated from patients in the Netherlands in 2004 and were travel associated. In one case travel history involved Turkey.

For all three individual sequence types of the three genotypes there were many sequences matching at 98% with various regions of origin, several different transmission modes, and from an extended time period. Therefore, if sequence matching is used for epidemiological linking, sequences should be matching more than 99%

TABLE 1
Number and proportion of death cases in hepatitis A outbreak in Latvia, November 2007 – December 2008 (n=17)

Months	Number of cases	Number of deaths	% of deaths
November 2007 – March 2008	96	0	0
April – June	276	1	0.36
July – September	1,149	6	0.52
October - December	1,296	10	0.77
Total	2,817	17	0.6

TABLE 2
Genotype analysis of hepatitis A virus isolated from cases in Latvia, 2008 (n=100)

Genotype	Number of strains isolated from Latvian patients	>99% matching sequence (accession number)	Origin of closest match	Match quality and resolution
IA	95	DQ114859 DQ650789	Turkey	0 differences in 177 nucleotide overlap
IB	1	DQ387553 AY343701 DQ387553	Morocco Morocco Ghana	1 difference in 177 nucleotide overlap
IIIA	4	DQ387610 19 others	Pakistan	1 difference in 177 nucleotide overlap

Source: National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

The single IB and the four IIIA strains were most likely introduced in Latvia by (returning) travelers, and spread to close contacts if at all. This is a pattern that can be seen in many European countries with a low level of hepatitis A endemicity. The IA strain that caused the outbreak may also have been introduced by a traveler but more importantly it was introduced into a group, in which it could spread more widely than just close contacts, thereby causing an outbreak.

Control measures

With the aim to contain the epidemic, the following measures have been implemented:

All cases of hepatitis A have been investigated by epidemiologists of the relevant local branches of "Public Health Agency". Family doctors have been informed about contacts. Control measures, such as medical observation of contacts and increasing of hygiene and restriction of contacts between children from different groups, have been implemented at places at risk – children establishments, food enterprises, as well as workplaces and households where two and more cases of hepatitis A were registered.

Monitoring of cases of hepatitis A has been enhanced - weekly and, if necessary, daily data are available at the national and local levels. Monitoring data are published on PHA website.

Detailed recommendations for different target groups (staff of food enterprises, children establishments, and general public) have been developed and distributed to different institutions at national and local levels. Recommendations had already been available on the PHA website. Survey data indicated that in October, PHA recommendations were available in 98% of schools.

Lectures for different target groups (health professionals association, school nurses, family doctors etc.) have been provided.

A special poster-sticker to stress the importance of hand washing has been developed and distributed to schools.

A special survey to identify risk factors for hepatitis A has been performed by PHA in schools. Local governments and administration of the schools were informed about the results of the survey, its conclusions and recommendations.

Intensive collaboration with mass media has been in place. PHA press releases on hepatitis A situation and recommendations have been developed and distributed weekly. Only in November there were 53 publications on hepatitis A in national and local mass media, and 13 interviews on this topic on TV and 17 on the radio.

Although vaccination against hepatitis A has not been provided free of charge, vaccination has been recommended to risk groups and contacts. A significant increase in the number of people vaccinated against hepatitis A has been observed since September 2008 corresponding to the spread of the epidemic.

Conclusion

The ongoing outbreak of hepatitis A in Latvia has not yet been fully understood, but a few working hypotheses may explain the spread of the epidemic. The increase in the number of cases in the beginning of 2008 can be related to the initial spread of infection among DUs and persons with low income level living in substandard hygienic conditions, as well as to several outbreaks (a school in Riga, a restaurant in Riga [2,3]). Increased circulation of the virus in highly susceptible population led, in the second part of the year, to a community-wide increase in the number of cases that demonstrated the typical seasonal activity of hepatitis A usual for endemic years in the past. The modes of transmission involved vary, including person-to-person transmission, contaminated food, and, possibly, swimming in bathing waters in summer.

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TABLE 3

Number of vaccinations against hepatitis A in Latvia, 2006-2008

Year	Month	Number of vaccinated with the first dose of vaccine
2006	January – December	1,815
2007	January – December	2,912
2008	January	292
	February	383
	March	247
	April	309
	May	415
	June	301
	July	187
	August	357
	September	1,054
	October	1,754
	November	1,631
	December	1,950
	January – December	8,880

Source: Monthly statistical data provided by clinicians

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Rapid communications

HEPATITIS A OUTBREAK IN A ROMA VILLAGE IN EASTERN SLOVAKIA, AUGUST-NOVEMBER 2008

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We describe an outbreak of hepatitis A in Lomnička, a village in the eastern part of Slovakia. The outbreak was limited to the village and did not spread either to other districts of Slovakia or to the neighbouring countries. The number of cases reported from 28 August to 30 November 2008 was 298. All cases but one occurred in the Roma population. The outbreak was associated with low socio-economic conditions which facilitated person-to-person transmission. No common source of the outbreak was verified.

Background

In Slovakia, hepatitis A is a mandatorily notifiable disease [1]. The national surveillance is coordinated by the Chief Public Health Officer of the Slovak Republic who is the head of the Public Health Authority of the Slovak Republic (PHA SR), an institution in charge of 36 Regional Public Health Authorities (RPHAs) covering 79 districts of the country.

The case classification is in accordance with the European Union (EU) case definition of hepatitis A [2].

Physicians and laboratory microbiologists are liable to report any confirmed or suspected case of acute viral hepatitis A to the epidemiologist of territorially relevant RPHA. Data from all regions are then collected in the National Central Register. The Epidemiological Information System (EPIS) is used for the purpose of data reporting, collecting, processing and analysing. It is a real

time system and thus new data about either sporadic cases or outbreaks can be evaluated every day.

The population of Slovakia was around 5.4 million as of 30 June 2008 [3]. According to the official population census, in 2001, the number of Roma inhabitants was 260,605. However, this seems to be an underestimate and the actual proportion of the Roma population is likely to be much higher. According to the World Bank study published in 2003, there were almost half a million Roma living in Slovakia [4]. The majority (about 57%) of the Roma population lives in eastern Slovakia.

Hepatitis A in Slovakia

The overall incidence of hepatitis A in Slovakia has shown a constantly decreasing trend in the last decades. The rates declined from more than 50 cases per 100,000 population in 1988 to the lowest ever recorded incidence of 7 cases per 100,000 in 2007. However, peaks of incidence have occurred in periodical intervals (every few years) since 1988, probably due to increasing numbers of non-immune children. Each peak, however, has represented a lower incidence than the previous one (Figure 1).

In recent years, mostly sporadic cases and rare small outbreaks have been reported. The outbreaks often affected the Roma population and were associated with low hygienic conditions and person-to-person transmission.

FIGURE 1

Incidence rates of hepatitis A per 100,000 population, Slovakia, 1988 – 2008 (as of 30 November 2008)

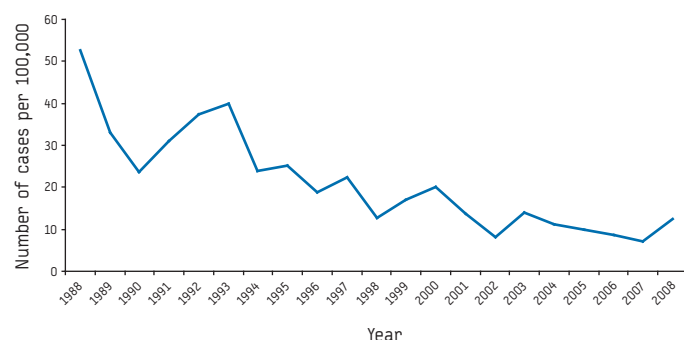
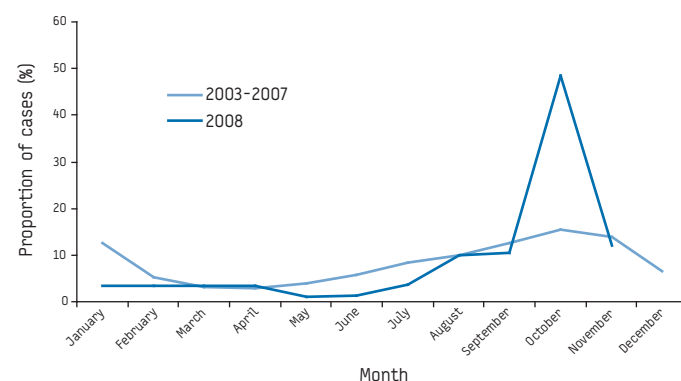


FIGURE 2

Seasonality of hepatitis A in Slovakia, 2003-2007 and January - November 2008



In 2008, a total of 667 cases of hepatitis A (incidence of 12.4 per 100,000 population) have been reported to the EPIS database, as of 30 November 2008. This includes nine outbreaks involving 485 cases.

The seasonal distribution of cases of hepatitis A in 2008 shows a typical pattern observed also in the previous years, with the highest proportion of cases reported in September, October and November (Figure 2).

The notification reports include information on whether the case is associated with “low” or “normal” hygienic standard. In 2008, 536 cases were recorded as “low hygienic standard” (80% of the total, incidence of 107.2 per 100,000 population) and 131 as normal hygienic standard (incidence of 2.7 per 100,000 population). Although the reports do not contain data on the ethnic origin of the cases, on the basis of available evidence, the Slovak public health authorities generally consider cases recorded with “low hygienic standard” to have occurred in the Roma population, reflecting the poor living conditions of this group.

Among the 131 hepatitis cases reported in 2008 and associated with “normal hygienic standard” (thus assumed to have occurred in the majority population), 14 were imported cases: nine from the Czech Republic, three from Egypt, one from Madagascar and one from Tunisia.

As many as 495 of the 667 cases reported in 2008 (74%) occurred in children below the age of 10 years, and the age-specific incidence rate was highest in this group. This can be linked with the fact that most cases were assumed to have occurred in the Roma population where children are exposed to the hepatitis A virus (HAV) at an early age, due to poor socio-economic living conditions.

The geographical distribution of cases reported in 2008 shows the highest incidence rates in two districts: Stará Ľubovňa with 720 cases per 100,000 population and Bardejov with 110 cases per 100,000 population, in comparison with the average incidence rate of 12 cases per 100,000 population for the whole country (Figure 3). The two most affected districts are situated in the Prešov region which has the highest density of the Roma population in Slovakia. Also, four of the nine outbreaks reported in 2008 occurred in these

two districts. The largest one involving 298 cases occurred in the village of Lomnička, district Stará Ľubovňa.

Hepatitis A outbreak in the village of Lomnička

Lomnička is a small village with population of 2,044 situated in the north-east of Slovakia, in the district Stará Ľubovňa. Almost all (2,034, more than 99%) inhabitants of the village are Roma, and close to 60% are below the age of 18 years.

The sanitation and living conditions in the village are very poor. There is access to running water service but the supply is often stopped in households that do not pay the fees. Waste and sewage water disposal is inadequate.

The first case in the outbreak was hospitalised on 27 August 2008, followed by further eight hospitalised in the next two days. An interval with none or very few cases was followed by an explosive wave with the peak on 17 October 2008 (26 cases hospitalised in a day). After that the occurrence of new cases gradually declined with none or single cases reported from 14 to 30 November 2008.

In all, 298 cases of hepatitis A, all of them hospitalised, were reported from the village of Lomnička between 27 August and 30 November 2008 (Figure 4). The most affected were children below 10 years of age. Of the cases, 148 were below 6 years of age, 142 were between 6 and 10 years old, seven were in the age group of 11-18 years, and only one was adult.

Control measures

The outbreak was officially declared on 28 August 2008, when the first four cases of hepatitis A occurred. Control measures were launched within 24 hours.

Control measures were carried out by the PHA SR and RPHA Stará Ľubovňa. The response action was coordinated by the Chief Public Health Officer and the PHA SR. He also called the regional anti-epidemic committee and the crisis committee, who announced an emergency situation in the district Stará Ľubovňa on 15 October 2008. This allowed potential restriction of movement of inhabitants to avoid spread of infection. The emergency situation was ceased on 22 October 2008. A temporal outpatient clinic was established in the village. The chief hygienist ordered to reprofile the hospital

FIGURE 3
Incidence rates of hepatitis A per 100,000 population, by region, Slovakia, 2008

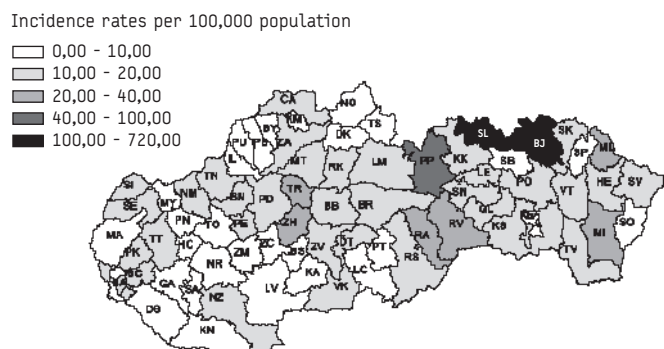
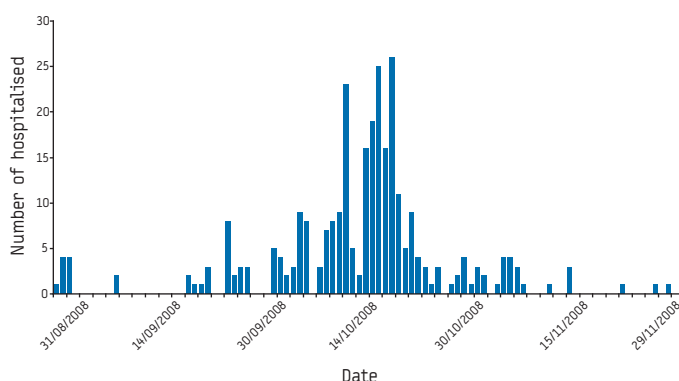


FIGURE 4
Number of hospitalised cases of hepatitis A in Lomnička village, Slovakia, 27 August – 30 November 2008 (n=298)



beds in the district of Stará Ľubovňa as well as in neighbouring districts to make them ready to receive more hepatitis A patients. The EPIS served as a very good communication tool for public health professionals, medical doctors and the general public [5].

Standard control measures to be applied in the foci of hepatitis A infection [6] were implemented - hospitalisation and treatment, contact tracing, medical supervision and disinfection. To prevent further spread of infection, control measures were implemented also in the food facilities, in the kindergarten, and in the primary and secondary school. Furthermore, water tanks as source of drinking water were provided.

Post-exposure and preventive vaccination was also administered. On 6 September, the day after two hepatitis A cases were reported in children attending the local kindergarten, mass vaccination started including family members of cases and children below 15 years of age attending the kindergarten and the elementary school in the village. As the next step, vaccine was provided to all young people up to 18 years of age and health professionals. In the end, the inhabitants of the neighbouring village of Podolinec were vaccinated. As of 30 November 2008, 1,814 children (almost 80% of all children below 15 years of age) and 742 adults have been vaccinated in the district.

Information on hepatitis A was disseminated via newspapers, radio and television. Educational leaflets on hepatitis A were distributed in public places (kindergarten, primary and secondary school, hospital, outpatient clinic, post office). Information was also available at the websites of the PHA SR and the RPHA of Stará Ľubovňa.

Conclusion

The outbreak of hepatitis A described in this paper was limited to one village. Since the end of November no further cases have been reported from the area. Considering the incubation period (max. 50 days), this shows that the control measures have proven effective and the outbreak has been contained. Nevertheless, this example shows that outbreaks of hepatitis A can affect relatively large number of people, particularly in susceptible populations.

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Rapid communications

CLUSTER OF CASES OF HEPATITIS A WITH A TRAVEL HISTORY TO EGYPT, SEPTEMBER-NOVEMBER 2008, FRANCE

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Since September 2008, 26 cases of hepatitis A with a history of travel to Egypt have been reported in France. Investigations indicate that a common source of contamination linked to Nile river cruises is the most likely explanation of the increase in the number of cases reported in France as well as in several other European Union countries.

Introduction

In France, hepatitis A is a mandatorily reportable disease defined by the presence of immunoglobulin M antibodies to hepatitis A virus (IgM anti-HAV) in the serum. From 1 September to 2 October 2008, 11 cases of hepatitis A with a travel history to Egypt (within two to six weeks prior to symptom onset) were notified by eight district health departments. This number was higher than observed in previous years: in 2006 five cases and in 2007 two cases with a history of travel to Egypt were notified for the period September to October.

An investigation was undertaken to identify the source of infection and implement appropriate measures.

Methods

A case was defined as any person with IgM anti-HAV who had stayed in Egypt between 2 to 6 weeks prior to symptom onset. All cases notified since 1 September 2008 were interviewed by telephone using a standardised questionnaire. Data were collected on age, sex, date of symptom onset, symptoms (jaundice, asthenia, anorexia, vomiting, fever), date of jaundice onset, date of IgM anti-HAV test result, hospitalisation, dates of travel to Egypt, type of travel (with a group or individual), description of the travel (Nile cruise, name of the ship, stay in hotel, name of the hotel), food consumption (raw vegetables, unpeeled fresh fruits, fresh fruit juices, ice creams, seafood, unbottled water, beverages with ice cubes) and vaccination against hepatitis A.

Sera from 11 hepatitis A cases who had travelled to Egypt and had positive results of IgM anti-HAV detected between 13 September and 23 October 2008 were analysed at the National Reference Centre for HAV. Phylogenetic analysis of HAV sequences was performed as described elsewhere [1]. For comparison, eight sequences from patients infected by HAV genotype IB who had travelled to countries other than Egypt were included in the analysis. We also included two strains involved in Belgian HAV cases with a travel history to Egypt in 2008 [2].

Results

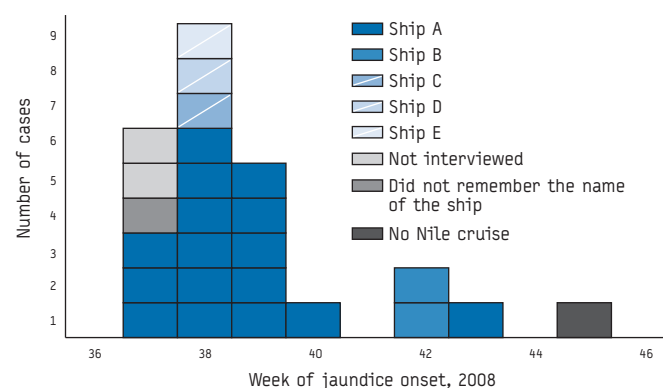
As of 9 January 2009, 26 cases were notified and 24 were interviewed. Among the 26 cases, 13 (50%) were men, and the age of the cases ranged from 10 to 65 years (mean age 32.8 years). Of the 26 patients, 25 (96%) had jaundice (Figure 1) and 17 (65%) were hospitalised. None died. None had been vaccinated against hepatitis A.

Of the 24 patients interviewed, 20 had travelled to Egypt in August, two in September and two in October. The length of their stay ranged from one to two weeks; 23 had participated in an organised tour to Egypt.

All cases except one had gone on a cruise on the river Nile and 15 had stayed in a hotel. Among the 24 cases, nine participated in a cruise only, 14 had gone on a cruise and stayed in a hotel and one had stayed in a hotel only. In 12 out of the 14 cases who stayed both on a ship and in a hotel, the stay at the hotel occurred after the cruise on the Nile.

FIGURE 1

Distribution of cases of hepatitis A with a history of travel in Egypt, by week of jaundice onset, September-November 2008, France (n=25)



Among the 23 cases who had gone on a cruise, 16 (70%) sailed on ship A, two on ship B, three on three different ships (C,D,E) and two did not remember the name of the ship (Figure 1).

Among the 16 cases who sailed on ship A, 11 (69%) travelled from 9 to 16 August and five from 16 to 24 August. The dates of the cruise on ship B were the same for both cases (6 to 13 September). The port of departure and arrival was Luxor for both ships.

Among the nine cases who participated in a cruise only, seven (78%) named ship A, one ship B and one did not remember the name of the ship.

Among the 15 cases with a stay in a hotel, 10 stayed in Hurghada (nine in the same hotel). The remaining five cases stayed in five different hotels in two different towns, Marsa Alam and Cairo. Twelve cases stayed in these hotels in August (11-30), one in September and two in October.

Cases reported consumption of the following food items during their stay in Egypt: raw vegetables (12/23, 52%), unpeeled fresh fruits (12/24, 50%), fresh fruit juices (8/24, 33%), seafood (1/24, 44%). None had ice cream, three (3/23, 13%) drank unbottled water and five (5/21, 24%) had beverages with ice cubes.

Molecular analysis of sera from 11 cases showed they had been infected by HAV genotype IB. A cluster of 10 sequences was

identified in the phylogenetic tree (strain 1) (Figure 2). These 10 sequences were identical over the 440 base-pair fragment analyzed. This cluster also contained the Belgian sequence 2008-Egypt-BEL-1 involved in HAV cases who also travelled to Egypt. All 10 patients have made a cruise on the Nile, though in different ships (six on ship A, one on ship B, one on ship C, one on ship D, one on ship E). The eleventh sequence was from a 12-year-old patient and differed from strain 1 by 7 nucleotides (strain 2) and was also different from 2008-Egypt-BEL-2, the second strain identified by our Belgian colleagues in patients returning from Egypt. IB strains from patients who had travelled in West Africa, South America or others countries in Northern Africa (n=8), were different from strain 1 and strain 2.

Discussion

In October 2008, an increase in cases of hepatitis A who had travelled in Egypt was observed compared to 2006 and 2007 surveillance data. The majority of cases had travelled to Egypt in August 2008 and had gone on a cruise on the Nile river. Among these, more than half had sailed on the same ship (ship A) during two different periods in August. Moreover, three quarters of the cases who only participated in a cruise during their stay in Egypt had travelled on ship A.

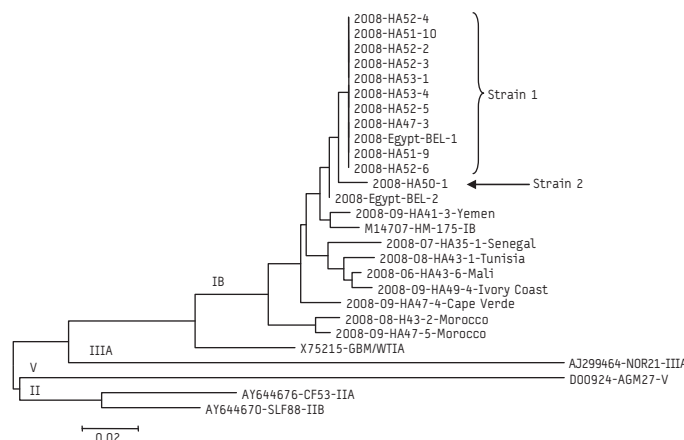
On 15 October 2008 France issued a message via the Early Warning and Response System (EWRS). Several European Union (EU) countries (Belgium, Germany, Ireland, Poland) reported single or clustered hepatitis A cases after cruises on the Nile river. None of the cases in these countries named the ships involved in the French investigation.

The excess of French hepatitis A cases may be explained by an exposure on ship A. However, the occurrence of cases who had travelled on others ships suggests that exposure to HAV infection was not limited to ship A, and a common source of contamination cannot be excluded (e.g. supplies to the ships, common stop-over). The genetic relatedness of the HAV sequence for all cases who had sailed on a cruise ship, regardless of the ship, supports this latter hypothesis. Several sources of contamination should be considered: consumption of foods or drinks on board of the ships or during stop-over contaminated by different food handlers excreting the virus, contaminated common supplies for the ships, baths in the ship swimming pool or in the Nile river.

Investigation limited to interview information was transmitted to the Egyptian health authorities. Hepatitis A is endemic in Egypt but we are not aware of an increase in the number of hepatitis A cases which could have contributed to an increase of contamination of food or water. The fact that other EU countries reported cases of hepatitis A among travellers who had been on cruises on the Nile river indicates that transmission of hepatitis A on board of ships involved in such cruises may be a relatively widespread problem. It is noteworthy that the main epidemic strains involved in French and Belgian cases are closely related. It is also likely that national surveillance systems in the EU have missed cases related to this exposure.

The French vaccination guidelines recommend hepatitis A vaccination for persons travelling in endemic area such as Egypt. This recommendation is often not followed; in 2007, 40% of hepatitis A cases reported in the surveillance system were imported [3]. Due to this outbreak, information on hepatitis A vaccination

FIGURE 2
Phylogenetic analysis of hepatitis A virus (HAV) strains from patients who had travelled to Egypt, September-November 2008, based on the sequences of the VP1-2PA junction of the HAV genome



Genotype and subtype are indicated for each branch. Reference sequences from GenBank are: genotype IA strain (X75215, GBM/WT), genotype IB strain (M14707, HM-175), genotype IIB (AY644670-SLF88), genotype IIIA (AY644676, CF53), genotype IIIA (AJ299464, NOR21), and genotype V (D00924, AGM27). IB sequences from eight patients who had travelled to countries other than Egypt are: 2008-09-HA41-3-Yemen; 2008-07-HA35-1-Senegal; 2008-08-HA43-1-Tunisia; 2008-06-HA43-6-Mali; 2008-09-HA49-4-Ivory Coast; 2008-09-HA47-4-Cape Verde; 2008-08-HA3-2-Morocco; 2008-09-HA47-5-Morocco. The outbreak strains found in Belgium are: 2008-Egypt-BEL-1; 2008-Egypt-BEL-2. A cluster of 10 sequences from the French outbreak is identified as "strain 1", and included the Belgian sequence 2008-Egypt-BEL-1.

was reinforced in public vaccination centres for travellers and in travel companies in France.

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Rapid communications

CLUSTER OF HEPATITIS A CASES AMONG TRAVELLERS RETURNING FROM EGYPT, BELGIUM, SEPTEMBER THROUGH NOVEMBER 2008

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Following a European alert by France, we detected a hepatitis A cluster in Belgian travellers returning from Egypt. Our investigation supports the hypothesis of a common source outbreak, linked to Nile river cruises. The outbreak also suggests the need to consider an intensification of the vaccination policy for travellers to hepatitis A endemic countries.

Introduction

Following the French notification in the Early Warning and Response System (EWRS) on 15 October 2008 about an increase of hepatitis A cases in travellers returning from Egypt and possibly related to cruises on the Nile, the Belgian health authorities were alerted in order to verify whether a similar increase had been observed in Belgium. The infectious disease control units, which are organised by region in Belgium and to whom mandatory notification of each hepatitis A case has to be sent by both physicians and clinical laboratories, analysed their data. At the same time their teams at the level of the provinces were asked to actively investigate each notified case for a potential link to travel in Egypt.

Hepatitis A virus (HAV), a single stranded RNA virus, is mainly transmitted by the faecal-oral route, either by person-to-person contact or by ingestion of contaminated food or water [1]. Only 2-3% of reported cases are identified as part of recognised foodborne outbreaks, though a considerable percentage of sporadic cases might actually be foodborne [2,3]. A large outbreak of hepatitis A among travellers to Egypt in 2004 has been described to be associated with the consumption of orange juice [4]. Hepatitis A is endemic in Egypt and import of hepatitis A from endemic countries is common in Belgium, as it was the case in at least 14% of the hepatitis A notifications in Flanders in 2008. Over the last twenty years the prevalence of hepatitis A in Belgium shifted from intermediate to low, which makes the population more prone to clusters or outbreaks. International and Belgian travel medicine guidelines recommend hepatitis A vaccination of travellers to hepatitis A endemic countries [1].

Methods

A confirmed case was defined as a clinically compatible case with IgM hepatitis A serology, with disease onset between 1 September and 30 November 2008 and with a history of travel to Egypt between 2 to 6 weeks prior to symptom onset.

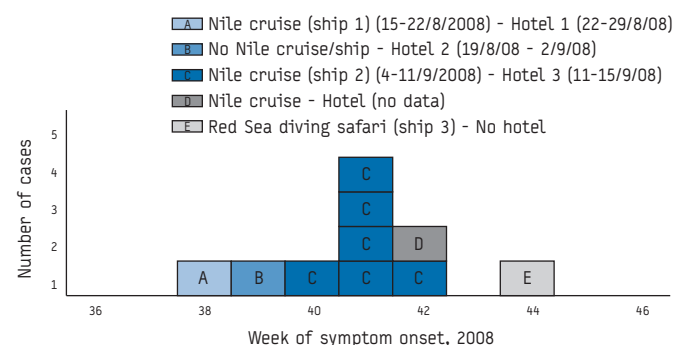
A limited epidemiological investigation was performed in order to verify a possible link between the Belgian cases and the possible sources (cruise ships), mentioned by name in the French alert. Data collection was done by telephone interview with the cases and their physicians. We collected information about age, sex, diagnostics, vaccination against hepatitis A, date of symptom onset, dates of travel and places of stay such as hotels and ships.

A virological analysis has been performed by the National Center of Viral Hepatitis (Scientific Institute for Public Health, Brussels). The HAV outbreak strain was characterised by sequencing a 350bp region, within the variable VP1/2PA junction of the HAV genome [5].

Results

At the time of the European alert, two cases of hepatitis A, suspected to be related to recent travel to Egypt, had been notified since 1 September 2008. By 30 November 2008, a total of 10 laboratory-confirmed cases of hepatitis A infection, with disease onset since 1 September and a history of recent travel to Egypt, had been registered (Figure 1). The median age of the cases was 41 years (range 23-59 years) and the male/female ratio was 3/7.

FIGURE 1
Distribution of cases of hepatitis A with travel in Egypt, by week of symptom onset, September-November 2008, Belgium (n=10)



One patient required hospitalisation but none died. None of the cases had been vaccinated against hepatitis A. They were living in four different provinces.

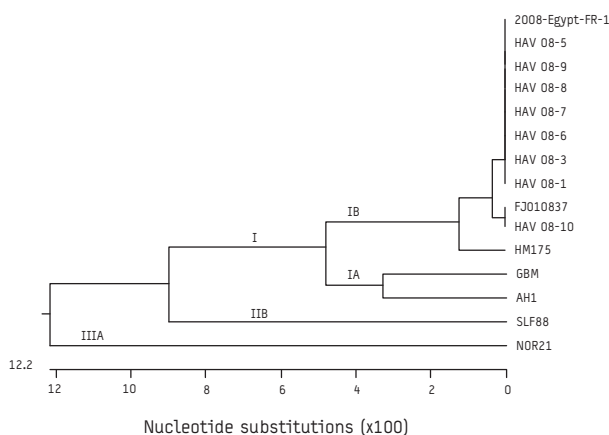
Eight cases had been travelling on a Nile cruise and one on a Red Sea diving safari. Those who took a Nile cruise had done this in combination with a hotel stay. At least three different ships and three different hotel accommodations were mentioned in the travel histories of the cases. However, none of these ships or hotels had been mentioned in the French alert and a bilateral contact by email with the French authorities did not reveal any common name.

One cruise ship was mentioned by six of the 10 Belgian cases. Although they had been travelling together, only two of them (friends who had been lodged in the same cabin on the ship) were aware of their common infection.

Virological analysis was performed on eight (not case D and E on figure 1) out of the ten confirmed cases. Phylogenetic analysis revealed that the HAV strains belonged to genotype IB, and were closely related to the Egyptian isolate (FJ010837). Among the eight patients, seven carried the same strain and the other one differed in only two nucleotides. The outbreak strain identified in France among ten patients who had travelled to Egypt (2008-Egypt-FR-1) showed 100% homology with the seven HAV sequences (HAV 08-1, HAV08-3, HAV08-5, HAV08-6, HAV08-7, HAV08-8, HAV08-9) (Figure 2).

FIGURE 2

Phylogenetic tree of the outbreak strains (HAV 08-1, HAV08-3, HAV08-5, HAV08-6, HAV08-7, HAV08-8, HAV08-9, HAV08-10) circulating in the Belgian travellers returning from Egypt, based on the 350bp region within the VP1-2PA junction of the HAV genome



The phylogenetic tree was generated using the MegAlign program (DNASTAR) by the CLUSTAL method, and the branch length represents the evolutionary distance expressed as the number of base substitutions per site. Genotype and subtype are indicated for each branch. Sequences from Genbank included: genotype IA strain (AH1, AB020564), genotype IA strain (GBM, X75214), genotype IB strain (HM175, M59808), genotype IIB (SLF88, AY644670), genotype IIIA (NOR21, M66695), and an Egyptian isolate strain (FJ010837). Among the eight patients, seven carried the same strain and the last one differed in only two nucleotides. The outbreak strain identified in France among ten patients who had travelled to Egypt (2008-Egypt-FR-1) was included in the analysis.

Discussion

A cluster of hepatitis A cases, related to travel in Egypt, has been identified in a group of Belgian travellers. Only active inquiry, prompted by the French EWRS alert, led to the identification of this cluster, indicating that similar hepatitis A clusters may often go undetected. As our findings come together with similar reports from France and Germany, a possible common source in Egypt, though not identified, cannot be discarded. The similarity of virological sequence analysis between cases in France and Belgium supports this hypothesis. Though travel medicine guidelines recommend hepatitis A vaccination of travellers to hepatitis A endemic countries, all of the identified cases were unvaccinated. In this perspective, and taking into account the increasing susceptibility of our population to hepatitis A, an intensification of the hepatitis A vaccination policy should be considered.

Acknowledgements

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Rapid communications

CLUSTER OF HEPATITIS A CASES AMONG TRAVELLERS RETURNING FROM EGYPT, GERMANY, SEPTEMBER THROUGH NOVEMBER 2008

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From September to November 2008, 34 cases of hepatitis A imported from Egypt were reported to the German public health authorities. Investigations point to a continuing common source of infection, most likely linked to Nile river cruises. The patients affected had not been vaccinated, which emphasises the need for more effective travel advice before trips to hepatitis A endemic countries.

Introduction

On 15 October 2008, the French Institute for Public Health Surveillance (Institut de veille sanitaire, InVS) issued a warning to the European Union member states via the Early Warning and Response System (EWRS) about a detected increase of hepatitis A cases with date of onset from 8 September onwards, and a history of travel to Egypt in August 2008. All cases had been on Nile river cruises, more than half on the same cruise ship. An association of hepatitis A infection with a Nile river cruise was therefore suspected. In following weeks, Ireland, Belgium and Poland also reported single cases or clusters of hepatitis A infections with history of Nile river cruises but on other ships than the majority of the French cases.

Hepatitis A (typical symptoms plus laboratory confirmation of acute infection) is mandatorily notifiable in Germany. At the time the warning was issued, a total of 10 cases of hepatitis A with date of onset from 1 September 2008 had been notified to German health authorities after travelling to Egypt. And further cases kept coming in. This compared to a mean of three cases (range 2-4) during the same time period in the previous three years.

Methods

The observed increase in case numbers prompted an investigation of all hepatitis A cases with date of onset from 1 September 2008 and travel to Egypt 15-50 days prior to symptom onset (case definition). In an email we asked local and state health authorities to obtain information on travel itineraries from the patients, including names of hotels and Nile cruise ships and dates of stay(s). This information was to be reported to the Robert Koch Institute, the German national public health institute.

Results

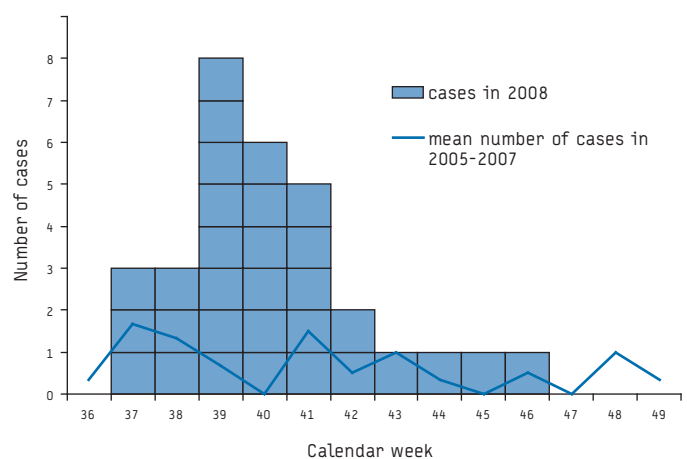
By 2 December, 2008, a total of 34 laboratory-confirmed symptomatic infections meeting the case definition were notified to

the German public health authorities (Figure 1). For three cases the exact date of onset was unknown but was later than 1 September. They are therefore not shown in figure 1. Weekly case numbers exceeded the mean case numbers notified in the three preceding years.

The mean age of cases was 40.1 years (range 11-69), and 20 (59%) were female. No case had been vaccinated against hepatitis A, 20 (59%) required hospitalisation, nobody died. Cases were from all across Germany.

Of the 34 cases, only four (14%) had not gone on a Nile cruise. For the others, the following information is indicated in Figure 2, if available: cruise ships, hotels, dates of travel and of symptom onset. Periods of stay on individual ships appear grouped in time. None had been on the ships implicated by French cases or by cases from other member states. In addition to cruise ships, many cases had also stayed in hotels in Hurghada, but named hotels varied much more than cruise ships.

FIGURE 1
Hepatitis A cases imported from Egypt to Germany, week 36-49 of 2008 (n=31)



Data as of 2 December 2008

Discussion

In summary, from September to November 2008 there was an increase in hepatitis A cases imported from Egypt to Germany. Whereas cases stayed in a plethora of hotels, some Nile cruise ships were named repeatedly, and cases' travel on them appeared to cluster in time.

To explain the excess of cases in Germany and elsewhere, a group of ships must have facilitated hepatitis A infections in tourists. The epidemic curve suggests a continuing common source rather than a point common source. Possible sources of infection might be contaminated food consumed onboard obtained from a common food catering company, contaminated tap water supplies for the ships' bunkers, or a common exposure on shore (e.g. a restaurant where tourist groups from various ships are being taken during day trips). As all of these ships continuously travel up and down a short stretch of the river (Aswan to Luxor and back) with standard must-see stops along the way, the cases possibly shared an exposure on land, which only intense additional study could reveal.

Both the long incubation period of hepatitis A (15-50 days) and long delays in collecting information on the individual cases precluded any rapid intervention on location.

International travel medicine guidelines and the German standing committee on vaccinations (STIKO) recommend hepatitis A vaccination for persons travelling to countries in which hepatitis A is endemic such as Egypt. Some health insurance companies reimburse the cost of the vaccination. In 2004 a large outbreak of hepatitis A among European tourists centred on a hotel in Hurghada [1], demonstrated that also package tourists ought to follow vaccination advice. Since then travel companies in Germany have become more active in recommending hepatitis A vaccinations to travellers to Egypt. Despite this, all cases described here were unvaccinated, emphasising the need for more effective travel advice before trips to hepatitis A endemic countries.

Acknowledgements

The authors thank the colleagues at the local and the state health authorities for collecting all information on the cases.

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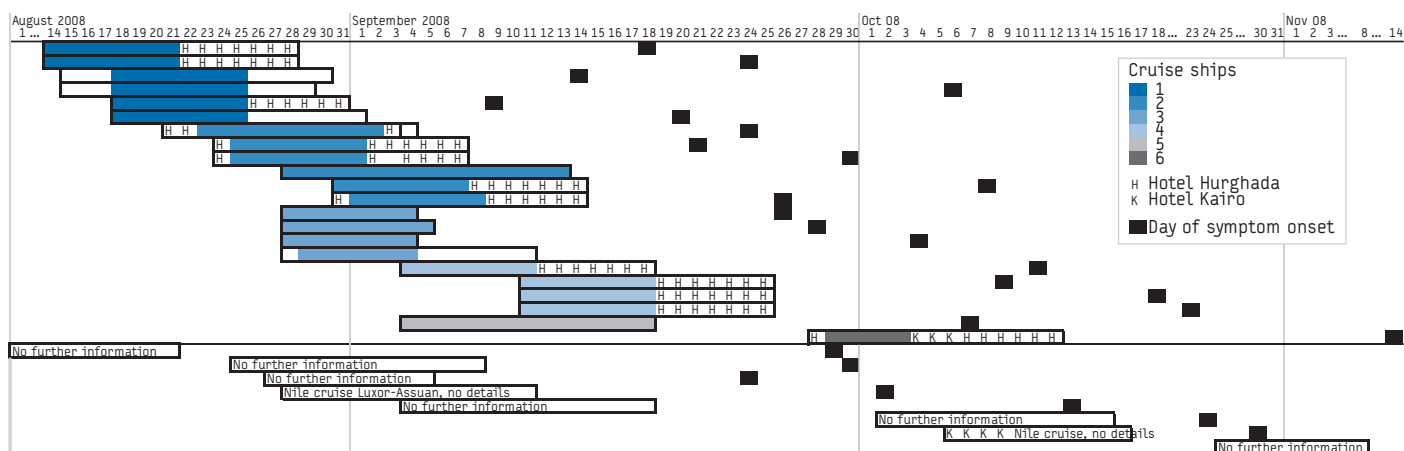
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FIGURE 2

Hepatitis A cases post travel to Egypt, Germany, September-November 2008 (n=30 cases with known date of travel)



Data as of 2 December 2008
Each line represents one case
Coloured boxes represent stays on Nile cruise ships and in hotels

A MEASLES OUTBREAK IN CROATIA, 2008

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We report an outbreak of measles in Croatia, involving 49 cases with onset of symptoms between end of April and June 2008. Cases occurred in Zagreb and Slavonski Brod but investigations indicated a common epidemiological link between these two geographically separate regions.

Introduction

Following an almost four-year period with zero indigenous measles cases notified in Croatia the disease reappeared during the second quarter of 2008. With the exception of an import-related outbreak in winter 2003-4, less than 10 measles cases have been reported annually since 2000, although none of them had been laboratory confirmed. Measles is a statutorily notifiable disease in Croatia since 1949.

In Croatia, all vaccinations offered to children within the universal immunisation schedule are mandatory and free of charge. Measles vaccination was first introduced in the national childhood vaccination schedule in 1968 at 12 months of age and was replaced by the combined measles, mumps and rubella vaccine (MMR) in 1976. A second dose was given at seven years of age as monovalent measles vaccine since 1968, and was replaced by MMR in 1994. During the period 1997-99, the second dose was recommended at 12 years of age. Vaccine coverage rates for the first MMR dose at two years of age in Croatia are estimated to have been above 90% since the mid-1980s, and more than 95% since 2004. Vaccination coverage estimates are done by administrative method based on data submitted annually by immunisation providers and verified by competent epidemiologists.

Sequence of events

The first notified case was a 27-year-old man who fell ill on 26 April 2008. He was hospitalized initially as a case of staphylococcal sepsis based on clinical suspicion, and identified as measles case only after his brother was hospitalized ten days later as a case of measles. Both patients are from the greater Zagreb area. Until 15 May, further five cases were notified from the same municipality as the index case, and included three community acquired cases and two visitors to the hospital where the index case was admitted (Figure 1). In the following weeks, the outbreak spread further in other municipalities of Zagreb area (community and nosocomial transmission), reaching a total of 29 cases (37 suspected), the last one with onset of symptoms on 20 June 2008.

In May, a measles outbreak was also reported in the province of Slavonski Brod, affecting 20 persons (32 suspected cases) of a

migrant Roma community (Figure 1). Members of this community had recently returned from abroad, most of them from Italy. Mistakenly, the Slavonski Brod outbreak was initially believed to be due to erythema infectiosum. The last case in Slavonski Brod fell ill on 30 May.

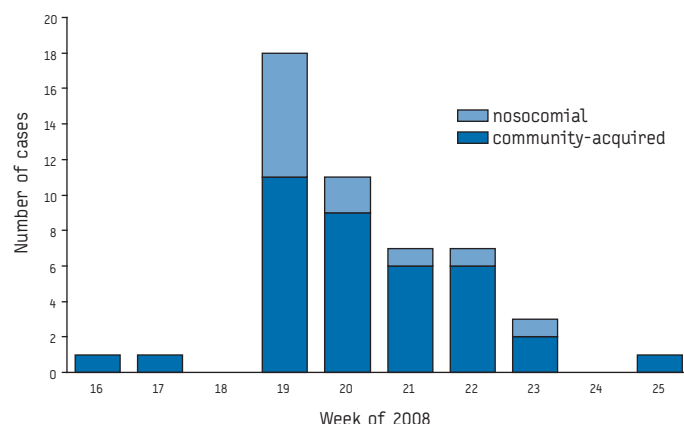
An additional case of measles was notified in a German tourist who stayed in Split and fell ill on 21 May, seven days after arrival to Croatia. However, there were no cases reported in connection with this case, so it was excluded from the analysis of the outbreak.

Laboratory and epidemiological investigations

Since the outbreak was first identified, 69 suspected cases of measles were notified. Of these, 40 (58%) were laboratory-confirmed using ELISA techniques on serum samples and/or polymerase-chain-reaction (PCR) analysis. Further four cases were epidemiologically linked and five were classified as clinical cases. The remaining 20 cases (35%) were discarded and excluded from further analysis.

Case classification was based on the European Commission case definition of measles [1]. In our classification a laboratory confirmed case corresponded to a confirmed case in the EC definition, an epidemiologically linked case corresponded to a probable case in the EC definition, and a clinical case corresponded to a possible case in the EC definition for which no laboratory information is

FIGURE 1
Cases reported in an outbreak of measles in Croatia, in 2008, by the week of onset of symptoms (n=49)



available. Discarded cases were clinical cases (possible cases in the EC definition) with negative serology and/or negative detection of measles virus/antigen from a clinical specimen.

Genotype D4 was identified in six PCR-positive cases all from cases reported in Zagreb area. There was no sequence diversity (unpublished data). Comparison of the isolated genotype with strains circulating in Europe is underway.

This outbreak was limited to greater Zagreb area (n=29, 59%) and the Slavonski Brod province (n=20, 41%). The mean age of all cases was 21 years (range 7 months - 50 years). The mean age of the cases from Zagreb was 27 years and that of the cases from the Slavonski Brod province was 12 years (Figure 2).

The vaccination status of cases was determined by interview and review of personal medical records. Overall, 32 cases had not been vaccinated against measles, three had received only one measles-containing vaccine (MCV), two cases had been vaccinated with two MCV doses and two had been vaccinated but the number of MCV doses was unspecified. In 10 cases the vaccination status was unknown. Of those with a known vaccination status there was a significant difference in the number of unvaccinated cases in the two affected areas: 16 out of 23 (70%) in Zagreb and all 16 (100%) in Slavonski Brod ($p < 0.05$)

Of the 49 cases included in the outbreak, 11 (22%) were hospitalised and five (10%) had an unknown hospitalisation status. In two cases complications with pneumonia occurred. Nosocomial transmission was reported in 12 (24%) cases, all from Zagreb area.

Further investigations

Upon further enquiry to identify a possible source of infection and reveal further contacts, it transpired that a Roma girl developed a rash whilst visiting the family of the 27-year-old man in Zagreb whom we had identified as the outbreak index case. The paediatrician who suspected rubella referred the child for laboratory testing to the University Hospital for the Infectious Disease in Zagreb. However, the child never attended the clinic, because she and her mother left Croatia. They were both Italian citizens who were visiting Serbia and Croatia on their way to Germany.

It was later revealed that this child and her mother had travelled from Italy to visit family members in the Slavonski Brod province before they visited the family in Zagreb. This led to the hypothesis that this child with an undiagnosed rash was the source of infection in both geographically separate areas of Croatia.

Control measures

After notification of this first generation of cases, a circular letter was sent to all health-care institutions notifying them of the outbreak and providing guidelines on reporting and investigating suspected measles cases.

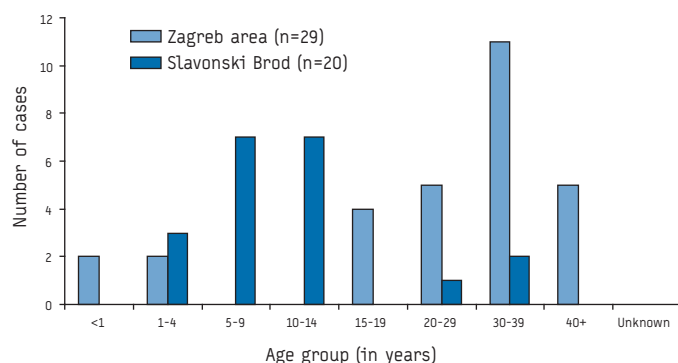
To control the outbreak, the Department of Infectious Disease Epidemiology performed contact tracing, vaccinated susceptible contacts and recommended voluntary quarantine of susceptible contacts to those who could not be vaccinated due to contraindications to vaccination or because it was too late to vaccinate them because their exposure had occurred more than 72 hours before. Paediatricians were instructed to invite parents to vaccinate all previously unvaccinated children above 12 months of age. Recommendations for vaccination were also issued to healthcare workers without evidence of immunity. It is not known, however, how many people were vaccinated as a result of these outbreak control measures.

Discussion

With the outbreak described in this paper, Croatia joins some other European countries that have recently experienced a resurgence of measles [2]. The presence of pools of individuals in the general population and amongst members of the Roma community susceptible to measles infection still exists in Croatia and is brought to light when the measles virus is imported from abroad. A serological survey carried out on samples collected in 1999-2000 showed a high susceptibility to acquire measles in those aged 16-40 years [3]. This conclusion is compatible with our findings, as the proportion of cases in this age group (particularly in Zagreb) was high. It is believed that the vaccination coverage amongst members of the Roma community living in Croatia, i.e. non-migrating Roma, does not differ largely from the coverage of the rest of the population, since they are well integrated into the primary health care system, which provides immunisation. However, migratory members of the Roma community, who spend a substantial time abroad do not benefit from the immunisation system, although the services are free of charge and available to everyone regardless of insurance status and citizenship. There is, therefore, a need to improve vaccination coverage using innovative ways in such groups that are hard to reach by normal vaccination programmes. In doing so, the herd immunity would be maintained at level conducive of measles elimination from Croatia.

The outbreak clearly demonstrates the role of nosocomial transmission in the spread of infection. Nosocomial transmission of measles virus has also recently been described elsewhere [4,5]. In the import-related outbreak in winter 2003-4, nosocomial transmission had also played a significant role (unpublished data). This shows the importance for health-care workers to be fully vaccinated against measles if they have no history of the disease. This investigation also demonstrates the potential of measles to be misdiagnosed as other infectious diseases presenting with a rash and fever. It is also evident that at least in some healthcare settings, health personnel's awareness of measles should be increased, in order to suspect measles in a timely manner. This also stresses the

FIGURE 2
Number of reported measles cases by area of notification and age group, Croatia, 2008 (n=49)



importance of laboratory testing of suspected measles cases and to identify the circulating measles virus genotype.

As part of the measles and rubella elimination plan by 2010 from the WHO European Region [6], the Croatian Ministry of Health has on several occasions sent circular letters to all healthcare workers urging all suspected cases of measles and rubella to be notified immediately, emphasizing the need for enhanced surveillance, and providing instructions on sampling and transportation of specimens to the national measles and rubella reference laboratories affiliated to the National Institute of Public Health.

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Rapid communications

MUMPS OUTBREAK ON THE ISLAND OF ANGLESEY, NORTH WALES, DECEMBER 2008-JANUARY 2009

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Twenty-three cases of clinical mumps in young people have been reported in North Wales over a five-week period since late December 2008. All cases have social links, and most of them have received two doses of mumps-containing vaccine.

Since 27 December 2008, the North Wales Health Protection Team of the National Public Health Service for Wales has been notified of 23 cases of clinical mumps. The onset dates are shown in the Figure. The age range was 9-37 years with a median of 15-16 years, and similar numbers of males and females were affected. The cases are all linked via family or social groups.

Epidemiological investigation

The first case reported on 27 December was a student in Manchester where, as confirmed by the Health Protection Unit, a number of mumps cases have occurred among students in recent weeks. They received an increase in notifications in the first week of December 2008 which peaked in the second week of December, and it is plausible that the student was infected at this time. Transmission from this case probably occurred at a Young Farmers party held on Anglesey on the 27 December 2008. Members of two local Young Farmers groups were invited, comprising around 50 young people aged 13 to 27 years.

An unusual feature of this outbreak is that 20 of the cases had received two doses of the measles, mumps, rubella (MMR) vaccine and two cases had had one dose. The only unvaccinated case was a 37 year-old patient who was too old to have been offered MMR as a child. Most cases appear to be mild, with no reports to date of orchitis or other complications. MMR vaccine was introduced into the childhood vaccination programme of the United Kingdom (UK) in 1988. The mumps strain currently used in the MMR vaccine is

Jeryl Lynn. However, some of the older cases (over 17 years-old) in this outbreak will have received MMR vaccine containing the Urabe strain which was used in the UK from 1988 to 1992.

Laboratory investigation

One case was admitted to the district general hospital where blood for serology was taken. This was reported as negative for IgM against mumps virus, but positive for IgG with no evidence of recent infection. This sample was taken the day after onset of symptoms, and would have been too early to capture an IgM response. A convalescent sample has been requested.

Another case, who is a healthcare worker, had paired sera taken by the Occupational Health Department, which showed a rising titre of mumps antibodies and was therefore confirmed as recent mumps infection by the regional virology laboratory.

Salivary swab samples of the remaining 21 clinical cases have been submitted to the reference laboratory at the Health Protection Agency Centre for Infections (CfI) at Colindale. The results for three cases have been received to date and are as follows:

Case 1.

The samples were IgM-negative and IgG-positive (very high titre), possibly indicating infection. A repeat salivary antibody test has been requested to ascertain whether IgM titres have subsequently risen.

Case 2.

The samples were IgM-negative and IgG-positive, although the titre was not particularly high. This is consistent with past immunisation, but does not allow confirmation of recent mumps infection. The patient's general practitioner notes that it was a very mild case of mumps.

Case 3.

The samples tested IgM-positive and IgG-positive (very high titre). Recent mumps infection is confirmed.

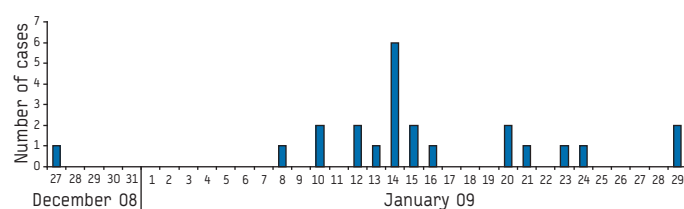
Two recent cases have been swabbed within 48 hours of onset, and their samples will be tested by PCR at the CfI reference laboratory.

Discussion and conclusion

Salivary swabs are usually submitted two weeks after notification of clinical mumps. In this outbreak blood samples have been taken in individual cases because of special circumstances. The

FIGURE

Epidemic curve, mumps cluster on Anglesey, North Wales, December 2008 – January 2009 (n=23)



laboratory results to date indicate that this is a genuine outbreak of mumps, although the timing of some of the samples may not have been optimal for capturing the antibody response.

This outbreak is different from the one described in Austria in 2008, where 49.1% of the young people affected had not been vaccinated [1]. However, in the Netherlands, a number of fully vaccinated individuals were affected as part of an outbreak in a predominantly unvaccinated community in 2008 [2].

Uptake of MMR vaccination has historically been high in Anglesey, and the majority of cases in the outbreak had received two doses. The lack of cases among unvaccinated individuals may reflect the high uptake of vaccine, and an investigation is ongoing to determine coverage rates for the birth cohorts involved. Current isolates from mumps cases in the UK have been identified as genotype G. Further tests are required in order to confirm that this is also the genotype for this outbreak.

The mumps component of the MMR vaccine does not provide the same high levels of protection as the measles and rubella components. One dose protects around 65% (62%-85%) of those who receive it [3]. A second dose raises the effectiveness to around 85%. This still leaves one in six recipients of two MMR doses vulnerable to mumps. This primary vaccine failure may be the explanation for this outbreak, but the contribution of waning immunity, secondary vaccine failure, must also be considered.

Infection control measures

Letters have been sent to the school many of the cases attend, advising that all children should ensure that they have received two doses of MMR vaccine. Letters have also been sent to general practitioners in the area alerting them to the fact that cases of mumps are occurring despite complete vaccination status, and preparing them for requests for MMR vaccination.

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MUMPS OUTBREAK AMONG THE MILITARY IN LUXEMBOURG IN 2008: EPIDEMIOLOGY AND EVALUATION OF CONTROL MEASURES

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In the last quarter of 2008, an outbreak of mumps occurred in Luxembourg affecting initially 10 young adults at a military centre. Following a mass vaccination campaign, no further clinical cases were observed. 90% of 136 vaccine recipients were IgG positive one month after vaccination compared to 54% before vaccination. Until 31 December 2008, 19 mumps cases were also reported from the community. The outbreak strain belonged to genogroup G.

Introduction

During the last three months of 2008, an outbreak of mumps occurred in Luxembourg with 29 suspected clinical cases reported until 31 December 2008. Prior to this outbreak, the last time a mumps case was reported to the health authorities was in 2005.

Mumps is an acute viral infection characterised by swelling of the salivary glands and particularly the parotid glands. Asymptomatic cases occur quite frequently (up to 30% of all cases) and symptoms can be flu-like. The most frequently observed complications include inflammation of genital glands (testicles or ovaries), pancreatitis as well as aseptic meningitis. Mumps can be prevented by vaccination which was introduced to the routine schedule in Luxembourg in 1986-7 with trivalent measles, mumps, rubella vaccine (MMR) for children aged 15 to 18 months. A recommendation for a second dose at the age of 5-6 years was released in October 1994.

Following the incidence of 10 cases in different units at a military centre in Luxembourg in September and October 2008, the Military Command, the Army Health Service and the Health Inspection decided to organise a vaccination campaign for personnel in all units working on this particular military site, which also included personnel and trainees of the Luxembourg Police Force. At the same time it was decided together with the National Health Laboratory to conduct a sero-epidemiological survey with the aim to determine seroprevalence against mumps virus in this army population and to study risk factors for being seronegative.

Methods

For the purpose of the outbreak investigation at the military centre, the following case definition criteria stated by the Centers for Disease Control and Prevention (CDC) were used [1]. A clinical case was defined as a patient with acute onset of unilateral or

bilateral tender, self-limited swelling of the parotid or other salivary gland(s), lasting at least two days, and without other apparent cause. Laboratory criteria for diagnosis were isolation of mumps virus from clinical specimen, detection of mumps nucleic acid by real-time PCR, or detection of mumps IgM antibodies.

Following the decision to hold a vaccination campaign, all army and police personnel working onsite were briefed about the cases and the current situation of the mumps epidemic, recommended to participate in the vaccination campaign (on a voluntary basis) and explained the reasons and usefulness of the sero-epidemiological study. The blood sample collection was organised at the Army Health Service onsite in collaboration with the National Health Laboratory upon receipt of written informed consent forms. The samples were immediately transported to the National Health Laboratory where they were prepared and stored for future analysis. A quantitative IgG and IgM assay (Genzyme Virotech, Rüsselsheim, Germany) was used to determine the presence of anti-mumps antibodies according to the manufacturer's instruction. A real-time PCR assay was implemented to detect mumps virus in throat swabs/oral fluid and followed by sequencing of the positive samples [2,3].

Results

The epidemic

Figure 1 shows the evolution of the mumps epidemic in Luxembourg up to the end of the year 2008. Following the vaccination campaign which began on 28 October 2008, no further clinical cases have been observed at the military centre, but several clinical cases were reported in the "civilian" population in Luxembourg.

The age distribution of reported cases shown in Figure 2 reveals that the large majority (23 of 29 or 79%) were aged between 15 and 34 years. Seven of the reported 29 (24%) cases were female.

Of 13 oral or throat swabs taken from suspected clinical mumps cases, six were positive by PCR (out of which five could also be cultured). Nucleotide sequencing showed that the strain belonged to genogroup G which has been observed recently in Bavaria, Germany (May-July 2008), the United States (2006) and the United Kingdom and Ireland (2004-05).

Detailed clinical data are available for the 10 cases reported at the military centre. Eight patients had a classical presentation with parotitis, predominantly right-sided. Of those eight cases, five had never been vaccinated, one had received a single dose and two had received two doses of a MMR vaccine. The two patients with non-specific symptoms and positive IgM test results had received two vaccine doses. Two patients hospitalised with suspected viral meningitis recovered without sequelae.

Sero-epidemiological study at the military centre

225 participants including 26 women (12%) agreed to give a blood sample prior to the vaccine administration by informed written consent. Of these, 134 (60%) had a positive IgG result, 37 (16%) had a borderline IgG result and 54 (24%) had a negative IgG result. The majority, 219 (97%) participants were IgM negative, five (2%) were IgM borderline, and one participant had a positive IgM result.

The IgG seroprevalence rate varied significantly with age – participants born before 1970 had higher seroprevalence (81%) compared to participants born after 1970 (53%, $p=0.006$).

For participants with a documented vaccination history, IgG seroprevalence did not vary significantly as a function of the number of received doses ($p=0.19$).

Of the 225 participants, 136 (60%) gave a second blood sample on average 31 days after administration of the Priorix® vaccine. 123 (90%) were IgG positive, six (4%) were IgG borderline and seven (5%) were IgG negative. Of 37 participants who were initially IgG negative, 24 (65%) became IgG positive, six (16%) were IgG borderline and seven (19%) remained IgG negative one month after vaccination. All 25 participants who were initially IgG borderline became IgG positive and all 74 participants who were initially IgG positive remained positive.

At the second sampling opportunity, four (3%) participants were IgM positive (they were initially IgG and IgM negative), three (2%) were IgM borderline (two had also been initially IgM borderline and one negative), and 129 (95%) participants were IgM negative.

FIGURE 2

Age distribution of reported mumps cases in Luxembourg, 2008 (n=29)

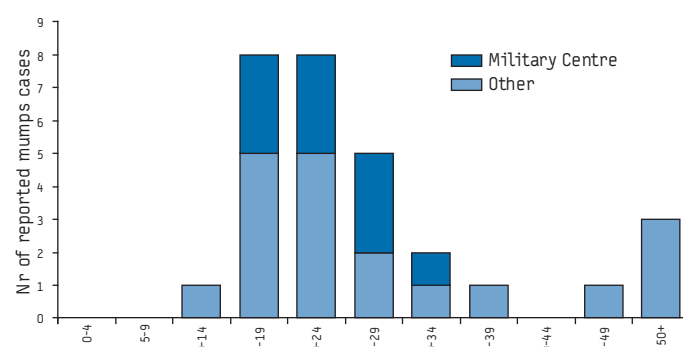


TABLE 1

Sero-epidemiological study of mumps at a military centre in Luxembourg, 2008. IgG results by year of birth (chi2 test, $p=0.006$)

Year of birth	negative	borderline	positive
<1970	6 (11%)	4 (7%)	44 (81%)
1970-83	19 (33%)	8 (14%)	30 (53%)
1984-86	15 (27%)	10 (18%)	31 (55%)
1987-90	14 (24%)	15 (26%)	29 (50%)
Total	54 (24%)	37 (16%)	134 (60%)

TABLE 2

Sero-epidemiological study of mumps at a military centre in Luxembourg, 2008. IgG results by number of measles, mumps, rubella (MMR) vaccine doses received before the onsite vaccination campaign (chi2 test, $p=0.19$)

Number of doses	negative	borderline	positive
0	24 (19%)	19 (15%)	81 (65%)
1	14 (39%)	5 (14%)	17 (47%)
2	14 (25%)	11 (20%)	31 (55%)
3	0 (0%)	1 (50%)	1 (50%)
Total	52 (23%)	36 (16%)	130 (59%)

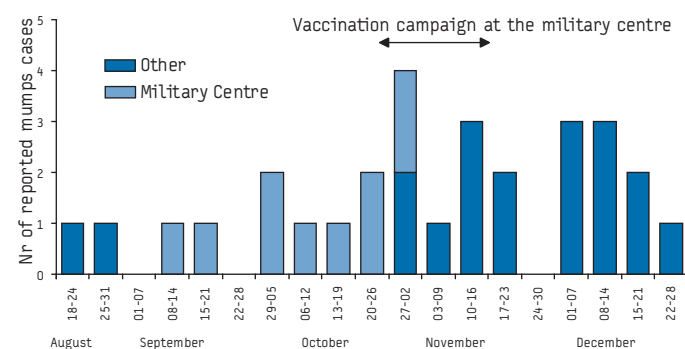
TABLE 3

Sero-epidemiological study of mumps at a military centre in Luxembourg, 2008. Comparison of IgG results before and one month after the vaccination campaign

IgG before vaccination	IgG after vaccination			Total
	negative	borderline	positive	
negative	7	6	24	37
borderline	0	0	25	25
positive	0	0	74	74
Total	7	6	123	136

FIGURE 1

Epidemic curve of reported mumps cases in Luxembourg, 2008 (n=29)



Discussion

Our study reveals that, following several years of absence, mumps virus has re-emerged in Luxembourg in the last term of 2008. This is not surprising as other countries in Europe and North America have also witnessed relatively sizable mumps outbreaks in recent years [4-13].

In our case, most reported cases occurred in young adults. More than half of the staff on the military site was born before the introduction of the combined MMR vaccine in 1986 and the majority of reported clinical mumps cases had never received vaccination. Whereas most persons born before 1970 have naturally acquired immunity, persons born between 1970 and 1985 have had less exposure to mumps virus (due to the reduction of mumps circulation after the MMR vaccine was introduced into the vaccination schedule in 1986) and have never been targeted by a "mop-up" vaccination campaign. Moreover, our data seem to suggest that a sizable fraction of persons born between 1986 and 1990 (49% of participants) have not received the recommended two doses of MMR vaccine. This could be explained by the fact that the official recommendation of a second dose of MMR was only issued in 1994, eight years after the introduction of MMR vaccine.

The vaccination campaign at the military centre appears to have led to a large reduction of viral transmission as no further clinical cases have been observed at the site. Following vaccination, 90% of the study participants were IgG positive compared with 54% before vaccination. Even if the sensitivity of our serological assay is slightly problematic (due to a high proportion of borderline results), a quantitative analysis seems to suggest that « borderline » results can be boosted by vaccination, from a mean of 10 Virotech units to 18 units.

Another interesting aspect of this incident is that the rapid implementation of the vaccination campaign at the military centre was an ideal real-life exercise for the influenza pandemic. Our experience suggests that in the right conditions, a doctor assisted by two technicians (one for the preparation of the vaccine and one for paper work) can administer vaccines to approximately 150 persons in half a day.

Although the sample size in our study is quite limited, our data suggest that a single dose of Priorix® vaccine could be immunogenic (i.e. induced a positive or borderline IgG result) in approximately 80% of previously seronegative adults. While some authors have suggested that waning immunity may contribute to mumps outbreaks in older vaccinated populations [6], the large majority of our cases have no history of vaccination. If waning of immunity was more prevalent, we would also expect the outbreak to spread to younger vaccinated generations (who go to secondary schools where a lot of mixing occurs [14]) and this has not (yet) been observed.

To stop the circulation of mumps virus in the long term in Luxembourg, we suggest that a MMR campaign aimed at all persons* born between 1970 et 1990 who have not received two doses of vaccine or who do not have protective antibody levels would be necessary to protect their health. Such a campaign could also have an additional advantage of increasing population immunity against rubella and measles which have been targeted for elimination by the World Health Organization (WHO) European Region by 2010. In addition, further measures are probably necessary to document and possibly raise levels of two dose coverage with the MMR vaccine in adolescents and children born after 1990.

*Note: While respecting vaccine manufacturer's contraindications, particularly in women: no current or pregnancy planned for 3 months after vaccination.

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A FATAL CASE OF LASSA FEVER IN LONDON, JANUARY 2009

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In January 2009, the eleventh* case of Lassa fever imported to the United Kingdom was diagnosed in London. Risk assessment of 328 healthcare contacts with potential direct exposure to Lassa virus - through contact with the case or exposure to bodily fluids - was undertaken. No contacts were assessed to be at high risk of infection and no secondary clinical cases identified.

Background

Lassa fever is an acute viral haemorrhagic fever (VHF) caused by Lassa virus, a member of the Arenavirus family. It is a zoonosis acquired from the multimammate rat (*Mastomys* species), which sheds the virus in its urine and droppings. The disease is endemic in many West African countries.

Person-to-person transmission of Lassa fever occurs once symptoms have developed or in the period of convalescence, and then only through direct contact with infected bodily fluids such as blood, urine, faeces, saliva or semen. The incubation period for Lassa fever is usually 7-10 days, although a range of 3-21 days has been reported. Approximately 15-20% of people hospitalised with Lassa fever will die, but overall only about 1% of infections result in death [1, 2].

While Lassa fever does not pose a significant public health risk in Europe [3], occasional travel-associated cases do occur. To date, all imported infections to the United Kingdom (UK) - ten cases between 1971 and 2003, with one fatality in 2000 - have derived from either Sierra Leone or Nigeria. None of these have resulted in further clinical cases in health staff or other contacts [1].

The incident

On 8 January 2009, a 66-year-old man was admitted to the Homerton University Hospital (HUH) in London with symptoms of fever, diarrhoea and confusion.

He had travelled on a flight from Abuja in Nigeria (where he had travelled south to Anambra state) to London on 6 January. He experienced fever, malaise, loss of appetite, and abdominal pain during the flight. He travelled from Heathrow airport by public

transport to his home in east London, and was described by a neighbour as being confused and feverish on arrival.

On 8 January, he was taken to HUH by ambulance, where he presented with a three-day history of fever, rigors, lethargy and mild diarrhoea. During his hospital stay, he was initially cared for in two open wards. He attended the radiology department on six occasions and an operating theatre once for lumbar puncture. Tests for a range of travel-associated infections (e.g. malaria, leptospirosis, dengue, yellow fever) were negative, and the case was managed in isolation at HUH as a possible typhoid case from 16 January. He was incontinent of urine and faeces at this time.

On 22 January, he was transferred to the Infectious Diseases Unit (Hospital for Tropical Diseases), University College Hospital, for further management, and on the same evening to the high-security infectious diseases unit (HSIDU), at the Royal Free Hospital, in a category 3 ambulance. The North East and North Central London (NENCL) Health Protection Unit (HPU) were alerted to the incident at this time.

A diagnosis of Lassa fever was confirmed by RT-PCR on 23 January, by the Novel and Dangerous Pathogens Laboratory (NaDP) laboratory at the Health Protection Agency (HPA) Centre for Emergency Preparedness and Response (CEPR), Porton Down. Lassa virus IgG antibodies were also detected in serum, and Lassa virus was subsequently isolated from blood and urine specimens.

The patient was commenced on ribavirin, and remained in isolation for the duration of his admission. He improved initially, but had a degree of nerve deafness - a feature consistent with Lassa fever [2,4]. Despite intensive nursing and medical care, the patient died on 29 January from complications exacerbated by pre-existing medical conditions. No post-mortem examination was undertaken.

Communication with agencies and the media

A series of immediate actions were implemented by an Incident Control Team (ICT). The incident was reported to the World Health Organization (WHO) under the International Health Regulations and followed up with the Federal Ministry of Health, Nigeria through the

WHO Country Office. The European Centre for Disease Prevention and Control (ECDC) was also notified.

A HPA press release was issued, confirming that there was no risk to the general public resulting from the case [5]. Information was cascaded to all general practitioners in the area (via the Primary Care Trust), to NHS Direct, and to all Emergency Departments in London. The incident was subsequently reported in national and local (online and print) media.

Risk assessment

All individuals with potential direct exposure to Lassa virus through contact with the case or exposure to bodily fluids required risk assessment. These contacts fell into a number of different professional and geographical groups:

- Other passengers on the flight
- The neighbour of the patient
- Ambulance staff involved in transporting the patient
- Medical, nursing and allied health professionals at the three hospitals

- Pathology staff handling specimens in several laboratories
- Radiology staff at HUH
- Domestic staff and porters at HUH

Each contact's risk of infection was assessed, and assigned into one of three categories (Table 1). Factsheets were produced on Lassa fever and the monitoring process (including advice for contacts going on holiday) according to risk category. These were available for dissemination to all contacts, most of whom were at HUH. The general factsheet (Category 1) was disseminated to HUH staff via the hospital intranet on 23 - 24 January.

From 23 January onward, members of staff were contacted either in person (at the hospital) or by telephone, asked about their contact with the patient, assigned to a category according to level of risk, and advised according to assigned category. No restriction was placed on work or movement for asymptomatic adults in any of the risk categories. A designated senior nurse was available 24 hours per day at the HUH to answer any queries.

TABLE 1

Level of risk related to exposure to a patient with Lassa fever, and action, by category

Risk Category	Description	Action
No risk (Category 1)	No contact with the case Casual contact (e.g. sharing a room with the case, without direct contact with a potentially infectious material)	Inform of absence of risk Give Category 1 (general) factsheet
Low risk (Category 2)	Close direct contact with the case (e.g. routine medical/nursing care, handling of clinical/laboratory specimens), but did not handle body fluids or wore personal protective equipment (PPE) appropriately	Self-monitor* for fever and other symptoms compatible with Lassa fever Report to the senior nurse if temperature $\geq 38^{\circ}\text{C}$, with further evaluation as necessary Give Category 2 factsheet
High risk** (Category 3)	Unprotected exposure of skin or mucous membranes (e.g. mucosal exposure to splashes, needlestick injury) to potentially infectious blood or body fluids, or unprotected handling of clinical/laboratory specimens	Record own temperature daily* and report this temperature to the senior nurse by 12 noon each day, with further evaluation as necessary Give Category 3 factsheet

* Contacts to be monitored for 21 days from last possible exposure to case

** Within this group, consideration for ribavirin prophylaxis, if any extreme exposure e.g. percutaneous injury

TABLE 2

Categorisation of contacts of a patient with Lassa fever at Homerton University Hospital, January 2009

Professional group	Risk category assigned				Total
	No risk (Category 1)	Low risk (Category 2)	High risk (Category 3)	Not contactable	
Medical	17	17	0	4	38
Nursing/ AHP ¹	49	71	0	16	136
Pathology	0	72	0	0	72
Domestic staff	12	0	0	5	17
Porters/ transport staff	32	4	0	3	39
Phlebotomy	4	8	0	2	14
Radiology/ other investigations	7	1	0	4	12
Total	121	173	0	34	328

¹Allied Health Professionals

Since the airline reported that there was no record of passenger illness or seeking assistance on the flight, the risk to other passengers on the flight was deemed negligible. The ECDC independently assessed the risk to other passengers on the flight and also concluded the case did not pose a significant risk to the citizens of the European Union.

Laboratories holding clinical specimens were contacted and asked to safely destroy these or transfer them for further testing or destruction as appropriate. Risk assessment of laboratory staff was carried out and there were no incidents reported at any of the laboratories involved in handling specimens. The neighbour of the patient was assessed and considered to be at low risk.

The funeral director was advised regarding the infectious state of the deceased who had already been placed in a sealed metal-lined coffin. It was advised that the coffin remain sealed and no viewing of the body take place.

Outcome of monitoring contacts

In total, 328 people at HUH were identified as possible contacts of the case. Thirty-four (10%) could not be contacted but attempts to do so are ongoing. The 21-day surveillance period (from date of last possible exposure) for HUH staff ends on 12 February.

To date, no contacts have reported any illness compatible with Lassa fever, and no high risk (Category 3) contacts have been identified (Table 2).

Discussion and conclusion

The risk for human-to-human transmission of Lassa fever is low. However, healthcare-associated transmission has occurred in areas where Lassa fever is endemic [6], and an instance of asymptomatic seroconversion was reported in a German physician in 2000 [7].

Clinical diagnosis of Lassa fever is difficult, and it is often confused with other more common infections such as severe malaria or typhoid fever [1]. A range of travel-associated infections was requested in this case. However, the diagnosis was only established two weeks after admission. Such a delay is not uncommon in imported Lassa cases [8]. In persons arriving from Africa, clinical histories should include careful assessment of travel to regions where uncommon diseases are endemic [6], including Nigeria, Sierra Leone, Liberia and Guinea for Lassa fever [9]. Early suspicion and diagnosis are vital to the successful management of these patients.

While ribavirin has been shown to be effective in early-stage arenavirus infections, particularly Lassa virus [2], in the absence of proven effectiveness for prophylaxis [3], oral ribavirin was not recommended for persons who might have been exposed to the case described here. Current advice would suggest restricting its use to contacts at highest risk [3].

Meticulous adherence to appropriate infection control practices to prevent unprotected exposure to blood or other body fluids is essential for the safe management of patients with possible Lassa fever [6], and the prevention of onward transmission, particularly given the non-specific presentation of Lassa fever and related VHF syndromes. In this incident, it is commendable that, even without knowing the diagnosis and the risks they were exposed to, all healthcare and other workers at the HUH who had contact with the patient before confirmation of Lassa fever diagnosis had worn appropriate personal protective equipment (PPE), and thus we did not identify any Category 3 risk persons.

* Authors correction

In the Abstract, the sentence "In January 2009, the seventh case of Lassa fever imported to the United Kingdom was diagnosed in London" was replaced by: "In January 2009, the eleventh case of Lassa fever imported to the United Kingdom was diagnosed in London". This was corrected on 13 March 2009.

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Rapid communications

THE FIRST CASE OF LASSA FEVER IMPORTED FROM MALI TO THE UNITED KINGDOM, FEBRUARY 2009

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This is the first case of Lassa fever to be imported from Mali to the United Kingdom. This paper discusses the investigations, the virological analysis, the surveillance and management of contacts undertaken following a case of Lassa fever.

In February 2009, the twelfth recorded case of Lassa fever, since surveillance records are available, was imported to the United Kingdom (UK). This is the second case to be imported to the UK in 2009 and the first reported case to have acquired infection in Mali. Risk assessment of 117 UK healthcare contacts with potential direct exposure to the patient's body fluids was undertaken. Seven contacts are considered to be at high risk of infection and are being actively monitored for 21 days.

Background

Lassa fever is caused by an arenavirus and is an acute illness of between one and three weeks duration. The incubation period is usually seven to 12 days but may range between three and 21 days. About 80% of human infections in endemic areas are asymptomatic. The overall case fatality rate is 1%, although it is reported to be 15%-20% in hospitalised patients [1,2].

The natural host of Lassa virus is the multimammate rat (*Mastomys spec.*) which sheds the virus in urine and droppings. Transmission of the virus to humans usually occurs via direct or indirect contact with rodent excreta. Person-to-person transmission occurs through direct contact with blood, saliva, urine, faeces or semen [1].

Lassa fever is known to be endemic in parts of West Africa, with most cases reported from Guinea, Liberia, Sierra Leone and Nigeria. People living in rural areas of West Africa are most at risk of Lassa fever. Imported cases to the UK are rare and occur almost exclusively in individuals who have worked in endemic areas in high risk occupations such as medical or development workers [4]. Although there is some evidence of endemicity in neighbouring countries [1,3-5], this is the first case of imported Lassa fever from Mali into the UK.

Clinical case description

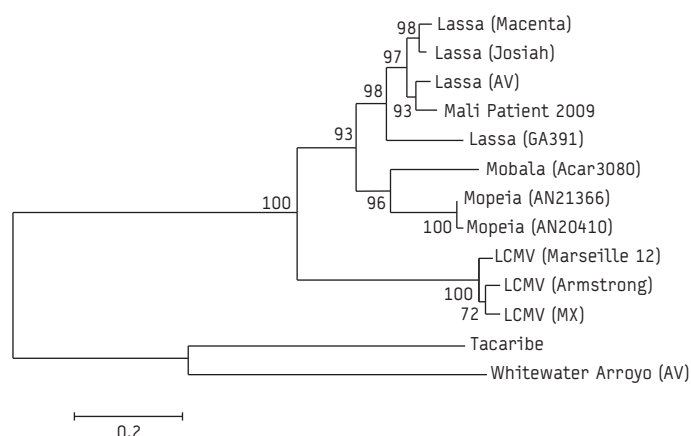
In February 2009, a man in his twenties was admitted to University College Hospital in London (UCLH) having been

medically evacuated from Mali with a 10-day history of fever and a diagnosis of falciparum malaria that did not respond to treatment. He had been in a village in southern Mali for four weeks, where he was working in remote rural conditions on the border with the Ivory Coast. He had travelled directly from the UK to Bamako, Mali and then travelled overland to southern Mali. Although precise details of possible exposure to rodents are not known, rodents including rats were seen regularly in the village.

On arrival the patient was alert and able to give a clear report on his medical history. However, he deteriorated rapidly and was transferred to a negative pressure room in the intensive care unit. He died of multi-organ failure later the same day. His malaria blood

FIGURE

Phylogenetic analysis of Arenaviruses, including the Lassa virus imported to the United Kingdom from Mali in February 2009



The tree was calculated by analysis of the 291 amino acids from the N-terminus of the lassavirus glycoprotein C using the neighbour-joining method. Bootstrap support (%) is indicated at each branch point. The virus from the patient described in this article (Mali patient 2009) is most closely related to the AV strain from Ivory Coast (Lassa AV) [7].

film and rapid antigen test were negative and a diagnosis of Lassa fever was confirmed the same night by PCR.

The patient was originally considered at low risk of Lassa fever because the disease has never been reported in Mali and is thus not considered to be endemic there. However, as he became more unwell his status was upgraded. Standard universal infection control precautions were followed and visors, but not full body protection, were worn during the attempted resuscitation.

Virological analysis

The diagnosis was confirmed in two different reverse transcription PCR (RT-PCR) assays targeting different regions of the genome and by sequencing of the 291 amino acids at the N-terminus of the Lassa virus glycoprotein C [6]. The detection of Lassa virus in two different RT-PCRs together with the characterisation of a unique part of the Lassa virus genomic sequence constituted a definitive diagnosis. Further studies including virus culture are in progress, and sequencing of the entire genome of the isolate is planned.

TABLE 1

Risk assessment for contacts of patients with Lassa fever

Risk Category	Description	Action and advice
Unclear	Not sure of contact	Reassure
		Inform to contact the infection safety officer should they recall any contact
		Give general factsheet
No risk (Category 1)	No direct contact with the patient or body fluids	Inform of absence of risk
	Casual contact e.g. sharing a room with the patient, without direct contact with body fluids	Advise to call if concerned following reading fact sheet
		No further action. Give category 1 (general) factsheet
Low risk (Category 2)	Direct contact with the patient (e.g. routine medical/nursing care, handling of clinical/laboratory specimens), not handling body fluids or wearing personal protective equipment appropriately	Self-monitor* for fever and other symptoms compatible with Lassa fever
		Report to the safety officer if temperature $\geq 38.0^{\circ}\text{C}$, with further evaluation as necessary
		Give category 2 factsheet
High risk** (Category 3)	Unprotected exposure of skin or mucous membranes (e.g. mucosal exposure to splashes, needlestick injury) to potentially infectious blood or body fluids, including unprotected handling of clinical/laboratory specimens	Record own temperature daily for 21 days following your last contact with the patient and report this temperature to the safety officer by 12 noon each day, with further evaluation as necessary
		Give category 3 factsheet
		Inform Health Protection Unit immediately if contact reports symptoms compatible of Lassa fever and further risk assessment is required

*Level of risk according to exposure and action and advice by category.

*Contacts to be monitored for 21 days from last possible exposure to case

** Within this group, consider ribavirin prophylaxis if any extreme exposure, e.g. percutaneous injury

TABLE 2

Categorisation of contacts in the United Kingdom, Lassa fever importation, February 2009

Contacts classification	Risk category			
	Category 1 (no risk)	Category 2 (low risk)	Category 3* (high risk)	Total
Intensive care unit staff	3	14	2	19
Accident and emergency (A&E) staff	17	12	0	29
Laboratory staff	21	45	3	69
Family	1	2	2	5
Colleague in Mali	0	1	0	1
UK ambulance service**	2	0	0	2
Totals	44	74	7	125

*Within this group, ribavirin prophylaxis was considered in the case of any extreme exposure e.g. percutaneous injury

** The air ambulance medics were still in attendance whilst transferring the patient to University College Hospital in London. The patient was wrapped in a tarpaulin sheet and the UK ambulance crew adopted universal barrier precautions, hence both were considered casual contacts and not at risk (category 1).

Phylogenetic analysis showed that the virus was distinct from other Lassa virus strains but grouped most closely with a strain of Lassa virus (Lassa (AV)) isolated from a case reported from Germany in 2000 [7] (Figure). The German patient had travelled through Ivory Coast, Ghana and Burkina Faso during the incubation period and the investigations could not determine where he had acquired the virus. The British case reported here had been working close to the border with Ivory Coast.

Surveillance and management of contacts

An Incident Control Team (ICT) meeting was called by UCLH early the following day to discuss risk assessment of contacts, safe decontamination of the environment and management of the body.

Risk assessment

The ICT identified 123 people who could have come into direct contact with the Lassa virus either through contact with the case or exposure to body fluids. Almost all of these contacts were UCLH emergency care and laboratory staff. All UK based contacts were assigned to one of three categories depending upon their level of risk (no risk, low risk or high risk, see Table 1) and were managed as reported recently [8]. Contacts will be monitored for 21 days from exposure.

International contacts

The German air ambulance crew are being followed up and managed by German authorities, and the World Health Organization (WHO) is supporting health authorities in Mali in conducting field investigations and in the implementation of control measures.

Risk assessment outcome and follow up

The outcome of the UK risk assessment is shown in Table 2. None of the category 3 contacts received ribavirin prophylaxis. The evidence base for the use of ribavirin prophylaxis is limited, but category 3 contacts were given information explaining its possible benefits and side effects and were left to make an informed choice.

Discussion

In the case described here, the reported diagnosis of malaria and the fact that Mali has not been considered endemic for Lassa fever made the clinical diagnosis difficult. As a consequence, the initial risk of Lassa fever was considered low. Only when the patient developed multi-organ failure six hours after admission was the risk of Lassa fever upgraded. Universal barrier precautions were used throughout, but not the high levels of protection currently recommended for viral haemorrhagic fevers [9]. As a result, 76 hospital staff were put at risk in the space of eight hours, and three of seven category 3 contacts were laboratory staff. Although transmission to healthcare workers from imported Lassa fever cases is very rare, this can cause considerable anxiety among contacts. There is only one reported case of transmission in a hospital setting in an industrialised country, and this was a seroconversion without clinical illness in Germany [10].

This is the first Lassa virus to be characterised from Mali. The virus is closely related to isolates from neighbouring countries and was amplified using a widely used diagnostic PCR test [6]. There is serological evidence that Lassa virus is present in Mali [3,5], but this is the first proven imported case and has implications for current risk assessment in travellers returning from this area.

Acknowledgements

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Rapid communications

BOTULISM IN INJECTING DRUG USERS, DUBLIN, IRELAND, NOVEMBER-DECEMBER 2008

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In November and December 2008, six cases of suspect wound botulism were reported in heroin injecting drug users, all residents in Dublin, Ireland. Patients were aged between 23-42 years of age; four cases were male; one patient died shortly after admission. The patients presented to four different hospitals across the city. Botulism in injecting drug users in Ireland was last reported in 2002.

On Monday, 24 November 2008, public health authorities were notified that an injecting drug user (IDU) had been admitted to a hospital in Dublin, Ireland, with neurological signs suggestive of botulism including progressive bulbar palsy, diplopia, dysarthria, and an electromyography (EMG) test consistent with a diagnosis of botulism. The patient was treated with botulism anti-toxin and supportive measures. Serum samples taken prior to administration of anti-toxin were sent to the Foodborne Pathogen Reference Unit, London, United Kingdom (UK) for detection of *Clostridium botulinum* neurotoxin by mouse biotoxin assay. Laboratory results confirmed the diagnosis of botulism and identified the causative toxin as *C. botulinum* toxin type B.

By Friday, 28 November, three additional suspected cases of botulism had been notified, all of whom received anti-toxin. These four patients were admitted to three different hospitals in Dublin. Although two patients lived in the same area, they did not know each other. All were IDUs, but there was no evidence that they shared the same drug supply.

No additional cases were notified to the Departments of Public Health in Ireland during the week beginning on 1 December. However, during the 36-hour period from 8 to 10 December, two further patients were admitted to a fourth Dublin hospital with signs suggestive of botulism (difficulty breathing and speaking, ataxia, double vision). Both patients received botulism anti-toxin. Unfortunately, one of these patients died shortly after admission from overwhelming sepsis. At the time of writing this report, one case is laboratory confirmed, two cases are probable and three cases are possible (see next section and Table for further details).

For the purpose of this investigation, a *possible* case of drug injection-related wound botulism was defined as a person in the Republic of Ireland with a recent history (within four weeks of symptom onset) of injecting drug use and with acute onset since 1 November 2008 of either of the following symptoms in the absence of any other obvious cause:

- symmetrical cranial nerve palsy,
- difficulty in swallowing or speech,
- unexplained stridor,
- difficulty in breathing,
- or descending flaccid paralysis.

A *probable* case was defined as having the features of a possible case and laboratory results suggestive of *C. botulinum* infection but unconfirmed by neutralisation.

A *confirmed* case was defined as having the features of a probable case, with a confirmed diagnosis of botulism (by detection of botulinum toxin in serum or isolation of *C. botulinum* from a wound or abscess site).

An outbreak enhanced surveillance form was designed (available upon request) to collect relevant demographic, clinical and drug use data. This form was filled out in as much detail possible by hospital staff. Specific attempts were made to identify links between the six patients in terms of area of residence, social networks and drug supply.

A summary of selected demographic and clinical data as well as information about drug use of the cases is presented in the Table.

The outbreak has been managed by an outbreak control team led by the Department of Public Health, Health Service Executive-East (HSE-E) in Dublin. In addition to HSE-East staff, personnel from the Health Protection Surveillance Centre (HPSC), Dublin, the HSE Drug Services, Dublin, and a clinical microbiologist from one of the involved hospitals were included in the outbreak control team. As is the norm in outbreaks of suspected clostridial infections, the HPSC alerted clinical staff throughout the country (emergency medicine physicians, neurologists, infectious disease physicians, microbiology services, drug services and public health medicine physicians) through email alerts. Press releases were also issued to

the national media. Internationally, the European Centre for Disease Control (ECDC) and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) were informed. HSE HPSC alerted other European countries using the Early Warning and Response System (EWRS), an internet based rapid alert system supported by the European Commission and run by ECDC. Advice in relation to 'skin popping' (subcutaneous injection of heroin) with potentially contaminated heroin was distributed to the drug using community through the drug services and the network of 13 local drugs task forces in Dublin.

Heroin used by one patient was obtained and has been sent to the Foodborne Pathogen Reference Unit for testing by the Irish police. However, *C. botulinum* could not be isolated from this sample. No other cases of wound botulism were reported in Ireland in 2008 apart from those reported here. There have been two cases of suspected wound botulism in drug users in different areas in the north of England since 25 December 2008. One of these is unconfirmed (serum sample collected several days after onset of symptoms), whilst testing for the second case is on going. Both cases had classic symptoms and have received anti-toxin. The authors are unaware of any other outbreak occurring in European countries in this time period.

Discussion

Since 2000, there have been three outbreaks of clostridial infections in IDUs in Dublin. In an outbreak of *Clostridium novyi* Type A in 2000, 22 patients were infected, of whom eight died [1-3]. A simultaneous outbreak occurred in the north of England and Glasgow, Scotland. In 2002, an outbreak of botulism involving three IDUs occurred in Ireland [4,5].

There is laboratory confirmation of a clinical diagnosis of botulism in approximately 40% of wound botulism cases, either by detection of botulinum neurotoxin in the patients' serum or by isolation of *C. botulinum* from wounds. Reasons for this low confirmation rate include delay in recognition of clinical signs and subsequent delays in specimen collection. Once toxin reaches the nerve endings, it binds irreversibly, thus reducing the amount of toxin in the serum to below detectable levels. In addition, inadequate volumes of serum sent for testing may reduce the sensitivity of the test, and the administration of systemic antibiotics prior to specimen collection

may reduce the viability of organisms in pus taken from associated wound abscesses.

The fact that only one case in this outbreak has been laboratory-confirmed, with the remaining cases classified as possible or probable, can be attributed to the difficulties mentioned in the paragraph above. Two of the cases were given anti-toxin before serum samples were obtained and thus toxin was not detectable.

Wound botulism became a notifiable disease in Ireland on 1 January 2004. Prior to this date, only food-borne botulism was notifiable under the disease category of *Acute Infectious Gastroenteritis*.

Wound botulism occurring among IDUs was first reported in the United States in 1982 [6]. Since then both sporadic botulism cases and outbreaks have been reported among this sub-population. A number of other European countries have also reported outbreaks among IDUs in recent years [7-11].

Heroin users who inject either subcutaneously or intramuscularly are at particular risk, as administration using this method is conducive to wound infection, abscess formation and generation of the anaerobic conditions for germination of *C. botulinum* spores and subsequent release of neurotoxin [7]. Spores of *C. botulinum* are often present in soil and may contaminate heroin or heroin taking equipment. Heating the heroin powder to solubilise it for subcutaneous injection does not kill the spores, and the acidulant used for solubilisation enhances tissue damage at the injection sites, facilitating the germination of botulinum spores and leading to release of neurotoxin.

Botulism (both food-borne and wound) is extremely rare in Ireland, unlike many European countries which routinely see food-borne cases each year. Wound botulism is much rarer, but both sporadic cases and outbreaks have been reported in European countries in recent years [7-11]. Maintaining high levels of awareness of the risk of botulism among the population of injecting drug users is vital to insure that they are aware of the risk and urgently seek medical attention if they develop any of the signs or symptoms associated with the disease. Alerting clinicians to botulism increases the likelihood of rapid diagnosis, early hospitalisation and appropriate treatment with anti-toxin and other supportive treatment of these patients, thus decreasing mortality and complications. Delays in

TABLE
Demographic, clinical and drug use history, reported botulism cases, Dublin, 2008

Case (No.)	Sex	Age (years)	Onset of symptoms	Clinical features/skin abscesses	Mechanical ventilation	Anti toxin given/date	Recent* heroin injection	Case classification	Outcome
1	M	33	20/11/2008	Dysarthria, indurated area	No	Yes 21/11/2008	Yes	Confirmed	Alive
2	M	23	21/11/2008	Respiratory problems	Yes	Yes 27/11/2008	Yes	Probable	Alive
3	F	34	17/11/2008	Respiratory problems	Yes	Yes 27/11/2008	Yes	Possible	Alive
4	M	39	21/11/2008	Respiratory problems/ abscess	Yes	Yes 27/11/2008	Yes	Probable	Alive
5	M	38	04/12/2008	Dysarthria	No	Yes 8/12/2008	Yes	Possible	Alive
6	F	42	10/12/2008	Dysarthria and ataxia/ abscess	No	Yes 10/12/2008	Yes	Possible	Died

*within four weeks of clinical disease onset

administration of anti-toxin treatment increase mortality, hospital stay and rehabilitation time. Raising the index of suspicion also increases the likelihood of additional cases being considered.

Our working hypothesis is that a contaminated supply of heroin is responsible for this outbreak, supported by the number of cases and focus in the Dublin area over a short period of time. However our investigation to date has failed to identify common links between cases. Such links were identified in the clostridial outbreak among IDUs in Dublin in 2000 [1-3], when common social networks and common drug dealers were identified for some of the cases. At that time, similar *C. novyi* outbreaks were occurring in northern England and Glasgow, Scotland, supporting the hypothesis of a common contaminated supply.

The fact that no further cases have been identified suggests that rapid dissemination of information and local action may have averted further cases. The authors would welcome hearing of any wound botulism cases seen in other countries around this time.

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Rapid communications

INCREASE IN INVASIVE GROUP A STREPTOCOCCAL INFECTIONS IN ENGLAND, WALES AND NORTHERN IRELAND, 2008-2009

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Increases in invasive and non-invasive group A streptococcal diseases are currently being seen in the United Kingdom. National enhanced surveillance is being launched to examine the clinical presentations, risk factors, outcome and clustering patterns of cases to further inform public health management strategies.

Following the increases in the number of scarlet fever cases identified across England during the 2007-8 season, further increases are being seen during the current 2008-9 season, accompanied by increases in invasive group A streptococcal (*Streptococcus pyogenes*) infections [1,2]. Although group A streptococcal infections typically increase at this time of year, the rises seen currently are above the seasonally expected.

Scarlet fever

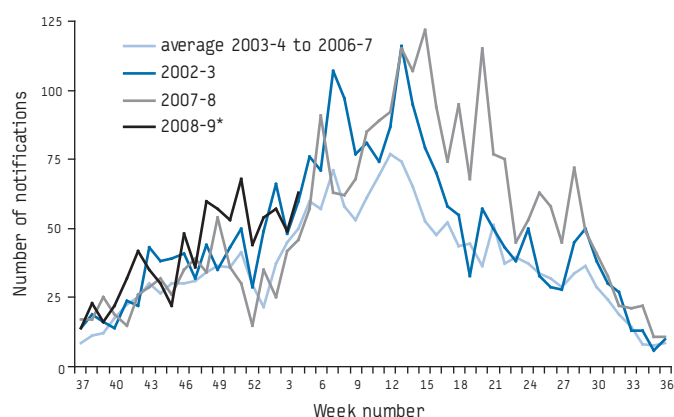
In the United Kingdom (UK), statutory notifications of scarlet fever, based on clinical symptoms consistent with scarlet fever, are submitted by diagnosing clinicians to the local public health officials. A total of 222 notifications of scarlet fever were made

during the last four weeks of 2008 by clinicians across England, compared to 134-141 notifications for the same period in 2004 to 2007, and 153 notifications for 2003, the last peak year for scarlet fever (Figure 1). Numbers of notifications were elevated relative to the period between 2003 and 2007 in all nine regions of England except the South West and Yorkshire and the Humber. Notifications for the first four weeks of 2009 showed a continuation of the high level of activity, with 223 notifications compared to 143-180 for 2004-2008 and 223 for 2003.

Invasive group A streptococcal infection

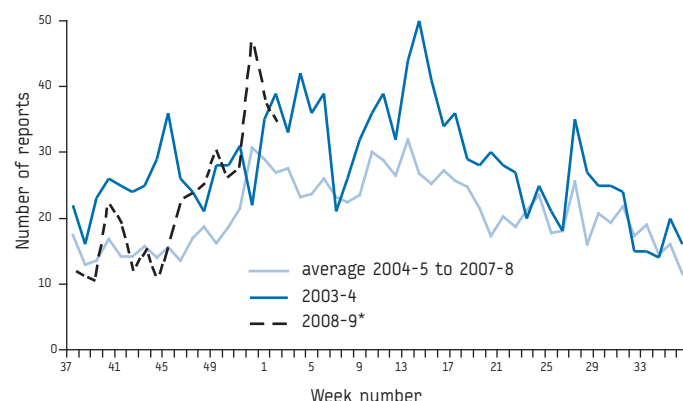
Cases of invasive group A streptococcal (iGAS) infection, defined through the isolation of group A streptococci from normally sterile sites, are identified through national routine laboratory surveillance and isolate referral to the national reference laboratory. Routine surveillance data identified 151 cases of iGAS in December 2008, with a further 98 reports made so far for January 2009, compared to 80-127 for December in the years 2003 to 2007 (Figure 2).

FIGURE 1
Scarlet fever notifications, England 2002-3 to 2008-9



* data available up to week 4, 2009

FIGURE 2
Weekly reports of invasive group A streptococcal infections, England, Wales and Northern Ireland 2002-3 to 2008-9



* reports received by 2 February 2009 up to week 2, 2009 (further reports expected)

Increases above the total seen in December 2003, the last peak season for invasive disease, have been seen in three of nine regions in England and Northern Ireland so far, whilst data for Wales remain within the seasonally expected range. Given delays inherent within routine laboratory reporting, further reports for 2008 can be expected. Overall, 2% (2/97) of iGAS isolates from December 2008 were reported as erythromycin-resistant. Age- and sex-specific rates of iGAS infection show highest rates in the elderly and infants (Figure 3).

iGAS isolates referred to the national reference laboratory from hospitals in England showed a substantial increase in December 2008 (n=143) compared to the same period in 2007 (n=86). The most common *emm*/M-types identified in December 2008 were *emm*/M1 (25% of all iGAS isolates), *emm*/M3 (25%), *emm*/M89 (9%) and *emm*/R28 (9%). Of the 100 GAS isolates received and typed so far for 2009, there has been a significant increase in *emm*/M3, with 50% of isolates typed belonging to this *emm* type.

Discussion

Periodic upsurges in iGAS have been reported in many countries across Europe and North America since the 1980s [3], with Finland the latest country to report an increase in iGAS from 2006 onwards [4,5]. The reasons behind these increases are poorly understood. Analysis of scarlet fever notifications in England over the last century suggest cyclical incidence patterns, with resurgences occurring on average every four years [6]. The last peak season for scarlet fever was 2002-3, although notifications were also high for 2003-4. A recent project started in the UK to examine the potential value of using syndromic indicators of superficial manifestations of GAS infection in forecasting rises in invasive disease, found that clinically diagnosed scarlet fever mirrored the pattern of iGAS [7], and as such the current increases in invasive disease may be attributable to a natural cycle in disease incidence.

The potential remains for changes in virulence of circulating strains or for increased incidence in particular risk groups, as seen in the UK during the early 2000s [8]. It is also possible that the significant influenza activity in the UK this winter may be contributing directly or indirectly by increasing transmission of GAS and/or rendering individuals with influenza more susceptible

to secondary infection with iGAS [9]. Analysis of isolates submitted to the national reference laboratory has not identified any unusual types circulating this season, although an increase in *emm3* is currently being seen. Further typing results are awaited to confirm this trend, which would be of concern given its association with a higher case fatality rate than most other *emm* types [10].

As a result of the current rise in iGAS notifications, national enhanced surveillance is being introduced in order to gain additional information on clinical presentations, risk factors, outcome and clustering. Alerts have been issued to regional health protection staff and consultant microbiologists, and a template letter outlining the current situation and reminding clinicians of possible early signs and symptoms of iGAS has been made available for cascade to hospital emergency departments and primary care services.

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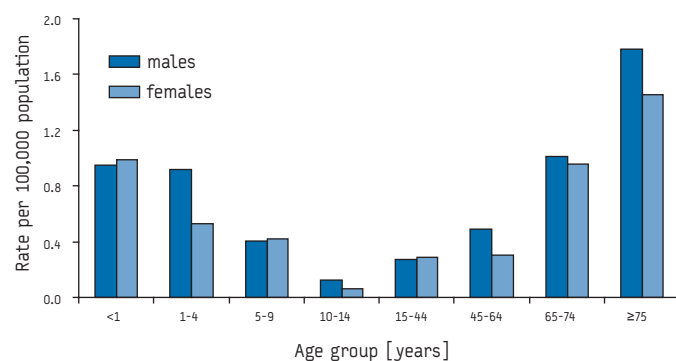
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FIGURE 3

Age- and sex-specific rates of invasive group A streptococcal infection*, England, Wales and Northern Ireland, December 2008-January 2009



* reports received by 2 February 2009 (further reports expected)

A series of horizontal lines for writing, spaced evenly down the page.

National Bulletins

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Mitteilungen der Sanitätsverwaltung
Bundesministerium für Gesundheit Familie und
Jugend, Vienna.
Monthly, print only. In German.
<http://www.bmgfj.gv.at/cms/site/standard.html?channel=CH0954&doc=cms1229425348542>

BELGIUM

Vlaams Infectieziektebulletin
Department of Infectious Diseases Control,
Antwerp.
Quarterly, print and online. In Dutch, summaries
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<http://www.infectieziektebulletin.be>

Bulletin d'information de la section
d'Épidémiologie
Institut Scientifique de la Santé Publique,
Brussels
Monthly, online. In French.
<http://www.iph.fgov.be/epidemiologie/epifir/episcoop/episcoop.htm>

BULGARIA

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Státní zdravotní ústav (National Institute of
Public Health), Prague
Monthly, print and online. In Czech, titles in
English.
<http://www.szu.cz/publikace/zpravy-epidemiologie-a-mikrobiologie/rocnik-18-rok-2009>

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Institut, Copenhagen.
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National Public Health Institute, Helsinki.
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Épinfo (az Országos Epidemiológiai Központ
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<http://www.oek.hu/oek.web?to=839,1572&nid=41&pid=9&lang=hun>

ICELAND

EPI-ICE
Landlæknisembættið
Directorate Of Health, Seltjarnarnes
Monthly, online. In Icelandic and English.
<http://www.landlaeknir.is>

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EPI-INSIGHT
Health Protection Surveillance Centre, Dublin.
Monthly, print and online. In English.
<http://www.ndsc.ie/hpsc/EPI-Insight>

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Notiziario dell'Istituto Superiore di Sanità
Istituto Superiore di Sanità, Reparto di Malattie
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Epidemiologijas Biļeteni
Sabiedrības veselības aģentūra
Public Health Agency, Riga.
Online. In Latvian.
<http://www.sva.lv/epidemiologija/bileteni>

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Užkrečiamųjų ligų profilaktikos ir kontrolės
centras
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<http://www.ulpkc.lt/ulpkc.laikraštis.php>

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zatruciach w Polsce
Panstwowy Zakład Higieny,
National Institute of Hygiene, Warsaw.
Fortnightly, online. In Polish and English.
http://www.pzh.gov.pl/epimeld/index_p.html#01

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Saúde em Números
Ministério da Saúde,
Direcção-Geral da Saúde, Lisbon.
Sporadic, print only. In Portuguese.
<http://www.dgs.pt>

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Info Epidemiologia
Centrul pentru Prevenirea si Controlul Bolilor
Transmisibile,
National Centre of Communicable Diseases
Prevention and Control, Institute of Public
Health, Bucharest.
Sporadic, print only. In Romanian.
<http://www.cpcbt.ispb.ro>

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Inštitut za varovanje zdravja, Center za nalezljive
bolezni, Institute of Public Health, Center for
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Monthly, online. In Slovenian.
<http://www.ivz.si/index.php?akcija=podkategorija&p=89>

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Boletín Epidemiológico Semanal
Centro Nacional de Epidemiología, Instituto de
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Fortnightly, print and online. In Spanish.
<http://www.isciii.es/jsps/centros/epidemiologia/boletinesSemanal.jsp>

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Communicable Disease Surveillance Centre,
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<http://www.cdscni.org.uk/publications>

Scotland
Health Protection Scotland Weekly Report
Health Protection Scotland, Glasgow.
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<http://www.hps.scot.nhs.uk/ewr/index.aspx>

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Folkehelseinstituttet, Oslo, Norway
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http://ec.europa.eu/health/index_en.htm

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