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Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009

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This article describes the characteristics of 574 deaths associated with pandemic H1N1 influenza up to 16 July 2009. Data (except from Canada and Australia) suggest that the elderly may to some extent be protected from infection. There was underlying disease in at least half of the fatal cases. Two risk factors seem of particular importance: pregnancy and metabolic condition (including obesity which has not been considered as risk factor in previous pandemics or seasonal influenza).

Introduction

To date, there are few data on risk factors, severe cases and deaths associated with pandemic H1N1 influenza 2009. Estimating and interpreting case fatality ratios (CFR) is difficult, mainly due to the challenge of accurately estimating the numerator (N deaths) and the denominator (N cases) [1], especially during a pandemic that is still evolving. Furthermore, many countries have abandoned individual case counts and systematic screening of all suspect cases. This article aims to describe the characteristics of reported deaths, to assess the CFR and high-risk profiles linked with underlying disease, while assessing possible bias.

Methods

The study is based on an analysis of available data until 16 July 2009, as compiled by the epidemic intelligence team at the French institute for public health surveillance (Institut de Veille Sanitaire, InVS), using a well-defined methodology [2]. The individual or aggregated data originated from validated official sources (Ministries of Health, local or national public health authorities, European Centre for Disease Prevention and Control, United States Centers for Disease Control and Prevention, World Health Organization), completed by informal sources when needed.

Results

The first (retrospectively) confirmed death occurred in Oaxaca State, Mexico, (onset of symptoms on 4 April 2009). As of 16 July 2009, InVS was aware of 684 confirmed deaths reported worldwide since the start of the pandemic (Figure 1) for a total of 126,168 reported cases (Figure 2). At this stage, no deaths had been reported and scarce data was available from African countries.

Data were available for 574 deaths associated with pandemic H1N1 influenza 2009: individual data for 449 cases in 26 countries (Table 1, Figure 2) and aggregated data for 125 cases in Mexico [3].

The quality and completeness of the data regarding age, sex, date of death and the notion of underlying disease varied greatly for each case. The overall 'computed CFR' (number of reported deaths per number of reported cases as of 16 July 2009) was 0.6% and varied from 0.1% to 5.1% depending on the country (and the accurate quantification of deaths and overall case counts) (Table 1).

Deaths by sex and age

Data on sex were available for 503 fatal cases worldwide (257 men and 246 women, sex ratio=1.04). Data on age were available for 468 fatal cases worldwide (343 with individual data and 125 with aggregated data). Data on both information (age and sex) were available for 448 fatal cases (Figure 3).

Although previous reports suggested that cases of pandemic H1N1 influenza 2009 occurred mainly in children [4], the mean and median age of the 343 fatal cases in our analysis were 37 years (range 0-85 years). Most deaths (51%) occurred in the age group of 20-49 year-olds, but there was considerable variation depending on country or continent (Table 2). Overall, 12% of deaths occurred in cases aged 60 years or more, but 36% of reported deaths in Canada (mainly female) and 28% in Australia occurred in this age group.

Underlying risks

Pregnancy

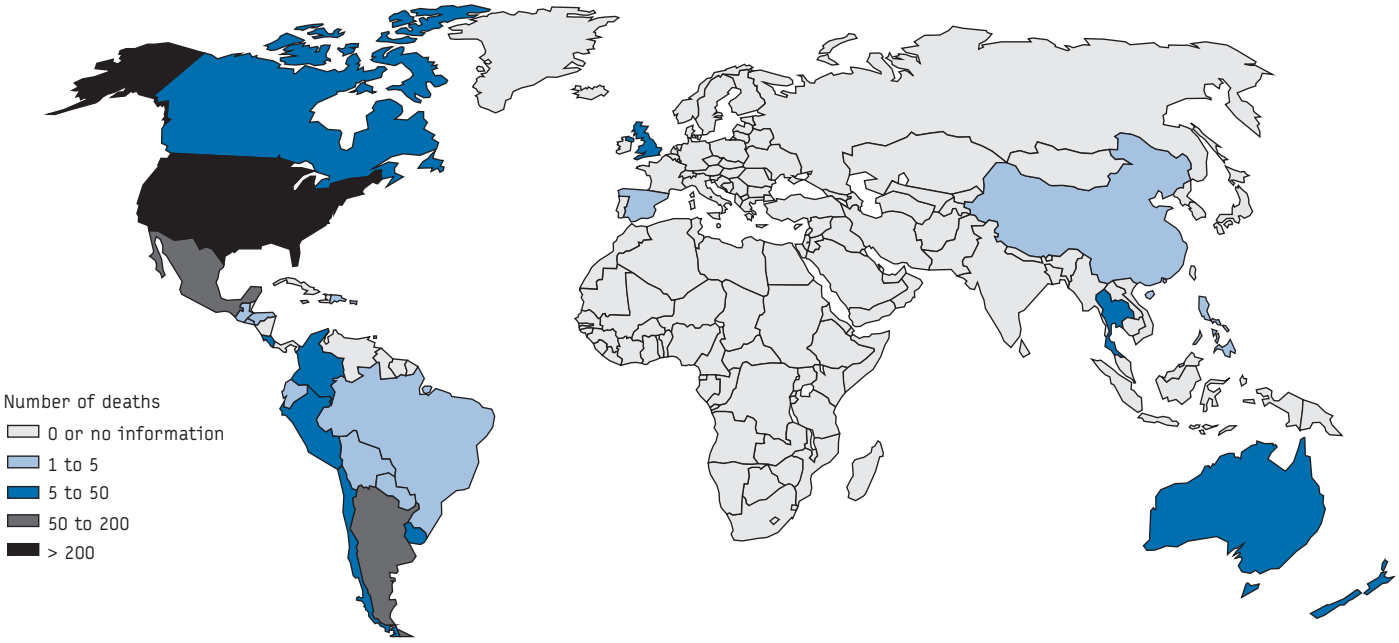
As of 16 July 2009, 16 women (10% of all individually documented female cases who died and 30% of the 20-39 year-old women who died) were pregnant or had delivered at the time of their death. Among these 16 women, at least eight had documented underlying health risks (obesity, heart disease or a respiratory disease such as asthma or tuberculosis). No information was available as to the underlying health status of the eight remaining women who died.

Underlying disease

A sub-analysis examined the 354 cases (241 cases with individual data and 113 with aggregated data) who died and were also documented for underlying disease and for sex and/or age (Figure 2). Presence or absence of underlying disease was documented for 241 of 449 (53% of the 449 cases with individual data) of deaths with individual data. Of these, 218 (90%) had documented underlying disease and 23 (10%) had documented absence of underlying disease. A further sub-analysis was conducted

Figure 1

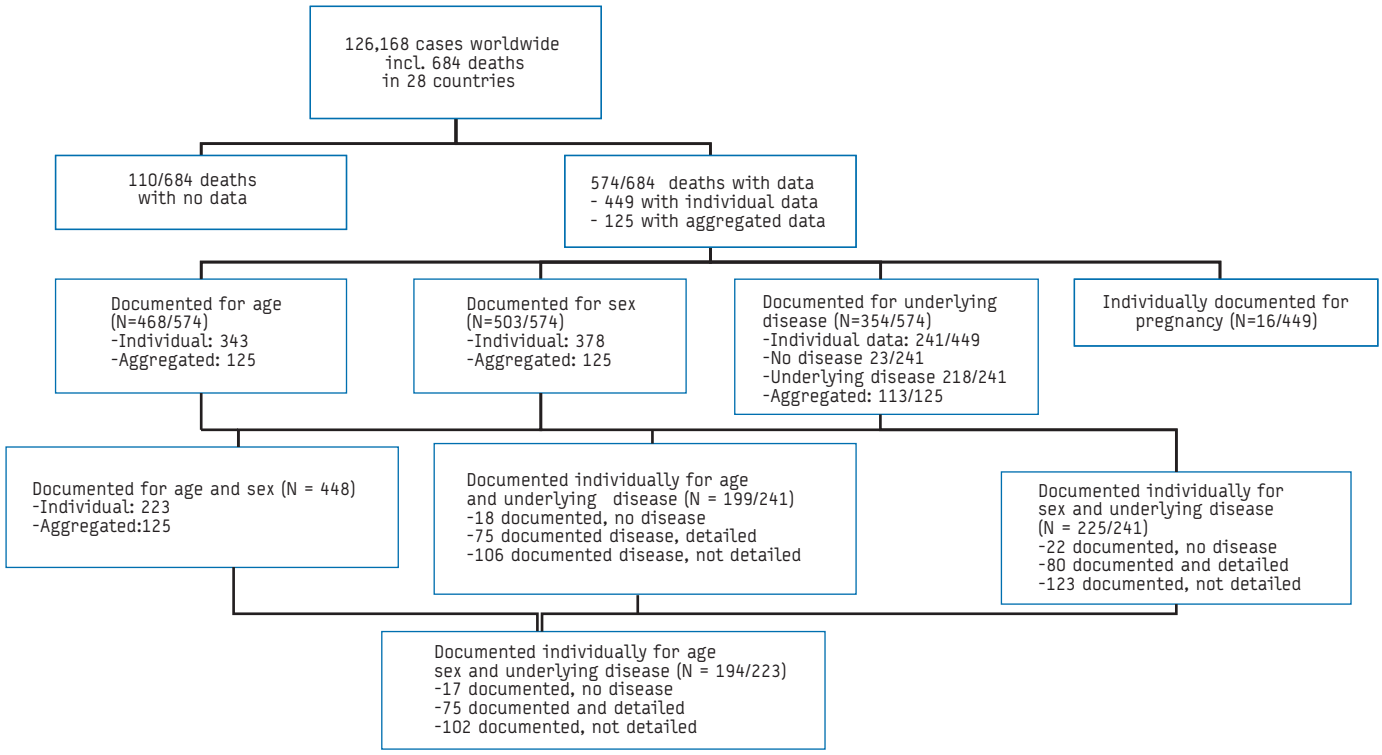
Deaths associated with pandemic H1N1 influenza 2009 reported officially worldwide as of 16 July 2009



Source: Ministries of Health, local or national public health authorities, European Centre for Disease Prevention and Control, United States Centers for Disease Control and Prevention, World Health Organization.
Map drawn with Philcarto (free software available from: http://philcarto.free.fr/)

Figure 2

Breakdown of fatal case counts used in our analysis



on 102 cases of known sex (80 with detailed underlying disease and 22 without disease) and 93 cases of known age (75 with detailed underlying disease and 18 without disease) (Figure 2). Underlying disease (or its absence) was equally distributed between the sexes, but understandably not among age groups (Figure 4). A high proportion of young children (27% of the 0-9 year-olds) and young adults (22% of the 20-29 year-olds) had no documented underlying disease, while 60% of people over the age of 60 years had heart or respiratory disease. Diabetes and obesity were the most frequently identified underlying conditions (Figure 5) and were found in fatal cases over the age of 20 years (the World Health organization defines "obesity" as a body mass index equal to or more than 30, but as the reporting format differed between sources and no standard definition of childhood obesity is applied worldwide, we cannot be sure the same definition has been applied for all cases). In the 13 fatal cases with individual detailed data on metabolic conditions, seven cases had obesity, five cases had diabetes, and one case had both. The available data for the other cases did not specificy whether the metabolic condition included obesity only, diabetes only, or both.

Discussion and conclusions

Most cases described during the three pandemics of the 20th century and during seasonal influenza involve transient illness not requiring hospitalisation. Most deaths are described in the very young or the elderly or those with underlying disease. The 1918-1919 pandemic, however, was characterised by a high mortality rate in healthy young adults and an estimated CFR of 2-3% [5]. Even with a low CFR, seasonal influenza epidemics cause significant morbidity and mortality with an estimated three to five million cases of severe illness and about 250,000 to 500,000 deaths worldwide [6].

To date, the CFR attributable to the current H1N1 pandemic has been estimated at around 0.4%, based on surveillance data from Mexico and mathematical modelling [7]. This CFR is higher than that of average seasonal influenza but remains of the same order of magnitude. Whether this will change before the expected epidemic peak in the northern hemisphere in the autumn is unknown.

Evaluating CFR during a pandemic is a hazardous exercise. Aside from the issue of whether or not a death has been caused by

Table 1

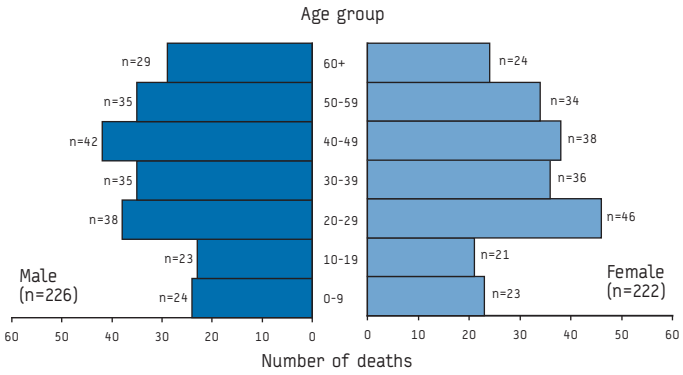
Available individual and aggregated data on cases of pandemic H1N1 influenza 2009 and associated deaths worldwide, by country, as of 16 July 2009

Country	Data reported in official bulletins* to 16 July 2009				N deaths with individual data available**
	N deaths**	N confirmed cases	Computed CFR	Mortality per million inhabitants	
United States	211	37,246	0.6%	0.66	242
Argentina	137	3,056	4.5%	3.37	13
Mexico	124	12,645	1.0%	1.12	0
Canada	39	9,855	0.4%	1.15	41
Chile	33	10,491	0.3%	1.93	10
Thailand	24	4,057	0.6%	0.35	23
Australia	21	10,389	0.2%	0.98	31
United Kingdom	17	9,739	0.2%	0.27	17
Uruguay	15	550	2.7%	4.45	8
Costa Rica	10	428	2.3%	2.16	6
New Zealand	9	1,984	0.5%	2.09	10
Colombia	7	185	3.8%	0.15	4
Peru	6	2,082	0.3%	0.20	4
Brazil	4	1,027	0.4%	0.02	13
Paraguay	3	125	2.4%	0.46	4
Philippines	3	2,668	0.1%	0.03	4
Ecuador	3	277	1.1%	0.22	3
Salvador	3	404	0.7%	0.48	3
Bolivia	2	585	0.3%	0.20	2
Spain	2	1,099	0.2%	0.04	2
Guatemala	2	339	0.6%	0.14	2
Dominican Republic	2	108	1.9%	0.20	2
Jamaica	2	39	5.1%	0.73	1
Puerto Rico	1	20	5.0%	0.25	1
Brunei	1	334	0.3%	2.46	1
China	1	1,362	0.1%	0.00	1
Honduras	1	123	0.8%	0.13	1
Hong Kong (China)	1	1,389	0.1%	0.14	0
Total	***684***	***112,606***	***0.6%***	***0.27***	***449***

CFR: case fatality ratio.
* As per national bulletins, ECDC and WHO.
** For some countries, the N value in the first column is higher than in the third column due to a time lag for official reports.

Figure 3

Deaths associated with pandemic H1N1 influenza worldwide by age and sex, as of 16 July 2009* (n=448)



* Individual data, except from Mexico where aggregated data originate from the Ministry of Health.

the influenza infection, cases tend to be detected initially among severely ill patients with a higher probability of dying. This leads to an overestimation of the computed CFR at the beginning of an outbreak. The computed CFR subsequently evolves as the case reporting strategy is adapted to the situation. When the situation no longer requires exhaustive reporting of cases, the computed CFR will inevitably increase and grossly overestimate the true CFR.

Specific investigations or modelling allow for a more accurate estimation of the number of cases. As of 27 May 2009, there had been 820 confirmed cases in New York City, of whom two had died, resulting in a computed CFR of 0.2%. A telephone survey estimated that in fact 250,000 cases had occurred in that city of 8.3 million inhabitants, resulting in an estimated CFR of 0.0008% [8,9]. In the United Kingdom (UK), there were 28 deaths reported for a documented 10,649 cases as of 16 July 2009 and a computed CFR of 0.26%. However, health authorities estimated that the cumulative number in the UK on that date was 65,649 cases and 28 deaths, which corresponds to an estimated CFR of 0.04% [10].

The pandemic, however, is far from over, and deaths will unfortunately continue to occur. As in previous pandemics, available

Table 2

Deaths associated with pandemic H1N1 influenza 2009*, percentage and mortality rate (per million inhabitants), by age group and by country or continent, as of 16 July 2009 (n=468)**

Country or continent	Age group [years]								Total	Missing data
	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60+		
Canada	0	3	2	1	2	6	4	10	**28**	13
%	*0%*	*11%*	*7%*	*4%*	*7%*	*21%*	*14%*	*36%*	***100%***	*32%*
Mortality rate	0.00	1.67	0.48	0.22	0.43	1.15	0.81	1.48	**0.83**	
USA	5	8	22	29	22	34	34	24	**178**	64
%	*3%*	*4%*	*12%*	*16%*	*12%*	*19%*	*19%*	*13%*	***100%***	*26%*
Mortality rate	0.23	0.38	0.51	0.65	0.52	0.76	0.81	0.42	**0.56**	
Mexico	11	8	5	30	25	22	17	7	**125**	0
%	*9%*	*6%*	*4%*	*24%*	*20%*	*18%*	*14%*	*6%*	***100%***	*0%*
Mortality rate	1.10	0.77	0.24	1.65	1.39	1.64	1.81	0.68	**1.13**	
Latin America	6	6	7	18	14	3	6	4	**64**	13
%	*9%*	*9%*	*11%*	*28%*	*22%*	*5%*	*9%*	*6%*	***100%***	*17%*
Mortality rate	0.17	0.16	0.09	0.25	0.23	0.06	0.16	0.10	**0.16**	
Europe	1	4	3	0	2	2	0	2	**14**	5
%	*7%*	*29%*	*21%*	*0%*	*14%*	*14%*	*0%*	*14%*	***100%***	*26%*
Mortality rate	0.17	0.70	0.25	0.00	0.13	0.12	0.00	0.08	**0.13**	
Asia	0	2	5	2	2	9	3	3	**26**	3
%	*0%*	*8%*	*19%*	*8%*	*8%*	*35%*	*12%*	*12%*	***100%***	*10%*
Mortality rate	0.00	0.13	0.17	0.07	0.08	0.44	0.20	0.21	**0.16**	
Oceania	1	1	1	4	5	6	7	8	**33**	8
%	*3%*	*3%*	*3%*	*12%*	*15%*	*18%*	*21%*	*24%*	***100%***	*20%*
Mortality rate	0.61	0.62	0.29	1.13	1.41	1.62	2.10	1.61	**1.28**	
Total	**24**	**32**	**45**	**84**	**72**	**82**	**71**	**58**	**468**	106
%	***5%***	***7%***	***10%***	***18%***	***15%***	***18%***	***15%***	***12%***	***100%***	*18%*
Mortality rate	**0.26**	**0.34**	**0.24**	**0.46**	**0.43**	**0.54**	**0.57**	**0.36**	**0.40**	

* Individual data, except from Mexico where aggregated data originate from the Ministry of Health.
** Latin America: Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Equator, Guatemala, Honduras, Paraguay, Peru, Dominican Republic, Salvador and Uruguay; Europe: Spain and United Kingdom; Asia: Philippines and Thailand; China is not included; Oceania: Australia and New Zealand.

data show that age groups are not equally affected. Compared to younger age groups, the elderly seem to be protected from infection to some extent, perhaps due to previous exposure to strains akin to influenza A(H1N1)v virus [11-13]. When infection does occur, however, the percentage of deaths in elderly cases seems to be higher than in others. Initial estimates available from Mexico for the period until 16 July 2009 showed that the risk of death in aged cases (over 50 years) was higher (6% deaths among cases) than in children (0-1% deaths among cases aged 0-19 years) and young adults (2-4% deaths among cases aged 20-49 years) [3].

There was documented underlying disease in at least 49% of documented fatal cases worldwide to date. Diseases most frequently associated with death were the same as those identified for death from seasonal influenza. Nevertheless, two risk factors are noticeable: pregnancy and obesity. Pregnancy is a well-documented risk factor for severe infection and death in seasonal influenza and in previous pandemics [14-16]. The role of obesity, however, remains to be further analysed in order to ascertain whether the risk is linked with complications of obesity during intensive care [17,18] or with a severe course of disease due to diabetes frequently associated with obesity [19], or whether obesity plays a specific role in the pathogenesis of severe influenza A(H1N1) v infection, for example by interfering with the host's immune responses, as has been shown in rodents [20].

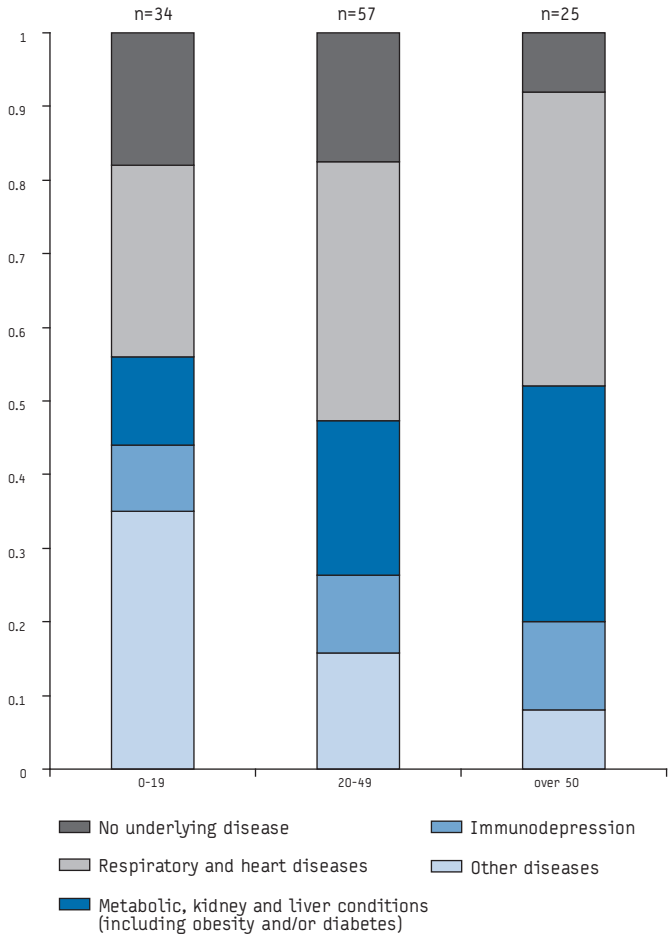
All the data presented here were from official sources and were carefully documented. Yet they are to be interpreted cautiously due to the variable quality of data regarding underlying disease (especially for pre-existing respiratory disease), small numbers, incomplete reporting using different formats, a mixture of individual and aggregated data, epidemic dynamics within the population (epidemics initially affecting school children or travellers) and population structure. For instance, we found that deaths in Canada seem to have been especially frequent in elderly women. Finally, the difficulty in determining whether the cause of death is attributable to influenza A(H1N1)v infection or to associated factors remains a major limitation.

The proportion of deaths with documented underlying disease must be interpreted with care due to a significant amount of missing data. There may be an information bias which overestimates the proportion of underlying disease since its presence may be reported more readily than its absence.

The analysis in this article is based on data collected only 10 weeks after the first international alert, and the pandemic is still in its very early phase. All evidence acquired so far remains to be completed and confirmed in the coming months, especially in view of the influenza epidemics currently ongoing in the southern

Figure 4

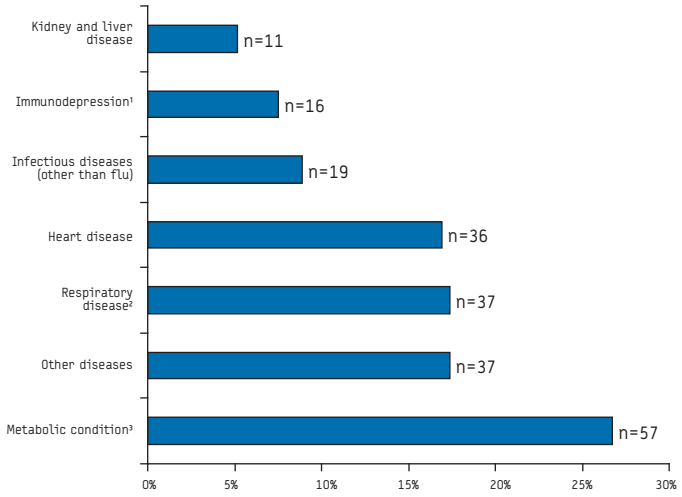
Distribution of underlying diseases in pandemic H1N1 influenza 2009-associated deaths by age, worldwide* as of 16 July 2009 (116 disorders documented in 93 fatal cases)



* Individual data, except from Mexico where aggregated data originate from the Ministry of Health.

Figure 5

Underlying diseases in pandemic H1N1 influenza 2009-associated deaths worldwide* as of 16 July 2009 (213 diseases documented in 193 fatal cases)



[1]Including tumour (n=5), transplantation (n=2) and auto-immune disease (n=3)

[2]Including asthma (n=8)

[3]Including obesity (n=7), diabetes (n=5), obesity and diabetes (n=1) and obesity and/or diabetes (n=41 for whom only aggregated data were available)

* Individual data, except from Mexico where aggregated data originated from the Ministry of Health.

hemisphere. Surveillance of the progression of the pandemic H1N1 influenza 2009 will focus more and more on severe cases. A more reliable CFR could be estimated through specific surveys, mathematical modelling, syndromic surveillance of influenza-like illness and of reported deaths in the population. Encouraging reporting in a common international format would also be useful.

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The importance of school and social activities in the transmission of influenza A(H1N1)v: England, April – June 2009

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During the containment phase in the United Kingdom (April to June 2009), a cluster of influenza A(H1N1)v cases was identified prompting further investigation and public health action by the Health Protection Agency. The first confirmed case, a pupil at a school in England, was imported. During the following two weeks, 16 further cases were confirmed with epidemiological links to the first imported case. In this cluster, we found that significant transmission occurred in two classes with attack rates of 17% and 7%. In each of the two classes a case had attended school whilst symptomatic. Other settings included a party and a choir. Minimum and maximum attack rates were 14% and 25% for the party. For the choir both the minimum and the maximum attack rate was 4%. We did not find any evidence of transmission on two school bus trips despite exposure over 50 minutes to a symptomatic case and over two periods of 30 minutes to a case during the prodromal phase (i.e. within 12 hours of symptom onset). Nor was there onward transmission in another school despite exposure over several hours to two cases, both of whom attended school during the prodromal phase.

Introduction

The first case of influenza A(H1N1)v in the United Kingdom (UK) was reported by the Health Protection Agency (HPA) in April 2009 [1]. Since then, the number of cases has been steadily rising. HPA data suggest that in England children under the age of 15 years are predominantly affected, with much higher rates of primary care consultation seen amongst the under 15 year-olds compared to the over 65 year-olds [2].

In the cluster of cases described below, the first confirmed case (X1), a pupil at school X, had acquired the infection whilst visiting a country with sustained human-to-human transmission of influenza A(H1N1)v. Over the following two weeks a further 16 people became ill and were confirmed as having influenza A(H1N1)v; they all had an epidemiological link to the same index case (X1).

Investigation by the HPA identified a number of school and social interactions amongst children and adults associated with three schools, including participation in a choir, use of school buses, and a party, where transmission may have occurred. Five of the 16 further cases were confirmed in pupils at school X, seven were pupils at two other schools (schools Y and Z), one was a sibling of a pupil at school Z and three were adult members of the choir.

Estimates of the risk of transmission associated with exposure in different settings and during the prodromal phase are scant in the literature to date. This paper describes the chains of transmission observed in a small but intensively investigated cluster in the early stages of the pandemic in the UK, and will contribute to the understanding of the risk of transmission as the pandemic continues.

Methods

During the investigation of this cluster, all cases were assessed using the HPA guidance algorithm in use at the time. Therefore, all possible cases who had either a history of travel to a country with sustained human-to-human transmission or an epidemiological link to a laboratory-confirmed case were tested using nose/throat swabs. Confirmed cases were investigated further and information on chronology, symptoms, travel history and any other exposures, as well as close contacts that may have needed prophylaxis were collected by the HPA.

For the purposes of this study, a line list was compiled of all laboratory-confirmed cases associated with the affected schools, the choir and the party. These confirmed cases were then analysed to elucidate probable chains of transmission based on day of onset of symptoms and association with different school or social settings.

Case definitions

A confirmed case was defined as an individual presenting with influenza like illness (ILI), in whom laboratory testing of a nose/throat swab had given a positive result for influenza A(H1N1)v. A secondary case was a confirmed case in whom onset of illness was between 24 hours and one week after direct contact with the index case (X1). A tertiary case was a confirmed case in whom onset of illness was between 24 hours and one week after contact with a secondary case and in whom there was no direct contact with the index case (X1).

Results

Chains of transmission

The epidemiological links observed between the confirmed cases (recorded by day of onset) are shown in the Figure. These are believed to be the most probable chains of transmission, taking into account information collected by the HPA.

School X

X1 attended school for approximately four hours whilst symptomatic with ILI on day 2 (but did not attend again until fully recovered). X1 had also attended school for the whole day on day 1. For some of that time X1 would have been in the prodromal phase,

which is defined for this study as the 12 hours prior to onset of symptoms. Over the next three days four further pupils (X2, X4, X5, X6) in the same class became symptomatic. Another pupil (X3), in the same year but different class than the index case, was also confirmed as a case. X2 and X3 were close friends.

The choir

Both X2 and X3 were members of a large choir comprising 107 adults (parents, staff, past pupils) and 62 children from schools X and Y. Choir members spent several hours together over the course of two days, during which time X2 became symptomatic. For some of that time, during day 2, X2 would have been in the prodromal phase. X3 was not symptomatic whilst at the choir. However for some time, during day 3, X3 may also have been in the prodromal phase. In addition to the two initial cases (X2, X3), a further six members became unwell with ILI and were subsequently confirmed as cases. Three of these six tertiary cases (P1, P2, P3) were adult members of the choir, and three (Y1, Y2, Y3) were pupils at school Y.

School Y

Two pupils, Y2 and Y3 attended school Y all day on day 5 whilst in the prodromal phase. Both became symptomatic on the evening of day 5 (symptom onset approximately 5 to 6 hours after school attendance). They did not subsequently attend school whilst symptomatic with ILI. There was no evidence of onward transmission at school Y.

A party

Two pupils from school X (X5, X6) attended a party of nine children, one of whom, the host's sibling, subsequently became unwell and was confirmed as the first case (Z1) in a third school (school Z). X5 was symptomatic on the day of the party which lasted for at least six hours. X6 became unwell the following day and Z1 two days after the party. It is possible that X6 was in the prodromal phase whilst at the party if infection had already been acquired from X1.

School Z

Z1 was symptomatic whilst at school for approximately four hours. Three further cases occurred at school Z. Two of these cases (Z2, Z3) were in the same year group as Z1. One additional confirmed case (Z4), in a different year group, was believed to be a result of sibling-to-sibling transmission (from Z2).

School buses

Case X1 used a school bus along with 42 other pupils from school X and Y for approximately 50 minutes whilst symptomatic. Two pupils from the bus subsequently reported ILI, but tested negative when swabbed.

Y3 also travelled on a school bus whilst in the prodromal phase on day 5. The journey was approximately 30 minutes in each direction with 17 other pupils from school Y. No child on the bus trip apart from Y3 reported ILI.

Other

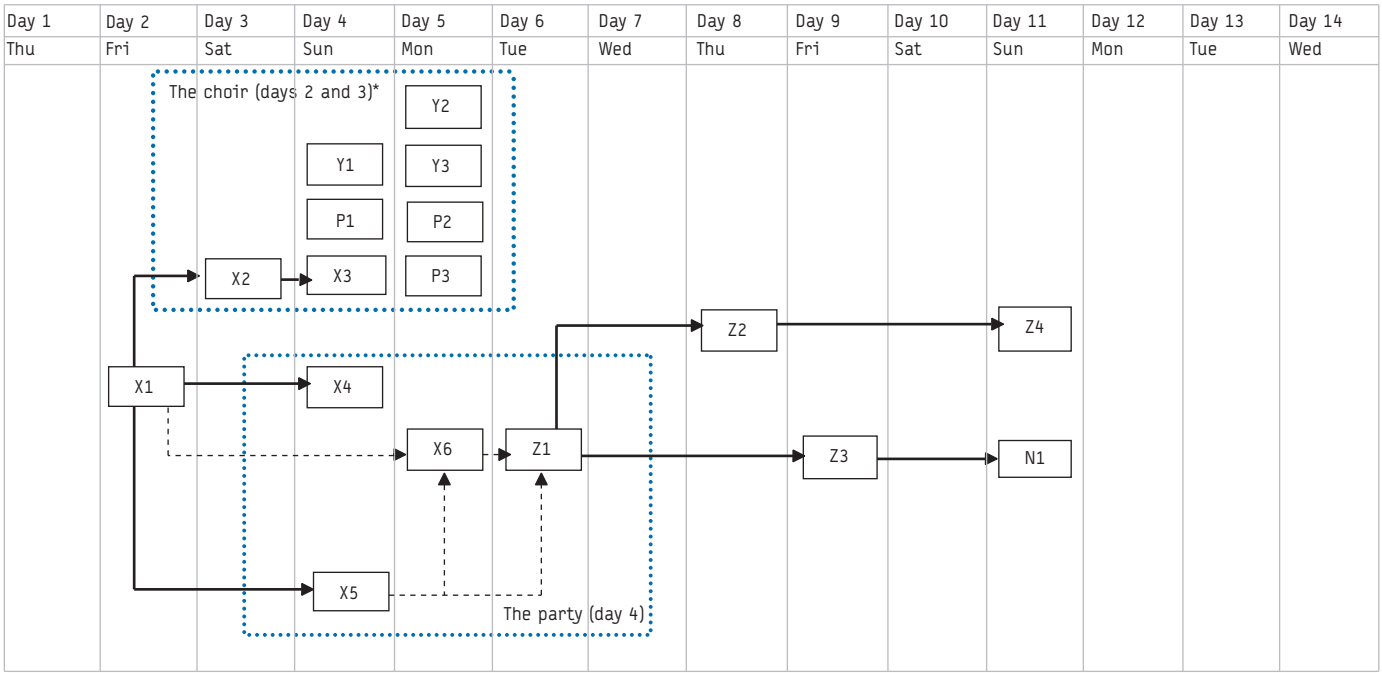
A further case (N1), who attended another school, was the sibling of Z3.

Attack rates

Attack rates have been calculated for each of the settings where cases were confirmed and are shown in Table 1. For school settings,

FIGURE

Probable chains of transmission amongst all laboratory-confirmed cases over a two-week period associated with the three schools (X, Y, and Z), the members of the choir, and a party according to day of onset of illness, England, April-June 2009 (n=17)



X1 – X6: pupils at School X; Y1 – Y3: pupils at School Y; Z1 – Z4: pupils at School Z; P1 – P3: adult members of the choir; N1: a sibling at another school

→ Single epidemiological link - - → One of two possible epidemiological links * No arrows show more than two possible epidemiological links

attack rates were calculated for the case's class, for other classes in the same year (excluding the case's class) and for the whole year. This is to reflect differences in cumulative exposure times. Both X1 and Z1 spent approximately four hours at school whilst symptomatic. During this time they were in contact with other pupils from their class. However, mixing with other pupils from the same year but different classes may occur for assembly and individual subjects. As a minimum, contact occurred during school breaks (morning break, lunch break) and in corridors between classes, with cumulative exposure times of at least one hour. For the choir and the party, both maximum and minimum attack rates have been calculated to reflect uncertainty around where and how infection was acquired and the possibility of co-primary infections. For example X3, who was close friends with X2, may have acquired the infection from X2 during the time spent together within the choir or outside the choir, i.e. in a different setting.

Attack rates were highest within the setting of the party and the classroom. The maximum attack rate for children at the party was 25% (2/8) and the minimum, 14% (1/7). Within the classes of X1 and Z1, attack rates were 17% (4/23) and 7% (2/27) respectively. These attack rates were substantially lower when the cases' year groups, rather than the class, were considered. The maximum and minimum attack rate for the choir was 4%.

There was no onward transmission on either of the two school buses, nor in school Y.

Table 1

Numbers affected and attack rates of laboratory-confirmed cases by setting, England, April-June 2009 (n=16, excluding index case X1)

Setting	Numbers affected		Attack rate(s) (%)	
School X				
Class of X1	4/23		17	
Other classes in the same year	1/96		1	
Total for whole year	5/119		4	
Choir	**Minimum**	**Maximum**	**Minimum**	**Maximum**
	6/167	7/168	4	4
Party	**Minimum**	**Maximum**	**Minimum**	**Maximum**
	1/7	2/8	14	25
School Z				
Class of Z1	2/27		7	
Other classes in same year	0/57		0	
Total for whole year	2/57		4	

Public health measures

At the time of this cluster, the UK was following a policy of epidemic containment. A risk assessment in line with HPA guidance was carried out in each setting to ascertain whether there was potential for transmission, and if school closure and the use of antiviral prophylaxis were indicated to prevent further spread of infection.

All three schools were advised to close for a period of one week, although in two cases this extended into scheduled school breaks. Antiviral treatment for cases and prophylaxis for contacts was provided as described in Table 2. In addition, all household contacts of confirmed cases were given antiviral prophylaxis. Advice was given to report any cases of ILI to the HPA, all of which were investigated with nose/throat swabs.

Table 2

Summary of public health measures that were implemented at each of the settings: schools X, Y, Z, the choir, school buses, and the party, England, April-June 2009

Setting/ Age group	Days between last exposure to case and prophylaxis	Group identified for prophylaxis	Proportion of group that were given prophylaxis	School Closure (if applicable)	Number of subsequent cases
School X/ Age 11-12	3	Year group of index case	100%	Closed for 10 days	5 in the same year (4 in the same class)
School Y/ Age 12-13	4	Year group of children who were prodromal whilst at school (i.e. within 12 hours of onset of illness)	93%**	Closed for 19 days (including half-term break)	0
School Z/ Age 7-8	3	Year group of first case identified at school.	100%	Closed for 21 days (including half-term break)	2****
Choir/ All age groups including adults	4	All choir members who attended events	78%***	Not applicable	6
Bus of X1/ Mixture of age groups	3	All children on the bus	100%	Not applicable	0
Bus of Y3/ Mixture of age groups	5	All children on the bus	100%	Not applicable	0
Party/ Mixture of age groups	3*		Not applicable	Not applicable	1

* Except for two who received prophylaxis one day and four days after the last exposure respectively.
** A number of pupils refused.
*** 35 members refused.
**** There were three more cases but not believed to be directly linked to the first case at school Z

It is possible that the patterns of transmission seen in this cluster were modified by the public health measures implemented, although, the same measures being applied in all settings, the direction of any effect should be the same across all settings.

Discussion

In this intensively investigated cluster of cases, high attack rates for influenza A(H1N1)v were observed in the classroom, at a choir and a party. In each of these settings there was cumulative exposure of several hours duration to a symptomatic case. Transmission of influenza A(H1N1)v was much lower amongst year groups of symptomatic cases who had shorter exposure times. There was no evidence of transmission on two school bus trips, despite exposure times of 50 minutes to a symptomatic case, and two periods of 30 minutes to a case who was in the prodromal phase. Nor was there any onward transmission in school Y despite exposure over several hours to two cases who had attended school during the prodromal phase.

Estimates of the risk of transmission of influenza A(H1N1)v in different settings and during the prodromal phase are scant in the literature to date. However, attempts have been made to model how children interact and thereby predict the likely patterns of spread in the event of a pandemic. One such modelling study [3] predicted that the school class and household were two of the most critical settings in terms of duration of contact and risk of transmission of infection. Events such as parties, though infrequent, were also associated with high predicted risk of transmission, as when they did occur, contact was prolonged. Other studies modelling the spread of respiratory pathogens have drawn similar conclusions, with school and social group activities generally involving closer contact of longer duration than travel activities [4].

The patterns of transmission anticipated by these modelling studies are partially borne out by our experience with this cluster of cases: higher transmission was seen amongst classmates and social groups compared with those sharing transport. On the other hand, very little transmission was seen amongst household contacts of confirmed cases. This may be due to effective antiviral prophylaxis which was administered to all household contacts as soon as a swab result tested positive for influenza A (before typing confirmed H1N1v).

Aside from duration of exposure, which in this cluster was a strong determinant of onward transmission, specific characteristics of the exposure setting may have contributed to the spread, particularly closeness of contact as predicted in certain social settings [3], and in the case of the choir, increased aerosolisation of respiratory secretions during singing. This has been documented with high levels of transmission of tuberculosis within choir settings before [5-6].

As part of the management of this cluster, all children, in the same year or sharing a school bus with a case who was within the prodromal phase, were given antiviral prophylaxis. This was in line with HPA guidance [7] at the time, during the containment phase. Policy with regard to school closure and use of antiviral prophylaxis changed later as the UK moved from the containment phase to the treatment phase.

In this cluster, we did not see any onward transmission of influenza A(H1N1)v from cases Y2 and Y3, both of whom were at school during the prodromal phase. Neither did we observe any transmission as a result of contact with Y3 on the school bus. This would indicate that risk of transmission during the prodromal phase is low. However, it is possible that the short incubation periods (of approximately 24 hours) observed before the onset of symptoms in X2 (following exposure to X1), and in those members of the choir who became symptomatic on day 4 (X3, Y1 and P1), may be accountable, in part, to exposure to cases (X1 and X2 respectively) during their prodromal phases.

Limitations

The patterns of transmission described are highly possible based on public health investigation of laboratory-confirmed cases. Given the small numbers described, caution in interpretation is needed. Although the HPA advised all individuals to report symptoms, there is a possibility that some individuals did not. Patterns of transmission are likely to have been modified by the public health response. Moreover we have no measure of the extent, if any, of asymptomatic carriage.

Conclusions

This study describes a small cluster in of influenza A(H1N1) v cases which was thoroughly investigated and epidemiological links characterised with reasonable precision. Our findings add weight to the argument that social activities are important routes of transmission which means that in the containment phase, school closure alone may not be enough to interrupt transmission. On the other hand, we did not find any evidence for transmission on school buses in this cluster. Given that the closeness and frequency of contact on public transport is likely to be less than amongst children using dedicated school buses, it may also be hypothesised that risk of transmission on public transport would also be low. Further work is warranted looking at the usefulness of social distancing measures in each of these settings (school, social groups, transport) in interrupting transmission of influenza A(H1N1)v.

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Epidemiological and clinical characteristics of influenza A(H1N1)v infection in children: The first 45 cases in Cyprus, June – August 2009

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Following the first imported case in a tourist in Cyprus on 2 June 2009, the influenza A(H1N1)v virus has spread on the island affecting mainly young adults and children. We describe here the first 45 cases in children. Fever, cough, rhinorrhoea and sore throat were the most common symptoms of infection. Half of the children had fever for one day or only for a few hours. Five children were hospitalised, and overall their symptoms were mild. Adherence to oseltamivir treatment was very high, with low frequency of gastrointestinal side effects such as nausea and vomiting. Camping places and summer schools played a significant role in spreading the infection among children of school age.

Introduction

Despite the rapid spread of the pandemic influenza A(H1N1)v virus [1,2], most cases did not have a serious course of disease. About 2-5% of people with laboratory-confirmed infection needed hospitalisation in the United States (US) and Canada [3]. Between half and two thirds of hospitalised cases had co-morbidities such as asthma, other chronic pulmonary disease, diabetes, and autoimmune disorders [3,4]. Fatalities due to pandemic H1N1 influenza have also occurred [5].

Based on seasonal influenza data, children under the age of five years and especially those under the age of two years, as well as those with underlying chronic conditions are at substantially higher risk of hospitalisation compared to older or otherwise healthy children. Pulmonary complications such as bronchitis or pneumonia, neurological complications (e.g. encephalitis or encephalopathy) or a sepsis-like syndrome in neonates have been reported even in previously healthy children [6]. Recent data support the development of neurological complications in children in association with the influenza A(H1N1)v infection in the US [7]. These data as well as the uncertainties about the severity of the evolving epidemic among children resulted in an Emergency Use Authorization decision of the US Food and Drug Administration supporting the use of the neuraminidase inhibitor oseltamivir during the current epidemic even for children under the age of one year [8].

On 2 June 2009, the first confirmed case of pandemic H1N1 influenza was reported in Cyprus. Here we describe the epidemiological and clinical characteristics of the first 45 cases of influenza A(H1N1)v virus infection among children under the age of 16 years, seen at the Archbishop Makarios Hospital in Nicosia.

Methods

Definitions of suspected, probable and confirmed cases were issued by the Department of Medical and Public Health Services at the Ministry of Health (MOH) in accordance with those issued by international organisations. All cases under 16 years of age seen from 4 July to 6 August 2009 at the Archbishop Makarios Hospital (AMH) in Nicosia are described. The AMH is the only referral hospital for mother and child care in Cyprus. For each child examined or admitted to the AMH, a questionnaire was obtained with information on age, residence, possible epidemiological link to A(H1N1)v influenza cases, symptoms, underlying risk factors for severe disease, treatment with oseltamivir and follow-up. Diagnosis was confirmed by testing respiratory samples (nasopharyngeal and pharyngeal swabs) with RT-PCR with specific primers for influenza A(H1N1)v virus. Cases were reported to the Department of Medical and Public Health Services with demographic information as well as clinical details.

Results

The first paediatric case was a 15 year-old boy who developed symptoms on 2 July 2009. He was a household contact of his older sister who had developed influenza-like illness after spending her holidays at a tourist resort in Cyprus. A few days later the third sibling also fell ill with similar symptoms. Two of the children tested positive for influenza A(H1N1)v virus in their respiratory secretions. By 6 August, a total of 45 laboratory-confirmed cases, all 15 years-old or younger, had been detected (Figure 1).

The confirmed cases were between 40 days and 15 years-old with a median age of nine years (Figure 2). Ten of these cases were five years-old or younger and four of them were under the age of one year.

Table

Clinical findings in children with laboratory confirmed influenza A(H1N1)v virus infection

Symptom	Number of children / all children for whom this information was available	(%)
Fever	44/45	98
Cough	43/45	96
Rhinorrhoea	34/43	79
Vomiting	8/39	21
Diarrhoea	7/40	18
Conjunctivitis	3/45	7
Sore Throat*	25/34	73
Malaise*	21/31	68
Headache*	17/30	57
Arthralgia*	8/34	24

* assessed in children over the age of five years (n=35)

Figure 1

Cases of laboratory-confirmed influenza A(H1N1)v in children, by day of symptom onset, Cyprus, 2 July – 6 August 2009 (n=45)

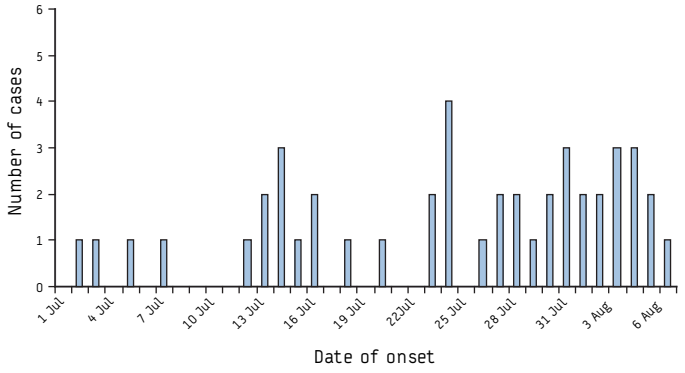
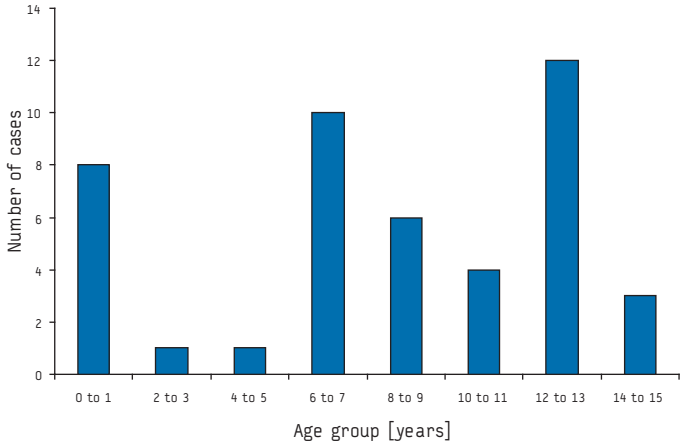


Figure 2

Cases of laboratory-confirmed influenza A(H1N1)v in children, by age group, Cyprus, 2 July-6 August 2009 (n=45)



As of 20 August 2009, no influenza-related fatalities have occurred in Cyprus. Five of the children were hospitalised, one due to very young age (40 days-old), one because of mild complicating pneumonia, and the remaining three children because of concurrent problems not necessarily related to influenza. Mean duration of hospitalisation was 3.4 days (range 1-7 days). Only two of the hospitalised children required treatment with oseltamivir. None of the hospitalised children had underlying chronic diseases.

Only three of the children diagnosed with influenza A(H1N1) v virus infection had underlying risk factors for severe influenza infection, all of them chronic asthma. One of them was additionally obese. All three received oseltamivir and made a quick recovery.

Cases generally presented with symptoms typical of influenza infection as described in the Table. Subjective symptoms such as headache or sore throat were only assessed in over five year-olds. Half of the 34 children with complete fever information had fever for only one day, in nine children the fever lasted for two days, and in eight cases it lasted for three or more days. The median duration of fever in laboratory-confirmed cases was one day.

Fourteen confirmed cases were linked to an index case though their household or a close friend, two of them were travel-related and the remaining 26 cases were linked to six different clusters. For three cases no epidemiological link could be identified. The six clusters were related to camping places (three clusters), summer schools (two clusters) and a handball team that had visited Italy (4 cases). In seven out of 10 cases in children under five years, the transmission was related to household members. For the remaining three, one was associated with family travel, one with a summer school cluster, while the transmission link for the last was unknown.

Policy for the management of cases and contacts

During the first few weeks of the outbreak, oseltamivir treatment was given to all suspected, possible and confirmed cases until confirmatory laboratory results were available. Contacts were traced and offered antiviral prophylaxis. Suspected, probable and confirmed cases were requested to stay at home and avoid contact with other people for at least seven days. Following new guidance from the Ministry of Health on 22 July 2009, treatment with oseltamivir was not offered to every paediatric case but only given to children who had severe symptoms or were up to five years old, and to those with an underlying risk factor that could contribute to severe disease. Furthermore, since no prophylaxis was given to the contacts, contact tracing for index cases was abandoned and only household members and close friends were advised to seek medical advice in case of fever or respiratory symptoms.

Treatment with oseltamivir, compliance and side effects

Nineteen of the confirmed cases were treated with oseltamivir. Seven children received oseltamivir because of the initial 'treatment for all' policy before 22 July 2009, three because of underlying chronic asthma, four because of persistent fever more than five days or because of complicating pneumonia, and five children because of their very young age (under two years-old). Compliance was assessed by telephone interviews during the follow up assessment of confirmed cases. Fifteen of 17 contacted parents reported that their child had taken the full course of treatment as prescribed. Only two of those who received the medication presented with side effects. Both of them developed gastrointestinal symptoms such as vomiting and nausea. In one of those cases vomiting was so severe that the antiviral treatment was discontinued. No children developed stomach pain or neuropsychiatric side effects.

Discussion

The H1N1 influenza pandemic started late in Cyprus as the first case was detected on 2 June. After the first case however, the disease spread quickly, initially among younger people who visited tourist resorts and entertainment clubs or school-aged children who stayed at camping places or summer schools. Most children of preschool age as well as infants and toddlers, who represent 22% of our cases, acquired the infection mainly through household contacts. Similar rates of household transmission were noted in the first descriptions of the outbreak in the United Kingdom (UK), although the UK rates were not based only on infants and toddlers [9].

The incidence rate of gastrointestinal symptoms such as diarrhoea among confirmed cases in children was found to be 17%. It is difficult to compare with similar series in other countries as no other paediatric series has been published as yet. In series not differentiating children, the frequency of diarrhoea ranged from 3% in Germany to 28% in the UK [9,10].

As observed elsewhere [11,12], the course of disease in our patients appeared to be mild, as half of them had fever for a maximum of one day. Despite the fact that five of the children in our series were hospitalised, only one of them had mild pneumonia as a complication related to influenza. The other children were mostly admitted for monitoring.

Compliance with oseltamivir treatment in our study was high with over 80%. Furthermore, the rate of side effects, two of 19 cases, was low. The only side effects seen in the children were nausea and vomiting, the most common side effects reported in the literature [13,14]. In a recent study on school-age children in the UK, who received oseltamivir for influenza prophylaxis, the rate of adverse effects was much higher, since 40% of the students developed gastrointestinal symptoms, and 18% had mild neuropsychiatric side effects such as poor concentration, sleeping problems, bad dreams and strange behaviour [15]. No patient in our series presented with any kind of neuropsychiatric side effects as described in that report.

Our study's limitations include the possibility that paediatric cases in the Nicosia district might have been underdiagnosed, since many children with viral upper respiratory illness and strong epidemiological link to influenza cases, including children who became ill in summer camps, did not visit the hospital for assessment, but preferred to visit their private family paediatricians. In addition, patients were only considered suspected cases and were tested for the influenza A(H1N1)v virus if they fulfilled the strict definition of suspected case and therefore fever was a necessary prerequisite. All but one case of confirmed influenza infection in our series (98%) had fever, whereas in various reports from other countries, fever was present in 90 to 95% of cases [4,9]. Finally, the number of patients with pandemic H1N1 influenza in Cyprus is relatively small in comparison to the number of cases reported in other countries. Therefore, our conclusions regarding the severity of the illness may change as the number of cases increases.

Conclusion

Influenza A(H1N1)v virus infection has spread rapidly in Cyprus. Symptoms among children were classic and the majority of paediatric cases had a mild clinical course. Treatment with antivirals appears to have not had any major adverse effects. Despite the summer season and the schools being closed, places such as summer schools and camps contributed significantly to the spread of the disease among children. Regardless of the above, we need to focus on the coming influenza season and apply different methods including the coming influenza A(H1N1)v vaccine in order to avoid severe cases, which may inevitably occur due to the low level of immunity to the pandemic virus strain or affect vulnerable segments of the population.

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

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

Introduction

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

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

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

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

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

Methods

S. suis strains

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

Susceptibility testing

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

Genotyping

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

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

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

Table 1

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

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

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

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

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

Results

Capsular (cps) and virulence-associated genes

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

Table 2

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

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

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

TABLE 3

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

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

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

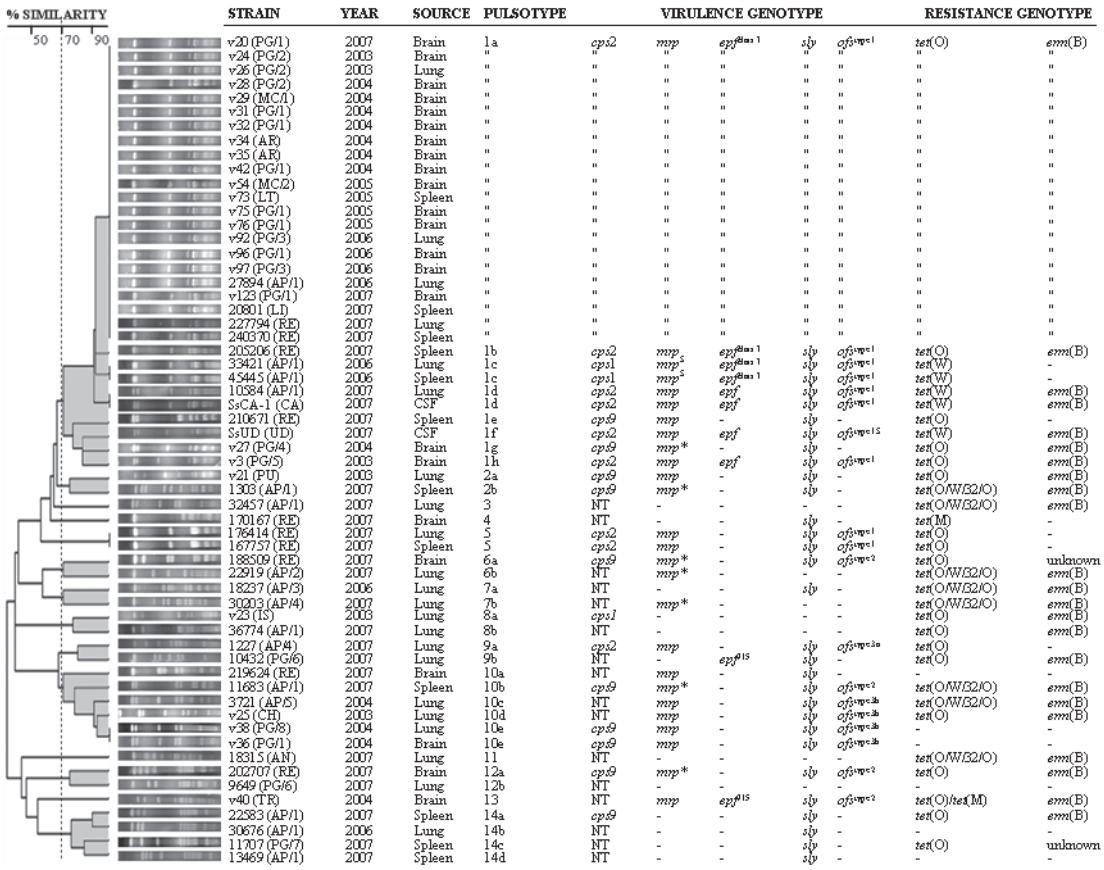
The *mrp* gene (three size variants: *mrp*; *mrp** and mrp^{S}) was detected in 47 strains (all 30 *cps*2 isolates, nine *cps*9, six NT, and two *cps*1 isolates); *epf* (three size variants: *epf*; $epf^{\text{class I}}$ and epf^{915}) was detected in 31 strains (27 *cps*2, two *cps*1 and two NT isolates);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

Susceptibility testing and detection of resistance genes

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

FIGURE

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



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

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

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

TABLE 4

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

Profile	Invasive	Non-invasive
cps2 isolates (*n* = 30)	23	7
mrp epf$^{\text{class I}}$ *ofs* $^{\text{type 1}}$ *sly arcA*	19	4
mrp epf ofs $^{\text{type 1}}$ *sly arcA*	2*	1
mrp epf ofs $^{\text{type 1S}}$ *sly arcA*	1	-
mrp ofs $^{\text{type 1}}$ *sly arcA*	1	1
mrp ofs $^{\text{type 3a}}$ *arcA*	-	1
cps1 isolates (*n* = 3)	1	2
mrp^{S} *epf*$^{\text{class I}}$ *ofs* $^{\text{type I}}$ *sly arcA*	1	1
arcA	-	1
cps9 isolates (*n* = 10)	8	2
mrp ofs* $^{\text{type 2}}$ *sly arcA*	3	-
mrp ofs $^{\text{type 3b}}$ *sly arcA*	1	1
mrp sly arcA	1	1
mrp sly arcA*	2	-
sly arcA	1	-
[a]NT isolates (*n* = 16)	5	11
mrp epf^{915} *ofs* $^{\text{type 2}}$ *sly arcA*	1	-
mrp ofs $^{\text{type 3b}}$ *sly arcA*	-	2
epf^{915} *sly arcA*	-	1
mrp sly arcA	1	-
mrp arcA*	-	2
sly arcA	3	2
arcA	-	4

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

PFGE typing and MLST

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

Clones

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

Discussion and conclusion

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

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

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

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

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

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

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

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

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

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

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

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

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

Introduction

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

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

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

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

Methods

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

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

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

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

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

Table 1

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

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

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

Table 2

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

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

CI: confidence interval.

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

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

Results

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

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

The pattern of antibiotic use

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

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

Use of hospital-specific antibiotics (HSA)

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

Indications for antimicrobial therapy

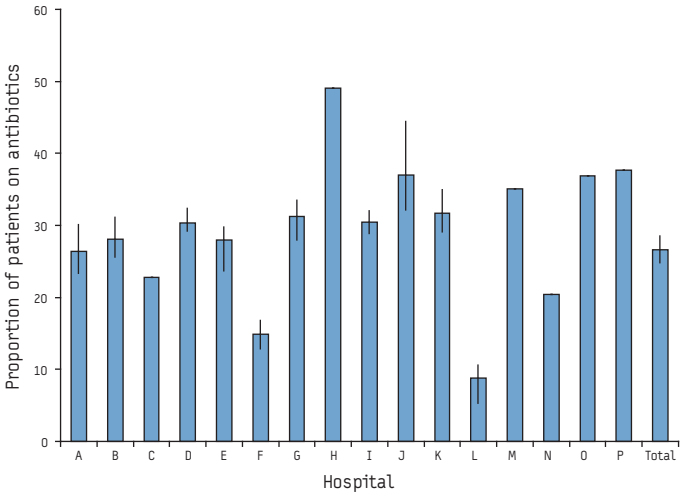
Infection

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

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

Figure 1

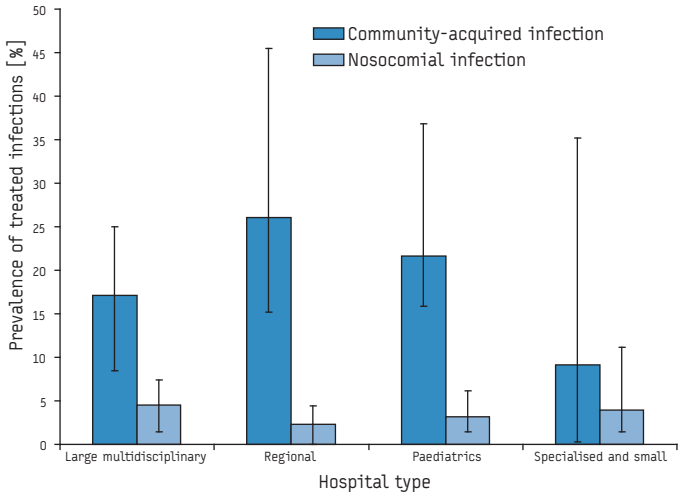
Mean number of patients receiving antibiotics with maximal and minimal annual variations, Latvia, 2003-2007



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

Figure 2

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



Surgical prophylaxis

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

Unclear use

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

The route of administration

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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