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Editorials

Building capacity in field epidemiology: lessons learned from the experience in Europe

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This issue of *Eurosurveillance* is devoted to training of field epidemiologists within diverse public health systems and highlights the contributions these programmes are making in Europe. The articles describe national field epidemiology training programmes (FETPs) [1], the European Programme for Interventional Epidemiology Training (EPIET) [2] and its transition to the European Centre for Disease Prevention and Control (ECDC) [3], how ECDC through its training activities is contributing towards building capacity in surveillance and response in communicable diseases, as well as the strengths and challenges of the various models of applied epidemiology training [4].

FETPs are two-year training programmes in applied epidemiology, based on a model of 'learning by doing'. They build public health capacity infrastructure by strengthening the public health workforce and surveillance systems. Key elements of these programmes enable their success and sustainability (Box) [5,6].

FETPs fill an important gap by increasing the number of competent field epidemiologists, but the programmes go beyond training: the fellows also provide services needed by the host country, such as outbreak detection and response. Furthermore, and perhaps most importantly, the programmes contribute to the strengthening of the public health system as a whole. The majority of graduates stay within the public health system, and many take on positions of leadership, changing the culture to one of using data for decision making [6-8].

EPIET, the national FETPs, and the EPIET-associated programmes (where fellows from national programmes participate in the classroom training with the EPIET fellows) described here are part of a larger community of FETPs, linked together in a global network, the Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET). Currently within TEPHINET there are 32 registered programmes (www.tephinet.org). Through partnerships with the host countries, the European Union (EU), the World Health Organization (WHO), TEPHINET, the United States Centers for Disease Control and Prevention (US CDC), multiple donors as well as private organisations, the number of FETPs continues to grow. The US CDC engage with 18 of these programmes outside Europe, providing a range of support from short-term technical assistance to placing a resident advisor from the US CDC within the ministry of health of the host government.

Within Europe, these applied epidemiology programmes are vigorously involved in public health surveillance and response activities, especially outbreak investigations.

Within Europe, these applied epidemiology programmes are vigorously involved in public health surveillance and response activities, especially outbreak investigations. Bosman *et al.* report that EPIET and EPIET-associated programmes produced 340 publications in peer-reviewed journals over 12 years, all derived from fellowship projects [2]. Measuring FETPs' successes must take into account their intent to both train the next generation of public health leaders in epidemiology and to provide service and strengthen the health systems of their host governments. Success indicators such as number of graduates, field investigations, publications, and international missions are easier to obtain, while tracking career choices after graduation, number of graduates in leadership positions in public health, and their impact on policy decisions and public health systems are much harder to quantify.

Although the various programmes are linked in their approach to train epidemiologists, they use different models based on the respective country's needs and the programme's objectives. Krause *et al.* provide an overview of five national FETPs and compares them to EPIET [1]. The authors address a number of challenges related to retention and sustainability. For example, teaching in the native language in national FETPs assures that more of the most qualified and appropriate candidates can participate and may improve retention of the graduates in the country, but lack of English proficiency often limits the ability of the fellows to participate in activities in the international scientific community. Recruiting into the programmes from within the public health service may also improve retention, but may limit the ability to attract new, young scientists. Providing a university degree upon completion of the

Box

Key elements of field epidemiology training programmes

1. Competency-based curriculum
2. Mentorship by a senior field epidemiologist
3. Majority of participant's time spent in field and in service to host government priorities
4. Recruitment and training of graduates as mentors as the programme expands
5. Translation of data for evidence-based decision making
6. Programme initiates sustainability planning at an early stage

programme may enhance recruitment, retention, and opportunities for promotion in some countries, but may jeopardise the quantity and quality of field work if rigid university requirements reduce the availability of fellows for field activities. Sustainability relies heavily on the ability to retain graduates, as the programmes cannot be sustained or expanded unless fellows serve as mentors and supervisors after their graduation. Finally, the need to train more field epidemiologists is constantly threatened by funding and administrative issues.

It requires substantial resources to start and maintain an applied epidemiology training programme. Bosman *et al.* estimate that the EPIET programme costs between EUR 2.3 and 3.2 million per year for cohorts 8 through 11 [2]. Bremer *et al.* report that since the transition of EPIET to the ECDC in 2007, 84% of the participants' salaries are funded by ECDC [3]. In the national FETPs, the country usually covers the costs of the participant's salary, since the participants are performing services for the government during their training. The majority of the costs are related to personnel required to supervise the participants and to supporting the introductory course and intermittent modular trainings.

Despite the relatively high costs, a demand for more qualified epidemiologists in Europe remains. Several articles appeal for the number of EPIET fellows to be increased, for strategies to facilitate return of these fellows to their country of origin, and creation of more FETP-like national programmes [3,4]. Krause *et al.* [4] suggest seconding an EU senior epidemiologist to new FETPs, much like the seconding of US CDC experts to the German and Italian FETPs. In some cases a regional approach might make sense. The cost of a national FETP in Europe is not presented, but the average cost of supporting a FETP by the US CDC is about USD 1 million per year, in the case where CDC remains fully engaged over a period of approximately five years. The costs decrease when the CDC resident advisor departs and the country takes over full responsibility for the programme.

Expanding the scale of FETPs within countries is another way of addressing the need for skilled epidemiologists. FETPs typically train 10 to 15 professionals in each cohort per year at the national level. Even with unlimited resources, there is an operational limit in the number of participants due to size of classrooms, number of supervisors and mentors, office space, etc. Having multiple FETPs within a country is an option, with each catering to different audiences. State-based FETP-like programmes exist in the US [9], and provincial FETPs are established in China. These programmes work together; for example, the national FETP in China sends fellows to the provinces for field experiences and the provinces ask the national FETP to assist with modular trainings. An annual scientific conference provides another opportunity for the provincial and national programmes to interact and learn from each other.

A key question is how many epidemiologists are needed. The Council of State and Territorial Epidemiologists (CSTE) in the US has recommended that the number of epidemiologists working in a state in the US be proportional to population size at the rate of at least one per 100,000 [10]. Based on this recommendation, the US currently has 30% fewer epidemiologists than recommended, even though the Epidemic Intelligence Service (EIS) programme has produced more than 3,000 graduates since 1951, with an additional 161 officers currently enrolled in the programme. Certainly not all epidemiologists working within a country need to go through FETP training. At different levels of the public health system, epidemiologists will need different skill sets. The Central America Regional FETP is an example of a comprehensive approach to training epidemiologists at multiple levels [8]. The curriculum is divided into a three-tiered training pyramid that corresponds to the needs at the local, district and central levels of the health system.

The articles in this special edition of Eurosurveillance disclose a vibrant network of applied epidemiology training programmes and epidemiology training activities, which are building public health workforce capacity in Europe. The health workforce is one of the six fundamental building blocks in the WHO health system framework [11], yet one of the greatest challenges to building effective public health systems globally continues to be the critical shortage of skilled public health workers [12]. Building sustainable health systems with a strong public health workforce and well-functioning surveillance and response systems will require commitment and support from all parts of the global public health community, based on the principles of the "Paris Declaration on Aid Effectiveness" calling for greater harmonisation of development resources [13]. By investing more strategically, donors and partner countries can not only achieve immediate impact through disease-specific programmes, but also contribute to the strengthening and the long-term sustainability of the health system. Within the global epidemiology community, we have a responsibility to address the critical needs through strengthening international and regional networks, evaluating programmes, piloting innovative approaches, sharing experiences and lessons learned, and determining the most effective approaches to support further investment.

Graduates from applied epidemiology training programmes, such as the ones described in this special edition, will play leading roles in defining and addressing crucial health problems in their countries and the international community.

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Editorials

The European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) - selected papers from the conference 2008

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1. Members of the scientific committee are listed at the end of the editorial

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This issue of Eurosurveillance has two focuses: a special issue on capacity building and training for applied field epidemiology in Europe [1] and a focus on the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) by featuring two papers based on presentations made at ESCAIDE 2008. The authors of these papers were invited by the Eurosurveillance editors to submit an article for peer-review after the abstract selection had taken place, because of their overall quality and the focus on information for action. In their contribution from Thailand, Pawun *et al.* report on a field-investigation of a nosocomial outbreak of bullous impetigo in newborns, caused by *Staphyloccoccus aureus*, in a hospital in northern Thailand [2]. The results from this investigation lead to the implementation of immediate measures that stopped the outbreak. Moreover, the awareness raised of the problems identified during the investigation triggered the implementation of measures to prevent similar outbreaks in the future. The second paper by Girardi *et al.* reports on the diagnosis of latent tuberculosis infection, an issue of considerable debate [3]. The authors compare sensitivity and specificity of interferon-gamma assays for latent tuberculosis infection by assessing the association of test results with tuberculosis occupational exposure in 115 health care workers by using latent class analysis. They found that the estimated specificity of in vitro assays was higher than that of Tuberculin skin tests (TST) also among individuals who were not BCG-vaccinated and from their data the authors conclude that when applied in healthcare workers, in vitro assays may provide a significant increase of specificity for tuberculosis infection compared to TST, even among non-vaccinated individuals, at the cost of some sensitivity.

The two papers presented serve as good examples for some of the unique features of ESCAIDE; the conference's focus not only on applied science and epidemiology (including field investigations), but on the direct, concrete application of study results for public health action. ESCAIDE is supported by (ECDC) European Centre for Disease Control and Prevention and jointly organised by ECDC, the European Programme for Intervention Epidemiology Training (EPIET), the EPIET Alumni Network (EAN) and the Training Programs in Epidemiology and Public Health Intervention NETwork (TEPHINET EUROPE). Besides sharing scientific knowledge, ESCAIDE provides an excellent opportunity for experts with a wide range of various backgrounds who are involved in epidemiology and infectious disease control and prevention to strengthen and expand networks and share experiences. The first ESCAIDE took place in October 2007 in Stockholm and was followed by a conference in Berlin in October 2008. At the time of publication of this editorial, the third ESCAIDE in Stockholm has just come to its end. From start, ESCAIDE has been a success with constantly well over 600 visitors and an annual increase of submitted abstracts of around 10 percent. Even if the focus of the conference is Europe, its' reach is global; in 2009, besides from Europe, participants came from Australia, Brazil, Canada, China, Hong Kong, New Zealand, Pakistan, the Philippines, Thailand, the Unites States and Vietnam. Pandemic H1N1 influenza has understandably been given some focus during the 2009 conference. However, as in previous years, many other topics were covered in the various sessions. Topics covered by plenary sessions ranged from ageing and infectious diseases to influenza vaccination and to new methods for analysing outbreaks. A new and special focus on this year's ESCAIDE meeting was the viewpoint from the laboratory and its role in public health, with a plenary session on what genotyping has to offer epidemiologists. More specific information on the conference can be found on a dedicated website (www.escaide.eu/) [4].

Besides sharing scientific knowledge, ESCAIDE provides an excellent opportunity for experts with a wide range of various backgrounds who are involved in epidemiology and infectious disease control and prevention to strengthen and expand networks and share experiences.

Given that ESCAIDE is both a forum for exchanging scientific knowledge and good practice as well as for networking and personal professional development, the two focuses of this Eurosurveillance issue stand well side-by-side: ESCAIDE and capacity building and training for applied field epidemiology in Europe.

Members of the ESCAIDE scientific committee are: Andrea Ammon, ECDC, Arnold Bosman, ECDC, Viviane Bremer, ECDC/EPIET, Johan Giesecke, ECDC (chair), Gérard Krause, ECDC Advisory Forum, Marion Koopmans, European Society for Clinical Virology , Davide Manissero, ECDC, Barbara Schimmer, EPIET Alumni Network, Ines Steffens, ECDC, Howard Needham, ECDC, Panayotis Tassios, European Society of Clinical Microbiology and Infectious Diseases.

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Euroroundups

Differences and Commonalities of National Field Epidemiology Training Programmes in Europe

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From 1994 to 2009, national field epidemiology training programmes (FETP) have been installed in Spain, Germany, Italy, France and Norway. During their two year duration, different components of the FETP are devised as follows: 63-79 weeks are spent on projects in hosting institutes, 2-26 weeks in outside projects, 9-30 weeks in courses and modules, and 1-2 weeks in scientific conferences. A considerable proportion of the Spanish FETP has is provided conventional 'class room training'. The content of the modules is very similar for all programmes. Except from the Italian programme, all focus on infectious disease epidemiology. The German and Norwegian programmes are so called EPIET-associated programmes as their participants are integrated in the modules and the supervision offered by EPIET, but salaries, facilitators, and training sites are provided by the national programme. These EPIET-associated programmes require strong communications skills in English. Alumni of all five FETP are generally working within the public health work force in their respective countries or at international level, many of them in leading functions. Although three new FETP have been installed since the last published 'Euroroundup' in Eurosurveillance on European FETP in 2001, the progress with respect to the establishment of national FETP or EPIET-associated programmes has been slow. Member States should be aware of how much support EPIET can offer for the establishment of national FETP or EPIET-associated programmes. However, they also need to be ready to provide the necessary resources, the administrative environment and long-term dedication to make field epidemiology training work.

Introduction

In March 2001, a special issue of Eurosurveillance presented reports on different field epidemiology training programmes (FETP) in Europe and the United States [1,2]. At that time, in Europe, national FETP were in place in France, Germany and Spain. These three programmes now look back on more than 10 years of experience and Norway and Italy have created additional national FETP since. This 'Euroroundup' aims to provide an overview of the existing five national FETP. It focuses on their respective history, their objectives and organisational details and discusses differences and commonalities with reference to the European Programme for Intervention Epidemiology Training (EPIET) as it is a multinational field epidemiology training programme in Europe. Furthermore, the analysis intends to provide a basis for further discussions of the strengths of FETPS for capacity building in Europe and the remaining challenges.

France

Since the late 1990s, changes occurred in the French public health arena: in 1998 the Institute of Public Health Surveillance (InVS) and its regional offices were created to reinforce the surveillance of and response to alerts and threats to public health and in 2002, in the context of bioterrorist threats, the French Field Epidemiology Training Programme PROFET (Programme de formation à l'épidémiologie de terrain) was launched. The programme was run in cooperation between the InVS and the National School of Public Health (EHESP) and built on a three-week intervention epidemiology course (IDEA) which had been ongoing since 1984 [3,4]. PROFET was set up with the aim to build capacity for preparedness and response in the field of public health, and in the development of public health surveillance. It intended to provide qualified professionals primarily to the national institute and its regional offices.

As most FETP, PROFET is based on the principle of 'learning by doing', fellows may carry out projects in the field of communicable diseases and environmental health, but also in occupational health, chronic diseases and injuries. They are expected to publish in the French national epidemiologic bulletin or in other national or international journals, and to give an oral presentation at an epidemiologic conference. During their two year training, the fellows attend six one-week training modules with specific topics: computer tools for outbreak investigation, risk assessment in environmental health, logistic regression, sampling, scientific writing, surveillance. The training is conducted in French by InVS epidemiologists and set up specifically for the fellows. However, some modules are open for external participants as well. At the end of the training, an assessment is made of the outcomes of the fellows but no formal diploma is awarded upon completion.

PROFET targets young public health professionals who are willing to get involved in field epidemiology in the French public health system. Candidates must have a master degree in the field

of public health, or equivalent. The programme is run jointly by two scientific coordinators from InVS and EHESP (respectively 0.7 and 0.3 fulltime equivalents [FTE]). Fellows are employed and paid by InVS with a specific trainee salary. The cost of the programme is mainly made up of salaries (90%) and of travel costs for training and conferences (9%). Costs directly related to the daily activities are included in the training site's budget. Since 2002, seven cohorts have been enrolled, amounting to 40 fellows (five cohorts of six fellows each and the two last cohorts of five fellows each). Trainees were mainly public health graduates (master in public health, or epidemiology), public health engineers, biostatisticians, pharmacists, public health nurses and veterinarians. Only one physician entered PROFET because medical students who want to specialise in field epidemiology generally apply for a residency at InVS during their public health medicine training. All 30 fellows of the five completed cohorts have successfully terminated the programme and all, except one, have been recruited in the public health network after this: 19 at InVS (11 at the national headquarters, 8 in regional offices) and 10 work for other public health partners in France.

After eighteen years of successful experiences with the IDEA course, the start of PROFET was intended to accompany the development and the regionalisation of the surveillance and response capacities in the French public health system. The cost of such training activities are usually seen as a challenge in setting up and maintaining programmes but an evaluation of PROFET carried out in 2008 showed that the training sites highly value the input of fellows, not only as a 'workforce' but also because of their organisational and methodological skills. The next challenge for PROFET will be to become part of the European network of training programmes. The collaboration of InVS with the European Centre for Disease Prevention and Control (ECDC) and its involvement in the European Programme for Intervention Epidemiology Training (EPIET) as well as the European focus of the EHESP are opportunities for PROFET to be addressed in the future.

Germany

In the 1990s the German Ministry of Health (MOH) initiated a number of measures to strengthen the federal capacity in the field of infectious disease epidemiology. One of these measures was the installation of a national FETP in 1996 [5]. The idea was that participants would upon completion of their training either join the Robert Koch Institute (RKI) or return to the peripheral health departments, from where most of them were initially recruited. The programme started with two participants and - due to various kinds of additional government funding - has in the meanwhile had up to six participants per cohort. In 2006 the programme was named Postgraduate Training for Applied Epidemiology (PAE).

From start, the PAE was organised as an EPIET-associated programme, which means that the PAE fellows participate in all EPIET modules and benefit from facilitation by EPIET coordinators. However, salaries for fellows, the German facilitators and coordinators within the EPIET programme and the training sites are provided by the RKI. This EPIET-associated FETP requires strong communication skills in English. In addition to the EPIET modules RKI is conducting a one-week introductory module and a laboratory module for PAE at the RKI laboratories (bacteriology and virology) as well as additional activities such as journal clubs and scientific seminars. In addition to the requirements for EPIET fellows [6,11], PAE fellows are expected to write at least one publication in the national weekly epidemiological bulletin, one chapter in the annual national epidemiological report and are involved in the regular quality control procedures of the national surveillance system. Usually PAE fellows also enrol as duty officer in the RKI 24/7 hotline for public health emergencies.

The PAE primarily targets individuals with fairly advanced training and work experience in a medical or related discipline. Besides a university degree, eligibility criteria include knowledge in public health or epidemiological methods, at least one year programme-related work experience and fluency in English and German. RKI closely cooperates with EPIET. The institute provides facilitators, locations and sometimes funding for some of the EPIET modules. For cohort 13/14 (2006-2009) RKI is training site for six PAE and two EPIET fellows. In addition four PAE fellows are currently being trained at the respective state public health agencies of Hesse, Lower Saxony, North Rhine-Westphalia and Baden Wuerttemberg. In 2009, two of the state agencies have also become EPIET training sites and one is now hosting an EPIET fellow.

Of the 42 fellows who entered the programme between 1996-2008, 36 had a medical degree, three a university degree in veterinary medicine, one a degree in biology, one in traditional Chinese medicine and one in public health. Most participants had worked outside the public health service upon entry to the programme, seven had completed a master degree in a public health-related field before starting the training, four obtained a master degree after termination of the PAE. Most fellows (38) had applied from outside RKI but within Germany, two applicants came from a neighbouring European country, two had no European citizenship. Forty of the 42 fellows admitted have successfully completed their training, two dropped out before completion of the programme (one because of another job offer, one for personal reasons).

Retrospectively, the main challenge in setting up the programme was to reach an acknowledgement at ministerial level that such a training programme is a necessary and fruitful investment. The PAE has undergone a remarkable expansion and stabilisation in the past years [7]. To have some of the PAE fellows trained in state public health agencies is maybe one of the most important achievements given the difficulties for such collaboration in a federal setting. As a result of close collaboration between RKI and the Charité Medical University in Berlin, the cohort starting 2009, will upon successful completion of the PAE also obtain a Master of Science degree in Applied Epidemiology (MScAE).

Italy

At the end of the 1980s, after several exchanges of experiences and health professionals with the US Centers for Disease Control and Prevention (CDC), Atlanta, the Istituto Superiore di Sanità (ISS, National Institute of Health) set up an experimental training programme to train some health professionals from the different regions in order to improve the preparedness to intervene essentially on outbreaks and to carry out epidemiological surveillance of infectious diseases. In 2000, the training programme for applied epidemiology PROgramma di Formazione in Epidemioloiga Applicata (PROFEA) was created. At present, most of the curriculum focuses on prevention for chronic diseases, even if a section of the training is devoted to infectious disease surveillance and outbreak investigation.

The curriculum contains 10 different modules followed by a field training assignment of one or two months. Each trainee has to achieve some formative objectives using exclusively data and information from his/her reality and is required to devote 1,500 hours during two years PROFEA, approximately 50% of the working time of a health professional employed by the National Health System. The training is held in Italian, even if the curriculum requires an article for a scientific journal and that all participants are invited to write their article in English. In 2002, PROFEA became a post-graduate Master course, through collaboration with the 'Tor Vergata' University in Rome.

In the past mostly medical doctors, veterinarians, biologists and statisticians have applied for PROFEA directly via the university. A particular condition to be eligible for PROFEA is a letter from the region or local health administration (LAH) of the applicant in which it confirms to financially support courses, workshops and fieldworks and assures that the candidate will be able to dedicate 50% of his/her working time to the training programme. Organised by the National Centre of Epidemiology (CNESPS), of the ISS, the training programme is carried out by teachers and tutors from CNESPS. So far, secured permanent funding has come from the Italian CDC (CCM from the Ministry of Health). All participants are already employed by regions or LHA and their employers cover financial costs of courses, travels, hotel and other costs generated from training or fieldwork activities.

Since 2001, six cohorts have enrolled the programme. Fifty participants now work in public health in Italy, many of whom were promoted to posts of greater responsibility, while others are involved in national and regional committees.

At the moment, PROFEA and the CNESPS face many challenges. Italy is becoming a federal republic and the national level is only entitled to establish essential levels of care for citizens, except in cases when emergencies or for health issues implicate several regions, but the strategies to achieve them are decided and implemented at regional level. For the new 'National Plan of Prevention', the CNESPS will be adapting PROFEA training modules to assure that health professionals acquire the skills and competencies necessary for these new tasks. In the future selection of candidates will be possibly carried out by the regions and the number PROFEA trainees could rise to 20 per cohort. The funds for the programme could come directly from the interested regions and not from the national level (Ministry of Health).

Norway

The Norwegian Field Epidemiology Training Programme (Nor-FETP) started in 2001 with the objective 'to strengthen Norway's capacity to prevent and control communicable diseases by training highly qualified physicians, veterinarians and public health nurses in surveillance, outbreak investigations, applied research, communication, and support for decision making'. The focus of the programme is infectious disease prevention and control. It has from the start benefited immensely from a close collaboration with EPIET and as such adopted the EPIET associated-programme model.

During the two-year training period, fellows are actively involved in field investigations, surveillance and related research activities, and get acquainted with laboratory methods relevant to epidemiological investigations. If feasible they also take part in the Nordic summer school of infectious disease epidemiology (two weeks), go on a site visit to another European department of infectious disease surveillance, to the ECDC or the World Health Organisation (WHO) for at least one week and attend an international scientific conference. The objectives of the Nor-FETP are the same as those of EPIET plus some additional Nor-FETP objectives, such as: becoming acquainted with the Norwegian Surveillance System for Communicable Diseases, the EpiNorth collaboration [8] and with one ECDC/EU network for surveillance of infectious diseases [9].

The main working language is Norwegian but most reports, presentations and publications are in English, depending on the target audience. The three most recent fellows to join the programme are in parallel involved in training for the medical specialty in public health medicine. Their Nor-FETP training will count towards this specialisation. Normally, one fellowship is awarded per year. Nor-FETP uses the same criteria for selection as EPIET plus: fluency in a Scandinavian language; the intention to work in public health in Norway and international experience, e.g. in research or NGO work.

The Nor-FETP is managed by the Norwegian Institute of Public Health. The daily administration is in the Department of Infectious Disease Epidemiology, where the fellows are trained. To fulfil the training objective of training other professionals, the Nor-FETP programme collaborates with the EpiNorth project, the International School of Public Health in Arkhangelsk, Russia and the Nordic School of Public Health.

Since its inception, four fellows have completed training while three are in the programme now and one has been selected for the upcoming cohort and there was no drop-out. Among these eight, four are physicians, one is a veterinarian with a PhD and three are registered nurses with a master degree in public health when entering the programme.

The main challenge when setting up Nor-FETP was to organise training modules for so few people. The collaboration with EPIET solved this and is crucial for the programme and which is expected to continue in its current form.

Spain

The Spanish Applied Field Epidemiology Training Programme (PEAC) was launched in 1994 by the Ministry of Health supported by the US CDC, Atlanta [10]. The programme is hosted by the National Centre for Epidemiology in close collaboration with the National School of Public Health, both at the Instituto de Salud Carlos III (ISCIII, National Public Health Institute). The mission of ISCIII is to provide and offer scientific and technical support, as well as high quality research and training, to the national health system and the society. Within this framework, the objective of the PEAC is to strengthen the capacity of response of the national surveillance system to epidemics and other health emergencies.

PEAC starts with a three-month introductory course together with the Spanish Master of Public Health course at the national public health institute. Additional modules include: data management and data analysis, outbreak investigation (general and special aspects), communication, infectious disease epidemiology, environmental epidemiology, occupational epidemiology, analysis of health situation and application of systems dynamics. Participation is obligatory for all modules which are all held in Spanish. The

Table

Structural and conceptual characteristics of five existing national Field Epidemiology Training Programmes (FETP) in Europe compared to the European Programme for Intervention Epidemiology Training (EPIET)

Country	Europe	France	Germany	Italy	Norway	Spain
Population	**505 million**	**64 million**	**82 million**	**60 million**	**5 million**	**47 million**
History and objectives						
Programme acronym	EPIET	PROFET	PAE	PROFEA	Nor-FETP	PEAC
Institution(s) in charge of the programme	National and regional public health institutes, European Centre for Disease Prevention and Control (ECDC)	French Institute for Public Health Surveillance (InVS) and National School of Public Health (EHESP)	Robert Koch Institute (RKI, Federal Public Health Agency)	National Centre of Epidemiology (CNESPS) Tor Vergata University, Rome	Norwegian Institute of Public Health (NIPH)	Instituto de Salud Carlos III (ISCIII); National Centre for Epidemiology
Exists since	1995	2002	1996	2001	2001	1994
Subject focus	Mainly infectious diseases	Mainly infectious diseases and environmental health	Infectious diseases	Chronic diseases	Infectious diseases	Infectious diseases
Competencies to be acquired (explicitly stated)						
Run/evaluate surveillance system	Yes	Yes	Yes	Yes	Yes	Yes
Investigate outbreaks	Yes	Yes	Yes	Yes	Yes	Yes
Design study protocol/ perform applied research	Yes	Yes	Yes	Yes	Yes	Yes
Communicate results	Yes	Yes	Yes	Yes	Yes	Yes
Teach epidemiology	Yes	Yes	Yes	Yes	Yes	Yes
Risk assessment	No	Yes	No	No	No	No
Conduct survey	No	Yes	No	Yes	No	No
Manage data	No	Yes	Yes	Yes	Yes	Yes
Conduct public health intervention	No	No	No	Yes	No	No
Curriculum						
Duration (years)	2	2	2	2	2	2
Weeks spent on projects in hosting institute	79	79	76	58	75	63
Weeks spent on project outside hosting institute	6	7	7	26	7	2
Weeks in courses	10	9	12	12	13	30
Weeks in conferences	2	2	2	1	2	2
National FETP/ EPIET-associated programme		National	EPIET-associated programme	National	EPIET-associated programme	National
Three week introductory course	Yes	Yes	Yes	Yes (2 weeks)	Yes	No

Obligatory modules	Computer tools, multivariate analysis, vaccinology, project review	Computer tools, multivariate analysis, risk assessment in environmental health; sampling, scientific writing, surveillance	Same as EPIET plus laboratory module	N/A	Same as EPIET	Same as EPIET, plus data management, outbreak investigation, communication, infectious disease epidemiology, environmental epidemiology, occupational epidemiology, analysis of health situation, application of systems dynamics to epidemiological analysis
Facultative modules	Two of the four following: time series analysis, communication and scientific writing, rapid assessment, laboratory essentials for epidemiology	None	Two of the three following: time series analysis, communication, rapid assessment	None	Same as EPIET	None
Teaching languages of modules	English	French	English, German	Italian	English	Spanish
Training sites (e.g. national, district, county level)	Mostly national level	National or regional level	National and state level	County level	National level	National level
Application requirements						
Long-term contract in public health system Required upon admission	No	No	No	Required	Required	No
Motivation to work in public health in the future	Yes	Yes	Yes	Yes	Yes	Yes
Specific nationality	EU-citizenship	No	No	No	No	No
University degree	Yes	Yes	Yes	Yes	Yes	Yes
Academic degree upon completion	No	No	Incoming cohort 2009 will complete with MSc in applied epidemiology	MSc in applied epidemiology	No	MSc in applied epidemiology
Language skills	English + 1 other EU language	French	German + English	Italian	Scandinavian + English	Spanish
Infrastructure						
FTE of supervisors dedicated uniquely to the training programme	4.4 FTE coordinators minimum 1 local supervisor per fellow	1 FTE	2 FTE	3 FTE	0.25 FTE	5 FTE
Number of project-supervisors involved in FETP projects	At least 40	At least 15	Approx. 20	2	6	3-6
Outcome						
Number of yearly admissions (min/actual/max)	6/19/19	5/5/6	2/8/8	8-20	1	5-10
The three most common degrees upon admission	Physicians, veterinarians, biologists	MPH/Epidemiology, public health engineer, pharmacists	Physicians, veterinarians, biologists	See above	Physicians, nurses with MPH, veterinarians	Physicians, veterinarians, biologists

Age of applicants upon admission min/ median/max	24/33/49	23/26/33	29/33.5/50	31/46/50	25/ - /35	
Percent women	64%	82.5%	52%	79%	88%	
Work place of fellows after training						
No work experience, no position, unemployed	0%	3%	0%	0%	0%	0%
Local / state / district public health	12%	51%	17%	86%	25%	82%
National public health service	43%	43%	38%	6%	75%	6%
International public health institution	33%	3%	31%	8%	0%	6%
Hospital / medical practice	3%	0%	7%	0%	0%	4%
Academia	3%	0%	0%	0%	0%	0%
Pharmaceutical company	2%	0%	0%	0%	0%	0%
Other/unknown	4%	0%	7%	0%	0%	2%

EPIET: European Programme for Intervention Epidemiology Training; FETP: field epidemiology training programme; FTE: fulltime equivalents; MPH: Master of Public Health; PAE: Postgraduate Training for Applied Epidemiology; PEAC: Programa de Epidemiología Aplicada de Campo; PROFEA: PROgramma di Formazione in Epidemiologia Applicata; PROFET: Programme de formation à l'épidémiologie de terrain.

programme mainly focuses on infections diseases. During the two-year programme, trainees have to evaluate or implement a surveillance system, develop an epidemiologic study and conduct an outbreak investigation and study at least one outbreak. At the end of the training, fellows obtain a master degree.

Application requirements for PEAC include a university degree in a health-related field, and professional experience of at least two years in public health. Every year the ISCIII offers at least five fellowships, complemented by at least one additional fellowship from the Spanish International Cooperation Agency for applicants from Latin America or Africa, and one fellowship from the Ministry of Defense for a member of the army. The cohort can also be completed with professionals currently working at the Autonomous Regions' health administrations. The PEAC coordination team consists of one academic director and two full time scientific coordinators. Scientific coordinators follow the development of the trainees' objectives, review all the draft projects and lead some of them. For some specific projects, senior epidemiologists from national and regional level are involved in the supervision and contribute to training modules.

PEAC is currently running cohorts 14 and 15 with seven and nine fellows respectively. Up to now 109 professionals have been trained, 4 to 10 fellows per cohort. Fellows are mainly physicians (78) followed by biologists (9) and veterinarians (9). The Spanish programme is also hosting normally one EPIET fellow per year. The programme has trained 10 professionals from Latin-America (Argentina, Colombia, Cuba, Haití, Nicaragua, Uruguay and Venezuela) and Africa (Mozambique and Cape Verde). Ninety-five percent of the PEAC graduates currently work in epidemiological surveillance, alert and response units or surveillance of non-communicable diseases. Over half of the PEAC graduates are working in leading positions in epidemiological surveillance in public health administration at local, regional, or central level in Spain and in other countries. Some are collaborating actively in training field epidemiologists in their administrations.

The PEAC was created in an institution belonging to the Ministry of Health, and it was oriented to cover the shortage of professionals trained in applied epidemiology at central and regional levels. The first trainees were professionals from within the public health administration and the curriculum was based on short courses with very specific goals tailored to their specific needs. Meanwhile, applicants have often less work experience in the public health service and use the programme as a way to enter the public health work force. In response to this change PEAC is now including core courses on general public health. In 2009, the programme was moved to the Ministry of Science and Innovation which has improved the facilitation of original research but has diminished collaboration with the autonomous regions and thus lessened the fellows' opportunities to participate in outbreak investigations. The challenge is now to intensify the cooperation with the autonomous regions again.

Conclusion

Our overview shows that the existing five national FETP in Europe are differently organised in the various countries, and it is not evident whether the methodological differences reflect a difference in training needs or rather are the result of historic opportunities and training traditions in the respective countries. However, we demonstrate that all national programmes fulfil one of their main objectives which is to strengthen the national capacity

in applied field epidemiology, in such that most people do work in public health in their countries after completion of the programme, many of them in leading functions. These findings are in line with those published in the paper by Bosman *et al*. in the same issue of this journal.

As concerns the particularities of the various programmes, the Italian FETP is very much a close system, while the German PAE seems to have been able to attract young professionals from outside the public health service, with a scientific background to dedicate and strengthen their skills for public health epidemiology. This may of course not be a result of the training programmes themselves but more a result of the overall flexibility of the staffing activities and penetration possibilities in the respective public health service, which in turn may become the most important determinant on how the public health work force in European countries will develop.

Looking back at the situation of FETP in 2001, some impressive improvements are visible. Three more programmes, the Italian, French and Norwegian FETP were created, the German FETP has become stronger and new EPIET-associated programmes were installed. In the editorial to the above mentioned overview in Eurosurveillance in 2001, Reingold has predicted Europe to face a bright future with respect to FETP [1]. Given the time that has elapsed since that statement, the indisputable progress with respect to the establishment of national FETP or EPIET-associated programmes is admittedly slow. Member States should be aware of how much support EPIET can offer for the establishment of national FETP or EPIET-associated programmes. However, they also need to be ready to provide the necessary resources, the administrative environment and long-term dedication to make field epidemiology training work.

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Perspectives

Contribution of EPIET to public health workforce in the EU, 1995-2008

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We analyse activities and outputs of fellows of the European Programme for Intervention Epidemiology Training (EPIET) between 1995 and 2008 and describe the employment history of graduates after the training to demonstrate the contribution of this programme and of national EPIET-associated programmes to the public health workforce in the European Union and Norway. Up to 2008, some 161 fellows entered the training: 121 in EPIET and 40 in EPIET-associated programmes. Of these 149 were awarded a diploma. Fellows engaged in projects in all areas of surveillance, in outbreaks and field investigations and produced 340 publications in peer-reviewed journals. Seventy fellows were sent to 98 individual assignments on 65 international missions. The vast majority of graduates (90%) take up a position and remain employed in applied public health, either on regional, national or international level. Several (27) are working outside the EU, all in public health, including 13 working in Switzerland for international organisations. Only three of the 12 EU Member States that joined the EU since 2004, employ EPIET graduates. A major challenge for training the public health workforce is the retention of professionals in countries with limited job opportunities or wages significantly below the EU average.

Introduction

In order to increase the capacity to respond to emerging and ongoing threats from communicable diseases the European Commission launched a call for proposals for a two-year training programme for intervention epidemiologists in the European Union in 1994. Responding to this, experts from several national institutes for Public Health came together and the 2-year European Programme for Intervention Epidemiology Training (EPIET) was set up, starting in 1995, taking the Epidemic Intelligence Service (EIS) training programme of the United States' Centers for Disease Control and Prevention (US CDC) as an example [1,2]. The EPIET curriculum is set up to deliver independent, mid level epidemiologists with skills in the areas of surveillance, outbreak investigations, field-based epidemiological studies, scientific communication and teaching. The programme was integrated into the European Centre for Disease Control and Prevention (ECDC) in 2007. The set up and specific training objectives are described elsewhere in this journal [3].

The first cohort of EPIET fellows started in September 1995 and soon after, in January 1996, the German National Field Epidemiology Training Programme (FETP) at the Robert Koch Institute (RKI) in Berlin was established as a national training programme associated with EPIET [4,5]. From the start of the German FETP (currently renamed into German Postgraduate training for Applied Epidemiology, PAE), there has been a strong interaction with EPIET, since the association includes sharing scientific coordinators and core teaching modules [4,5]. After this, other countries: Norway, Austria, Finland, Slovenia, followed linking national training activities to the EPIET programme which are referred to as EPIET-associated programmes [3]. These programmes are required to employ fellows in an acknowledged EPIET training site and to use selection criteria and daily working activities that are similar to the EPIET.

In December 2008 the European Commission published a Green Paper on the European Workforce for Health highlighting the problem of shortages in health professions, including public health, now and in the near future [6]. The strengthening of public health capacity through training has been defined by the ECDC as a strategic target in the multi-annual programme 2007-2013 [7].

In order to demonstrate the contribution of the EPIET and EPIET-associated programmes to the public health workforce in the EU Member States and Norway, we analyse activities and outputs of fellows from cohorts 1 to 12 (October 1995- September 2008), and describe the employment history of graduates after the training. Since there are strong links in programme content, philosophy and scientific review between EPIET and EPIET-associated programmes, we chose to analyse these programmes together.

Material and methods

We used the EPIET programme office archives to compare the curriculum of the programme, including training objectives and composition of short training modules throughout the cohorts. The concept of 'site' also needed defining. A site is considered acknowledged by EPIET when it employs at least one senior epidemiologist that participated in training-of-trainer activities, including facilitation at the three week introductory course for new fellows. Information on training-of-trainers and the number of external participants to EPIET training activities was extracted from the database described below.

The contribution of the EPIET and EPIET- associated programmes was defined and measured in terms of the number of people trained, the number of peer-reviewed publications published

on work performed during the training, the number of participations in international missions and the type of employment taken up after training. The output of all fellows has been registered in a 'pedagogical database', including publications (in the categories peer reviewed journals, bulletins, reports, abstracts and other), attendance to modules and projects, participation in international field missions, graduation results and abstracts presented at conferences. Information regarding publications was reported by the fellows using the quarterly reports or incremental progress reports. This information was complemented with a PubMed® search for publications of work performed during the fellowship. Data on publications were stored in EndNote® version X.0.2.

To track current employment, we used data on employment after graduation as registered in a database by the EPIET Alumni Network (EAN). These data were provided by alumni themselves using a structured form in MS Excel. Missing employment information was collected using web-based social networks such as LinkedIn® and FaceBook® and using affiliation information from publications retrieved through Medline®.

We also analysed the costs of the EPIET programme, using budget data from the 2002-2005 financial reports sent by the budget holder to the European Commission. Finally we used information from the ECDC budget for training 2006-2009 to calculate the costs to train one person during a one-week course. Data were analysed using MS Excel and MS Access.

TABLE 1

Training modules developed within the EPIET curriculum

Name of the module	Currently in use
Communication and dealing with the press	
Communication and scientific writing	**x**
Computer tools in outbreak investigations	**x**
Data management	
Geographical information systems (GIS)	
Logistic regression	
Time series and logistic regression	
Multivariable analysis	**x**
Rapid assessment and deliberate release threats	
Rapid assessment in complex emergencies	**x**
Time series analysis	**x**
Training-of-trainers	
Vaccinations	**x**

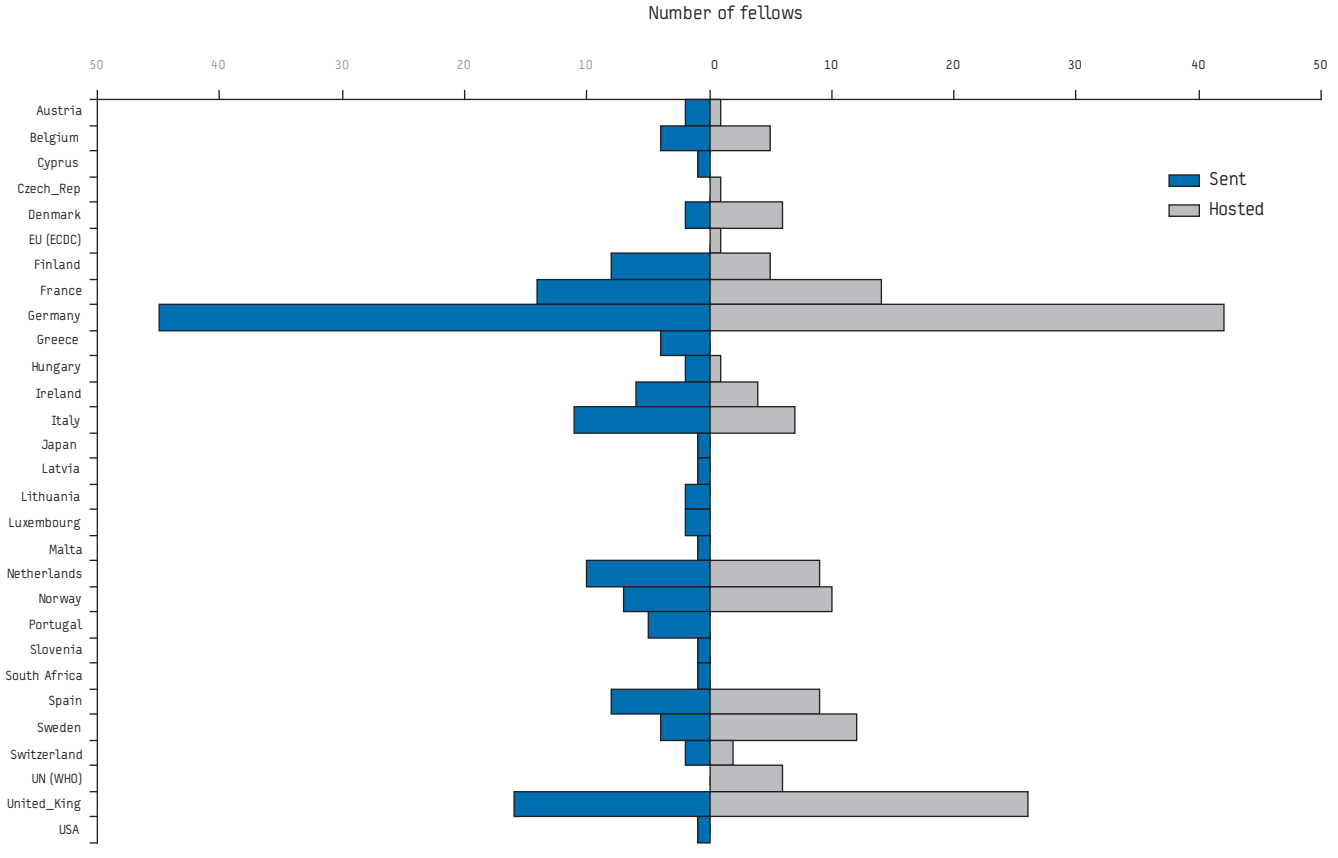
Results

EPIET curriculum through the years

The ratio of theoretical teaching versus supervised training has remained unchanged throughout the years; a maximum of 10 weeks

FIGURE 1

Number of fellows sent and hosted in EPIET and EPIET-associated programmes, by country, cohorts 1-12, 1995-2008 (n=161)



of teaching in modules and courses versus 22 months of supervised work at the training site or during field missions.

In total 13 short training modules, of which six are currently included in the curriculum, were developed for the EPIET between 1995–2008 (cohort 1-12) (Table 1). All training materials and training module curricula developed within the EPIET network are available to FETP-like training programmes. Since the migration of EPIET to ECDC, these modules have served as templates to develop short courses for EU Member States [8].

EPIET training sites and trainers

In 2008, twenty-four training sites in 16 different countries were acknowledged by EPIET: Austria, Belgium, the Czech Republic, Denmark, Finland, France (3 sites), Germany (3 sites), Hungary, Ireland, Italy, the Netherlands, Norway, Spain, Sweden, Switzerland and the United Kingdom (5 sites). Recently however, the sites in the Czech Republic and Hungary were inactivated as supervisors moved to other employment.

During the first 12 cohorts 268 professionals from 66 different organisations participated as facilitator in EPIET modules and courses. On average, a facilitator returned twice to teach. Most facilitators (189) were employed at public health institutes at national, regional or local level in the EU who participated without requiring teaching fees. A minority of facilitators were private consultants (23) hired to teach highly specific technical topics. The remaining facilitators (56) were employed by public health institutes outside the EU, universities, NGO's or the ECDC and also donated their time and expertise for free. Approximately one third of the trainers in EPIET started teaching through a 'training of trainers' activity such as the preparation week of the introductory course, or through supervised teaching by more senior trainers in specific modules.

Cohorts 1-12, 1995-2008

Fellows, projects and publications

In cohorts 1-12, a total of 161 fellows entered the training: 121 in EPIET and 40 in EPIET-associated programmes. Of 27 EU countries plus Norway, 22 have sent fellows to the programmes and 15 have hosted fellows in acknowledged EPIET training sites. In addition, fellows have been trained at EPIET sites in Switzerland, at the ECDC, at the World Health Organization (WHO) Lyon office and at the WHO Headquarters Geneva (Figure 1). The EPIET diploma was awarded to 149 fellows. Reasons for not receiving the diploma included failure to achieve the EPIET training objectives and terminating the training prematurely.

The European Commission (DG SANCO) funded 61 of the 121 EPIET salaries, nine were funded by ECDC, four by the WHO and one by Switzerland. The remaining 46 salaries were funded by Member States.

Fellows engaged in projects in all areas of surveillance, in outbreaks and field investigations have produced 340 publications in 71 different peer-reviewed, Medline-listed journals (Figure 2). These publications appeared in Eurosurveillance (114), Epidemiology and Infection (47), Emerging Infectious Diseases (22) and the Lancet (11). A number were published in general infectious diseases journals (35) and in national journals (23). Eleven articles were published in journals in the domain of microbiology.

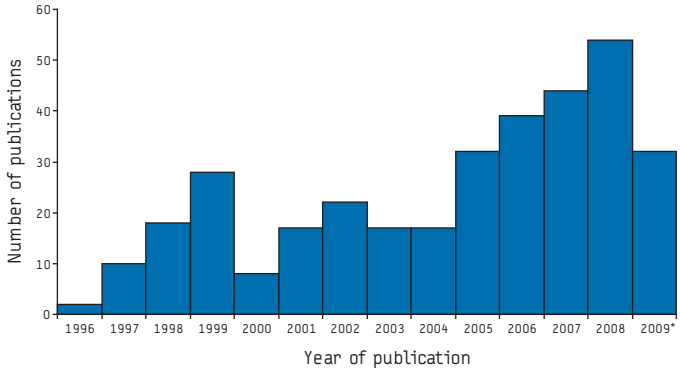
The top 10 topics of the 340 publications include mainly food- and waterborne diseases and vaccine preventable diseases (Table 2).

International missions

Fellows were requested to participate in missions by various international organisations: WHO (regional office Europe [EURO], Geneva Headquarters and Regional Office for the

Figure 2

Publications in Medline from EPIET and EPIET-associated programme fellows from fellowship projects, January 1996-April 2009 (n=340)



*Publications until 10 April

Table 2

Top-10 topics in peer-reviewed publications from EPIET and EPIET-associated programme fellows from fellowship projects, 1996-2009 (as of 10 April)

Topic of the study	Number of publications
Salmonellosis	33
Measles	16
Norovirus / Norwalk-like agent	13
Hepatitis A virus infections	12
Campylobacteriosis	11
Meningococcal disease	11
Influenza	10
Shigellosis	9
E.coli O157	7
Mumps	7

Table 3

Level of employment of EPIET and EPIET-associated programme graduates, in first and current employment, cohort 1-12, 1996-2008

Level of employment	First job (N=140)	Current job (N=139)
International public health	29%	33%
National public health	46%	44%
Regional public health	14%	13%
Private sector	4%	5%
Other	6%	5%

Eastern Mediterranean [EMRO], Caribbean Epidemiology Center [CAREC]), the ECDC, Epicentre, the Nordic Council, US CDC and the Norwegian National Institute for Public Health (FHI). To date, 70 fellows have been sent to 98 individual assignments 65 missions on behalf of the EU. Assignments included 32 outbreak investigations, one risk assessment, 17 surveillance projects, nine epidemiological surveys, four teaching and two other types of missions in 45 different countries, seven EU and EEA/EFTA, seven other European, 17 African, 10 Asian and four in South America. The pedagogical coordination of these missions was managed by the team of EPIET Scientific Coordinators, on occasion jointly with programme directors of the national field epidemiology training programmes in Canada, Germany and Spain.

Career track after graduation

We retrieved information on the first employment after graduation for 140 of the 149 graduates from cohorts 1-12 who received an EPIET diploma. For 139 alumni we were also able to retrieve the current employment. The vast majority of graduates (90%) take up a position and remain employed in applied public health, either on regional, national or international level (Table 3). Jobs in the private sector include consultancy and working with epidemiology in pharmaceutical companies. The category 'other' jobs include teaching.

Overall, 65% of the graduates currently have the same employer as immediately after their graduation. Of the 139 EPIET graduates where information on current employment is available, 27 are working in the public health sector outside the EU, including 13 working in Switzerland for international organisations (such as WHO, the United Nations High Commissioner for Refugees [UNCHR] and Médecins Sans Frontières [MSF])(Table 4). In terms of organisational position, one graduate is director of an international public health organisation, two coordinate EU disease specific networks, six are scientific coordinators of various FETP's and six are heads of unit.

Costs of EPIET

The costs per cohort of EPIET based on analysis of four cohorts (8-11, 2002-2005), ranged from 2.3 (cohort 8) to 3.2 million EUR (cohort 11), totalling 10.8 million EUR. These costs included 4.96 million EUR contributed by EU Member States in the form of salary costs for facilitators and supervisors and by hosting EPIET modules and courses. These contributions of the Member States were not

Table 4

Geographical location (country/continent) of current employment of EPIET graduates and EPIET-associated programme graduates, cohort 1-12, 1996 -2008

Country of employment	Public health			Private industry	Other	Total	Number of sent fellows
	International	National	Regional				
Austria		1				1	2
Belgium				1		1	4
Denmark	3	3				6	2
Finland		4				4	8
France	5	7	2	3		17	13
Germany	2	12	5		2	21	42
Greece		3				3	3
Hungary		1				1	2
Ireland		1	2			3	6
Italy	1	2				3	11
Lithuania		1				1	2
Luxembourg	1					1	1
Malta		1				1	1
Netherlands	1	3	1			5	10
Norway		5				5	5
Portugal	1	2	1			4	4
Spain		1			1	2	7
Sweden	11	1	1	1	1	15	4
United Kingdom	2	4	6	2	3	17	16
Subtotal EU	**27**	**52**	**18**	**7**	**7**	**111**	**143**
Africa	1	2				3	
Asia	4	4				8	
Caribbean		1				1	
Europe	13					13	
North America		2				2	
South America		1				1	
Subtotal non-EU	**18**	**10**				**28**	

reimbursed from the EPIET budget, yet they were a condition in the grant agreements on EPIET with the European Commission: Member States were expected to contribute approximately 40% of the total costs for EPIET.

From cohorts 8-11, 62 EPIET fellows were trained and external participants joined for 226 person-weeks in EPIET modules and courses. The average cost per year to train an EPIET fellow therefore is 88,300 EUR. This amount includes the total salary costs, which are on average 60,000 EUR per year, including all additional costs for the employer such as taxes, social security fees and insurance. This means that the annual costs exclusively attributed to the training of one EPIET fellow, when excluding salary, is 28,300 Euro. This includes participation to modules and courses (travel, accommodation, per diem, calculated salaries of the facilitators), costs of the salaries for EPIET scientific coordinators, EPIET Programme office and the salary of the supervisors on site.

In comparison, the average cost to train a participant during a one-week ECDC course is approximately 2,700 EUR, including trainer fees, flights, accommodation, meals and per diem.

Discussion

We present the result of an objective exploitation of available data to describe the contribution of EPIET to public health workforce. A thorough impact analysis of the programme will be provided in the near future through an external evaluation of EPIET, which will focus on elements of the programme such as quality, appropriateness, required capacity to train, costs, administration and organisation.

The curriculum of EPIET has remained focussed on structured, supervised skills development (learning by doing). The knowledge-based teaching (modules and courses) has evolved through the years with the development of specific teaching modules, which possibly reflects the ability of the programme to adapt to changes in the competence requirements of intervention epidemiologists.

The high proportion of graduates working in public health in the EU reflects the successful achievement of the programme's objectives. EPIET contributes to the key objective of the Green Paper on Workforce for Health [6] to 'achieve self sufficiency at EU level' and to 'promote circular movement of staff moving to another country for training and returning with additional experience and skills'.

Our data show that the top-five countries benefitting from employment of the highest numbers of EPIET graduates are Germany, France, United Kingdom, Sweden and Denmark. This most likely reflects a mix of factors such as nationality of those who entered the programme ('fellows sent'), availability and number of EPIET training sites and job opportunities. Germany heads the list, probably because of the national PAE, which is included in this analysis. In addition, the United Kingdom, Germany and France have the highest number of EPIET training sites within in the country, which may also be an indicator of employment opportunities after graduation. Three countries employ less than one third of the number of EPIET fellows they have sent to cohorts 1 to 12: Belgium, Italy and Spain. There is no obvious explanation for this observation, though this may also be linked to relatively fewer employment opportunities for EPIET graduates as compared to other EU Member States. So far, only three of the 12 EU Member States that joined the EU since 2004 employ EPIET graduates. Since cohort 12 (2006), an additional two 'new' EU Member States opened EPIET acknowledged training sites, but two operating sites were inactivated since cohort 12 due to trained supervisors taking up other employment. Even though the current cohorts in training include fellows from nine of the 'new' Member States, it will still take a while before job opportunities for EPIET graduates will be at the level of 'old' Member States.

One of the major challenges for training the public health workforce is the retention of professionals in countries with limited job opportunities or wages significantly below the EU average. Strategies to fill this gap may include development of more EPIET-associated programmes in new Member States and increased efforts to identify new supervisors to join the EPIET training-of-trainers programme. The number of fellows that needed to be trained each year to address the needs of public health in the EU will be addressed in the external evaluation of EPIET. At this stage we observe that the size of the latest EPIET cohort, cohort 15 consisting of 29 fellows including fellows from EPIET-associated programmes, is less than half the number of EIS officers recruited yearly in the US programme, while the EU population is significantly larger.

The increase of scientific output of the EPIET fellowship keeps the pace of the increase in size of the cohorts, with the areas of food- and waterborne diseases, vaccine preventable diseases, influenza and meningococcal disease among the most frequently published topics. The majority of articles were published in the 'Eurosurveillance' and 'Epidemiology and Infection' journals. We are aware that scientific publications provide a very limited indicator of a programme's performance, however this was the most convenient and complete set of data available for analysis. For future analysis it would be useful to look into citation indices and impact factors of the journals. In addition, it could be considered by the programme to create an indicator of public health actions that were the consequence of the work performed by fellows.

The costs to train one EPIET fellow should be seen in the light of the programme approach, which is learning by doing. The fellow works at an institute at least at the level of a junior scientist and is available for 90% of the working time when corrected for absence for modules and conferences. Therefore, the salary costs of an EPIET fellow should not be considered as costs for training but as similar to the cost for employing a public health professional.

In addition to the measurable outcomes of the EPIET training as mentioned in the results, the side benefits of the EPIET training are to be found in the training-of-trainers approach of the programme towards new facilitators and supervisors and the opportunity for external participants to training modules and courses when spare seats are available. For each fellow, at least three external participants were accepted in EPIET modules without charge and the fact that 24 training sites cooperate with the scientific coordinators to deliver consistency in methods of applied epidemiology, thus achieving 'one professional language' and tangible professional bonds between institutes. This 'professional bonding' is considered an important outcome of the programme, which is difficult to measure [9].

In conclusion, we believe that the EPIET programme is successful in achieving the programme objectives by developing a European Network of Intervention Epidemiologists practicing uniform methods, by developing a capacity to respond to public

health crisis in and beyond Europe and by strengthening the workforce in communicable disease surveillance and control in EU Member States.

Though many countries around the world have national FETP, the character of EPIET is rather unique in the sense that it is shared by 27 Member States as a joint effort for capacity building through training. After the two-year training, graduates are able to apply the relevant competencies in cross-border activities, addressing the specific challenges that communicable disease control poses at the European level. The fact that such a network of epidemiologists has been trained in one language (both professionally as linguistic) offers a great advantage in the joint response to disease control in Europe.

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Perspectives

New perspectives after the transition of EPIET to ECDC – the future of the programme

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Strengthening capacity in intervention epidemiology is key to the overall goal of responding to the challenge to detect and counter threats posed by outbreaks of infectious diseases in the European Union (EU). Since its founding in 1995, the European Programme for Intervention Epidemiology Training (EPIET) has become a core resource in training in intervention epidemiology in the EU. EPIET was integrated into the European Centre for Disease Prevention and Control (ECDC) on 1 November 2007 and this has resulted in an increased sustainability of the programme, allowing for long-term planning. Also, a new training programme, the European public health microbiology training (EUPHEM), was set up in 2008 to increase the response capacity for microbiology. Collaboration with EU Member States and other training programmes has been further intensified. Merging EPIET and other training activities in the ECDC training section has created the opportunity to develop an integrated multilevel approach to training in applied field epidemiology. An integrated approach to training activities on EU level, and increasing the number of EPIET and EPIET-associated fellows are essential to respond to the training needs of EU Member States, particularly new Member States. An external evaluation of EPIET in 2009 will provide guidance for a future strategy for the programme. This article examines the achievements of the EPIET programme after its transition to ECDC and provides an outlook on its future.

Introduction

The European Programme for Intervention Epidemiology Training (EPIET) was created in 1995 [1, 2]. The aims of EPIET are to develop a European network of intervention epidemiologists using commonly agreed methods, to build a response capacity inside and beyond the European Union (EU) and to strengthen communicable disease surveillance and control in EU Member States and at Community level. The programme is aimed at EU health professionals with previous experience in public health and a strong interest in epidemiology. The purpose of the programme is for EPIET fellows to gain practical experience in intervention epidemiology [1].

The programme lasts two years and is competency-based [3] with a 'learning by doing' approach. It starts with a three-week introductory course in infectious disease epidemiology. Following the introductory course, fellows spend 23 months at a training site at a national or regional centre for surveillance and control of communicable diseases in an EU Member State or Norway [4, 5], different from the country of origin of the fellow. Ten percent of the time of the programme is used for formal training courses and the remainder for supervised activities at a training site, where fellows are considered as a part of the public health workforce and are required to perform outbreak investigations as well as to carry out projects in the area of surveillance and do research on relevant public health issues. In addition, they are expected to present the results of their work to the scientific community during the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) and publish in peer-reviewed journals.

EPIET was integrated into the European Centre for Disease Prevention and Control (ECDC) on 1 November 2007 [6]. Prior to its integration, the European Commission and EU Member States were funding the programme and the salaries of the fellows on a project basis. The Swedish Institute for Infectious Disease Control administrated the budget and hosted the EPIET programme office responsible for all logistical and administrative issues between 2002 and 2007. Representatives of the training sites provided guidance on the programme strategy through the annual meeting of the EPIET Steering Committee. This article examines the changes within the EPIET programme after the transition to ECDC and provides an outlook on the future of the programme.

Evolution of EPIET after transition to ECDC

Administration

Since November 2007, EPIET is part of the section for Epidemiological Training of ECDC's Preparedness and Response Unit (PRU) and has a secured budget since its integration into ECDC. The EPIET programme office at ECDC continues to handle logistical and administrative issues of the fellows. The EPIET chief coordinator is also based at ECDC in Stockholm in the Section for Epidemiological Training. A framework partnership agreement between ECDC and four European national institutes for public health (Robert Koch-Institute, Institut de Veille Sanitaire, Health Protection Agency, Instituto Carlos III) has allowed the placement of the other EPIET scientific coordinators in Germany, France, the UK and Spain, also after the transition to ECDC. The fellows' contracts, salaries, removals and travel arrangements are handled by ECDC's Administrative Service Unit.

One year before the transition, starting in October 2006, ECDC took over the funding of EPIET fellows previously paid by the EU Commission. ECDC recruited fellows of the cohorts 12 to 14 and placed them in the training sites. Salaries offered by Member States were used to fund additional fellows. Since 2009, all salaries were transformed into individual grants. The former EPIET steering committee was replaced by the EPIET Training Site

Forum to allow continued input from the Member States after the transition to ECDC. All national training sites, a representative for the fellows currently in training and the EPIET alumni association are represented in the EPIET Training Site Forum. The Forum provides feedback on the functioning of the curriculum and current programme, identifies training needs for trainers, and participates in the recruitment of fellows and facilitators.

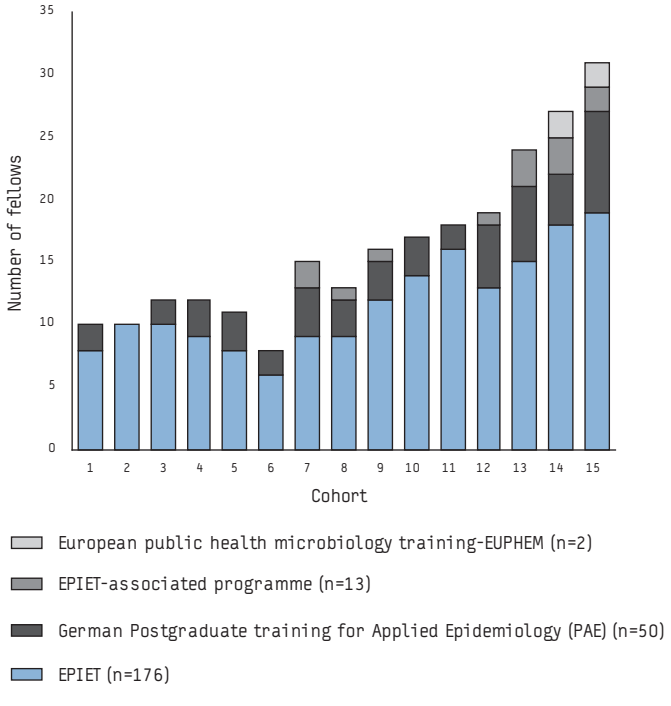
Growth of EPIET

The number of salaries provided for trainees increased from nine in 2002 to 19 in 2009. Before the transition, the number of salaries funded by Member States needed to equal at least those funded from the EU budget. This condition has been removed and in 2008 most salaries (84%) were funded by the ECDC. In addition to the increase of fellows funded by ECDC and Member States, a rising number of Member States started training fellows at national EPIET training sites. These fellows participate at EPIET modules and EPIET scientific coordinators review their progress, using the same appraisal criteria as for EPIET fellows. After successful completion of the training, these fellows also receive the EPIET diploma. This type of training is referred to as an "EPIET-associated programme". In 2008, four fellows recruited by Germany for the Postgraduate training for Applied Epidemiology (PAE) and one fellow recruited by Finland, Slovenia and Norway, respectively, were included into EPIET [6]. Thus, a total of 26 fellows have been included in the 14th EPIET cohort which started in September 2008 (Figure).

Compared to 2002 (cohort 7/8), the number of fellows currently in training (cohort 13/14) has increased from 28 to 47, corresponding to an increase of 68%.

Figure

Number of EPIET, German Postgraduate training for Applied Epidemiology (PAE), EPIET-associated and EUPHEM fellows, 1995-2009 (n=141)



Following the growth of the number of fellows, the number of EPIET scientific coordinators has increased from four to six, which corresponds to an increase of 2.8 to currently 4.4 full time equivalents.

Public Health Microbiology training programme

A laboratory component has been introduced by some field epidemiology training programmes in recent years [7]. In 2008 two EPIET salaries were used for the first time to recruit two fellows for the newly created European public health microbiology training (EUPHEM). The aim of this two-year pilot training is to develop a European network of public health microbiologists, a response capacity for microbiology inside and beyond the EU and to strengthen communicable disease surveillance and control through an integrated laboratory-field epidemiology network for outbreak detection, investigation and response EUPHEM fellows are placed in national public health laboratories and carry out outbreak investigations, surveillance and research activities in close collaboration with epidemiologists. Another aim of the placement is to develop skills in laboratory techniques and understand the specific methods, challenges and requirements for public health laboratories. EUPHEM fellows follow some of the modules of the EPIET programmes and are currently monitored by EPIET scientific coordinators.

International collaboration

Since the start of EPIET, the programme has relied strongly on the contribution from Member States. Fellows are currently hosted and trained in 21 training sites in the EU Member States and Norway. Estimating an average of four hours of supervision per week, these training sites contributed a total of 8,000 hours in 2008. EPIET continues to recruit facilitators for its modules from the pool of senior epidemiologists and EPIET alumni working in national or regional public health institutes. In 2008, they contributed a total of 37 weeks of facilitation to EPIET modules and a large proportion of these was provided by the Member States hosting EPIET-associated fellows.

EPIET aims to intensify its collaboration with the Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET), which is a professional alliance of field epidemiology training programmes (FETPs), located in thirty-two countries around the world linking all existing field epidemiology training programmes [8]. Among other activities, EPIET/ECDC exchanged trainers and organised joint events with other independent FETPs, for example with the French and Spanish programmes, PROFET and PEAC as well as the Canadian Field Epidemiology Program.

Conclusions and recommendations

Integration of EPIET into ECDC

The transition of EPIET from an EU funded project to ECDC has resulted in increased sustainability of the programme. This opens the possibility for long-term planning of training in field epidemiology in the EU. In addition, merging EPIET and other training activities in the ECDC training section has created the opportunity to develop an integrated multilevel approach to training in applied epidemiology. An integrated approach to all training activities is essential to address training needs of EU Member States at national and regional level and should be pursued further.

Future growth of EPIET

Training more EPIET fellows is necessary in order to respond to the need for public health epidemiologists in Member States. Strengthening capacity in intervention epidemiology is key to the overall goal of responding to the challenge to detect and counter threats posed by outbreaks of infectious diseases in the EU. Even though the number of fellows increased substantially over the past six years, it is still insufficient to fulfil the needs in all 27 Member States. Large Member States need to recruit a large number of fully trained epidemiologists at local, regional or national level. The majority of the twelve new Member States do not yet have any EPIET alumni who have returned to work in their country of origin. Finally, two thirds of EPIET alumni currently work in Member States at either national or regional level, while the remainder started working at international level, in the private sector or outside Europe [10].

Therefore the number of EPIET fellows needs to be increased further to respond to the needs of Member States. Especially training of fellows from new Member States is of outmost importance. In addition, ECDC and Member States need to consider developing strategies to facilitate the return of EPIET alumni to their countries of origin. The creation of more FETP or EPIET-associated programmes might contribute to build local capacities, as fellows trained in their own country are more likely to remain there after graduation and contribute to intervention epidemiology [10].

EUPHEM will contribute to create a network of professionals who will be able to collaborate with epidemiologists in the field of surveillance, outbreak investigation and applied research and this increased cross-sectoral cooperation will strengthen the capacity of outbreak investigation. Similarly to EPIET, EUPHEM requires a network of trainers available for supervision and coordination of the programme.

International collaboration

EPIET will continue to rely on the existing excellent collaboration with training sites in the Member States which are identified through a structured appraisal process by the EPIET scientific coordinators. Up to now, only few training sites are located in new Member States. With a growing number of fellows, there is a strong need for new training sites with experienced training site supervisors, teachers and facilitators. The number of experienced trainers available to teach highly specialised topics in intervention epidemiology is limited. Therefore, the training of future trainers is of high importance to ensure the quality of the EPIET programme. ECDC has started to address this issue by coordinating four workshops organised by the EPIET alumni association, TEPHINET, the Canadian Field Epidemiology Training Programme and the Robert Koch Institute. These workshops were specifically aimed at trainers and arranged around ESCAIDE. These efforts need to be continued to assure that a sufficient number of experienced trainers will be available.

Most of the EPIET scientific coordinators work at Member States' level and this has helped to maintain strong links with Member States. EPIET has reinforced the links to national institutes which host EPIET-associated programmes by increasing the number of facilitators originating from them. This collaboration, as well as maintaining strong links between EPIET and independent FETPs such as the French Programme de formation à l'épidémiologie de terrain (PROFET) and Spanish Programa de Epidemiología Aplicada de Campo (PEAC), is extremely useful to facilitate the sharing of resources and the development of joint training materials. TEPHINET has the potential to become the platform for these exchanges. EPIET should therefore take a more active role in TEPHINET, especially on the European level.

In addition to the collaboration with the Member States, ECDC's technical units for Preparedness and Response, Surveillance, Scientific Advise and Health Communication are increasingly offering activities corresponding to the EPIET objectives [11, 12, 13]. Therefore, EPIET will promote the involvement of its fellows in projects carried out by ECDC.

Challenges

After the integration into ECDC, the EPIET has developed into the most important source of training in intervention epidemiology in the EU. In the past it has played a central role in building a public health capacity in surveillance, outbreak investigation and applied research in the EU and it will continue to do so in the future. Whether linking the successful completion of EPIET to an academic title would help to increase the programme's visibility and reputation has been discussed repeatedly. For example, the PEAC is tied to a master degree [14]. Similarly, the German PAE cohort starting in 2009 will be awarded a Master of Science in Applied Epidemiology at the end of their training. EPIET modules will count as an integral part of their theoretical training [15].

An external evaluation of the programme has been commissioned that will take place in 2009. It will provide strategic advice and guidance for the future development of EPIET and address the future role of EPIET-associated programmes.

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Perspectives

Applied epidemiology training in Europe: quite a success - but more to be done

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This article describes the development of training in applied epidemiology in Europe and outlines the current situation in Europe with a view of how the system can be improved to meet future challenges.

Applied epidemiology training is often being referred to as training in field or intervention epidemiology. Field epidemiology has been characterised as "quick and appropriate" meaning that it addresses important public health problems in the community in a timely manner and employs the appropriate resources and epidemiologic methods to probe causality to the degree sufficient to identify the source or aetiology of the problem and to establish immediate and long term control and prevention accordingly [1].

The origin of training in field epidemiology

The first structured programme deliberately focussing on applied epidemiology training was the Unites States Centers for Disease Control and Prevention (US CDC) Epidemic Intelligence Service (EIS). It was founded in 1951 by Alexander Langmuir as a two-year on-the-job training at the CDC. [2]. Although the scope of topics to be covered and some of the methods have further developed since, the hallmark of the EIS remains the combination of a three-week introductory course followed by a two-year public health assignment interrupted only by a few specialised training modules. Due to increasing demand from foreign applicants and also in order to stimulate a common international methodological and conceptual training approach, the CDC started supporting the creation of "Field Epidemiology Training Programmes" (FETP) in many other countries [3-5]. In that context CDC seconded staff as long term consultants, temporary supervisors or course facilitators to other countries and provided training material. The Training Programmes in Epidemiology and Public Health Interventions NETwork (TEPHINET) was founded in 1997 and aims to improve networking between the FETP [6]. Today some 42 FETP are officially members of TEPHINET. Others exist independently from TEPHINET.

The development of field epidemiology training in Europe

The European Programme for Intervention Epidemiology Training (EPIET) was founded in 1995. It is a special form of FETP as it was set up from the very beginning to have a collaborative, multinational approach [7]. It has been a principle of EPIET that participants coming from one country of the European Union (EU) be assigned to a training site of another EU country, so as to increase networking on the European level.

National FETP also exist in the EU. They generally assign national participants exclusively to training sites within the country, and training is done in the national language. A variation of this are the EPIET-associated programmes in which fellows are assigned to a training site inside their country of origin but attend the modules and receive supervision organised by EPIET. In the following discussion, the FETP, the EPIET and the EPIET-associated programmes will be referred to collectively as the Applied Epidemiology Training Programmes (AETP).

The AETP in Europe generally have similar training objectives. They aim at enabling participants to apply epidemiological tools in the practical public health context. Outbreak investigations, surveillance activities and epidemiologic research represent the core approaches to rapid infectious disease control and are the main focus of the projects to be completed during the programme. European AETP have a lot in common with the EIS as most of the architects of EPIET and heads of the departments hosting the French, the Italian and the German FETP, as well as various facilitators and supervisors, are EIS alumni.

Country-specific aspects of AETP in the EU

The existing European FETP have different approaches [7]. The Italian programme has a very strong focus on non-communicable diseases and highlights the programmatic and preventive aspects of public health instead of the surveillance and intervention aspects in infectious diseases which largely characterise the other national FETP.

The European FETP also have different strategies for capacity building. The Italian FETP places emphasis on "in house capacity building" where public health workers who already have permanent positions in peripheral health departments are recruited to strengthen their skills in their established functions. The German FETP on the other hand attempts to "attract and specialise external workforce" placing elevated application requirements with respect to prior academic degrees, work experience and language skills in order to attract young scientists from various academic disciplines into the public health workforce. The French and Norwegian programmes are somewhere in between those approaches and the

Spanish FETP is currently moving from the "in-house capacity building" strategy towards "attracting external workforce".

The Italian, French and Spanish programmes are purely national in that all modules and training activities are carried out within the country without direct interaction with the EPIET or the other FETP. The advantages of offering modules and courses in the national language are that applicants selected for training do not have to be proficient in English. This in turn may attract applicants who are more likely to remain working in the national public health workforce instead of moving on to (possibly more attractive) positions in other countries. On the other hand, for the time being, English remains the lingua franca in medical science: a literature review, foundation of any epidemiological study, requires reasonable English reading skills at least; and sharing epidemiologic findings within the scientific community will in many instances be most effective if done in international scientific networks, journals and conferences. Given the new International Health Regulations and multiple networks within the EU, the ability to communicate in English has become a daily necessity on national level. This will inevitably and increasingly hold true also for local public health officers. One very important and successful characteristic of EPIET is to require proficiency in English and at least one other European language. During the EPIET fellows have to learn the language of their hosting country. This sometimes represents a tremendous challenge. However this challenge has many benefits. Being exposed to other languages and cultures, EPIET fellows become better equipped to negotiating and networking at the European level. Because of these very reasons it would therefore be desirable that English language proficiency also be required and developed in national FETP, so that fellows and alumni of national FETP can also be active members of the European epidemiologists network as EPIET and EPIET-associated programmes' fellows already are.

Academic recognition and accreditation

Applied epidemiology training differs from university-based training such as the Master in Public Health (MPH) or Master of Science in Epidemiology programmes. Master studies are usually characterised by a typical "class room" kind of curriculum. Applied epidemiology training is typically organised as a two-year full time programme in which over 80% of the time is filled with supervised on-the-job training. Lectures, seminars, case studies and other training formats common in academic training only make up for less than 20% of the time [8].

The Spanish FETP (PEAC) has a strong "class room" approach requiring fellows to attend a three-month introductory course at the national local school of public health (Escuela Nacional de Sanidad, ENS). The French, German and Italian FETP also cooperate with universities to varying degrees but without it affecting the on-the-job training approach.

Graduates of the Italian and Spanish FETP receive a MPH. Similarly the German FETP is now providing a Master of Science in Applied Epidemiology upon completion. Those formal titles have immediate implications on career chances and salaries in many European countries. Other AETP such as EPIET or the French FETP do not result in academic diplomas. The fellows that attend those programmes can however individually use the teaching modules and practical work conducted during their training to gain academic credits with specific European universities. Many alumni believe that, given the quality of the AETP, it should be appropriate that successful completion of the two-year programmes be acknowledged accordingly. Others recognise that pursuing an MPH and an AETP at the same time could jeopardise the quality of both.

It should be noted that the EIS, in over 50 years of its existence in the United States (US), never needed to be recognised with an academic degree. The visibility of the EIS programme and the career boost that it represents relies mainly on the quality of the work performed during the two-year training. Most EIS alumni complement their practical training with an MPH or a PhD degree obtained before or after the EIS programme.

In Europe academic diplomas do not automatically imply professional accreditation or board certification in public health medicine or epidemiology. Such accreditation is lacking in many countries and at the European level. However it must be recognised that the combination of an MPH and an AETP with an EU professional accreditation would provide a good basis for a career in field epidemiology. Applied epidemiology training is therefore not redundant to public health or preventive medicine training but should rather be seen as complementary.

Role of AETP in epidemiology training capacity in the EU

FETP and EPIET have been commended for the high level of training quality and the successful integration of alumni in the European public health workforce [9]. In the last 15 years EPIET and FETP fellows have participated in most of the major outbreak investigations conducted at the national and EU level as well as in the response to major international outbreaks [10]. They constitute a force of intervention within Europe and to some extend beyond it although the involvement of the European Centre for Disease Prevention and Control (ECDC) in activities outside EU is limited.

While EIS officers and most FETP fellows are regular staff members of the respective institutions, EPIET fellows are currently funded through a scholarship, in order to overcome specific administrative obstacles within the EU regulations. This scholarship status however runs the risk that EPIET participants are seen and see themselves as students, without the privileges and duties of regular staff members. EPIET and especially the respective training sites must therefore take care that EPIET fellows be visible as full members of the European workforce in intervention epidemiology.

While the expansion of EPIET in the recent years is impressive, the needs in terms of human resources are not met. The European training capacity lags behind the US EIS as far as the number of trained experts is concerned [11]. In the US with a population of around 305 million people the EIS has currently around 80 EIS officers per cohort, that is to say it is training about one expert per 3.8 million inhabitants [12]. In comparison, in the EU and EFTA countries with a population of about 505 million people, EPIET and all FETP taken together have around 50 fellows per cohort which would result in one expert per 10.1 million inhabitants. Furthermore this very rough comparison does not take into account three additional factors: first, the need for field epidemiologists is not only determined by the size of the population but also by the number and complexity of administrative levels; second, the long existence of EIS has already generated a solid basis of a field epidemiologist workforce; and third, a number of states in the US have their own complementary field epidemiology training programmes which have not been included in the calculation above.

For all these reasons it seems safe to say that the European training capacity for applied epidemiology should be increased.

AETP are very resource-intensive. They usually operate on the borderline of the mandates of ministry of health and ministry of research and education and generate conflicts regarding their funding by national, regional or local governments. This mixture leads to a situation in which the need of such programmes is easily agreed upon yet the organisational and financial responsibilities are often being disputed between various entities. Most of the five existing FETP in Europe have undergone critical phases when the source of funding was uncertain and other administrative problems impeded their functioning. For many years Poland and Hungary have tried to initiate FETP. Yet the lack of logistic capacities, especially in terms of human resources, made it impossible so far.

Role of AETP in European integration

Most countries that accessed the EU after 2004 have large, centralised public health systems, which have undergone several reforms, and different models of public health training have been in place. The main obstacle in capacity building in the new Member States - although not necessarily limited to these countries - is the poor availability of experienced epidemiologists, mostly due to still limited university training. Especially the local public health departments lack professionals who can apply epidemiological methods, perform epidemiological studies, publish their results, and generally use a "language" common with their Western colleagues.

Well-trained epidemiologists from the new Member States often choose a career in Western Europe, the US or in international organisations, due to much higher salaries and an environment more suitable to their professional development. This situation creates barriers for the development of FETP programmes in these countries since the few epidemiologists working there are not available as supervisors. The role of EPIET in this matter is also limited as only few EPIET alumni from the "new" EU countries have returned to their home countries to help in capacity building.

Future perspectives of applied epidemiology training in Europe

The capacity building in applied epidemiology in Europe is likely to be more successful if new FETP and EPIET-associated programmes are created and integrated in a European Network of national FETP rather than increasing the size of EPIET alone. According to Article 9 of the founding regulation of ECDC it is one of its tasks to "assist Member States to have sufficient numbers of trained specialists, in particular in epidemiological surveillance and field investigations, and to have a capability to define health measures to control disease outbreaks" [13]. Therefore it seems it should be a priority for ECDC not only to run EPIET and offer training courses (which it is already doing) but also to assist Member States in creating FETP and to support the concept of EPIET-associated programmes.

It should be acknowledged that the Spanish, German and Italian FETP benefitted from the secondment of US CDC experts to those countries [3]. Following this example, seconding EU senior epidemiologists to European countries willing to develop an FETP is a practice that needs to be further developed and accepted by Member States. With ECDC hosting a stable and ever increasing EPIET, the conditions have never been as good and the steps to be taken never as clear to actually foster cooperation between existing FETP and to create new ones in Europe.

National ministries of health need to assume responsibility in generating and assuring an internationally compatible workforce in applied epidemiology, including the creation of national applied epidemiology training programmes while EPIET should function as a breeding ground for these programmes.

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Syndromic surveillance: the next phase of public health monitoring during the H1N1 influenza pandemic?

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In this edition of *Eurosurveillance*, Coory and colleagues describe the use of a deputising medical service for influenza-like illness (ILI) surveillance in Australia [1]. They validate these novel surveillance data against a traditional general practitioner (GP) sentinel network. The use of sentinel GP surveillance networks is considered the gold standard of influenza surveillance in many European countries and formed the basis of the European Influenza Surveillance Scheme (EISS), which tracked seasonal influenza across 30 European countries from 1996 to 2008 [2]. Coory *et al.* demonstrate that the data collected from the deputising medical service were comparable with the sentinel GP data, thus illustrating the potential of these novel surveillance data to track influenza.

We are now in the midst of the first influenza pandemic the world has experienced for over 40 years. The pandemic influenza A(H1N1) v virus spread surprisingly quickly: the initial cases detected in North America and Mexico during the first few weeks of April 2009 [3,4] were quickly followed by detection in other countries, and by the end of April, the virus had spread to over 123 countries. To date (25 October 2009), it is estimated that there have been over 440,000 laboratory-confirmed cases [5]. Despite initial fears regarding the relatively high mortality rate in Mexico, the pandemic H1N1 influenza infection has so far generally presented with relatively mild acute respiratory symptoms. During the early stages of the pandemic the majority of deaths occurred in the Americas, with the only other recorded deaths in Australia, the Philippines, Spain, Thailand and the United Kingdom (UK) [6]. Currently (25 October 2009), the estimated number of deaths is at least 5,700; these deaths now are more widespread across the globe, however the main burden still lies in the Americas [5].

There are several ways of tracking the spread of influenza and estimating the burden of disease within the community. Monitoring confirmed laboratory reports, GP-diagnosed episodes of disease, emergency department (ED) attendances, hospital admissions and excess deaths are all methods employed by public health authorities. Laboratory-confirmed case reporting of influenza was used to track the initial pandemic H1N1 influenza cases during the first months of the outbreak. However, in some countries the number of cases then increased markedly, resulting in a change of policy from 'containment' to 'treatment'. In these situations, the large number of cases makes it impractical to use laboratory testing to confirm each case and therefore, the use of syndromic surveillance takes precedence as the primary means of estimating the community burden of pandemic influenza infections.

The origins of the recent increase in the use of syndromic surveillance can be traced to the United States (US), where the use of data from secondary healthcare facilities for sentinel surveillance is relatively common (though few systems are national). The response to the threat from (bio)terrorist activities since the events on 11 September 2001 has increased the frequency of such systems which are now common in individual states [7-10]. One of the first syndromic surveillance systems to evolve from the anti-terrorist response started in New York City, where ED patient attendances with 'chief complaints' are monitored on a daily basis [11].

Syndromic surveillance monitors disease patterns using syndromic indicators, which are primarily based upon clinically diagnosed (but not confirmed) episodes or symptom presentation.

Although the US have been the main focus of syndromic surveillance (predominantly ED systems), other international groups have developed similar systems, now including the current paper by Coory *et al.* in this edition of *Eurosurveillance* [1]. A French syndromic surveillance system (Oscour®) was developed in response to the European heatwave in summer 2003 [12]. Amongst a range of infections, this system has been utilised to monitor influenza and norovirus activity, and has also been used to report on potential heatwave-related morbidity in France [13]. Although the main focus of these systems has concentrated on monitoring respiratory [13,14] and gastrointestinal infections [15-17], the systems have in some cases included linkages with mortality data [13].

In the UK, a combination of sentinel GP surveillance and data from telephone-health lines comprise the current national syndromic surveillance capability, although it is hoped that this will be expanded to use other sources such as ED attendances and GP out-of-hours provisions. Sentinel GP networks have been in operation for over 40 years in the UK: the Royal College of General Practitioners (RCGP) Weekly Returns Service (WRS) has provided continuous weekly reporting of GP-diagnosed ILI incidence rates in England and Wales since 1967 and monitored the 1968-1969 influenza pandemic which impacted on the UK

during the winter 1969-1970 [18]. QSurveillance® is a UK-based GP system that, since 2005, operates on a larger scale (in terms of both geographic coverage and patient population) compared to the RCGP WRS [19]. NHS Direct is a nurse-led telephone helpline run by the National Health Service (NHS) in England and designed to triage callers based on presentation of symptoms [20]. The syndromic surveillance system operated by NHS Direct and the Health Protection Agency (HPA) uses these symptom-based telephone call data to provide real-time daily monitoring of influenza, and other seasonally occurring communicable diseases such as norovirus infections [21,22]. The main advantage of these systems is the provision of data in real-time, i.e. daily reports, thus providing a much more responsive surveillance system which allows early warning of potential problems. All NHS Direct data can be aggregated into specific age bands and broken down by region (including postcode analysis), which enables recognition of potential regional hot spots that might not be detected using traditional methods [23].

In the UK, there are surveillance programmes that undertake the integration of microbiological investigation into syndromic surveillance systems. Since 1992, the RCGP WRS sentinel GP system has, in collaboration with the HPA, undertaken virological investigation of a sample of patients diagnosed with ILI [24]. Results from this scheme are vital in providing the earliest community-based influenza virus isolations during an influenza season, providing information on the circulating influenza virus types/subtypes, potential virus-vaccine mismatch, vaccine effectiveness and the emergence of antiviral resistance. In addition, community-based respiratory samples from this system have been used retrospectively to assess the impact of newly discovered pathogens, e.g. human metapneumovirus [25]. In recent years the NHS Direct/HPA syndromic surveillance system has also been used to obtain clinical samples from patients calling the helpline. The novel aspect of this system is the self-sampling protocol which involves sending swabbing kits to patients who then take nasal swabs themselves and return the samples to a central laboratory [26]. Results from this pilot study were encouraging, and this has now been rolled out in the current pandemic situation in England to assess the frequency of community-based pandemic H1N1 influenza infections [27].

A potential disadvantage of using syndromic surveillance systems is the lack of specificity of the data collected. Laboratory reporting of confirmed cases provides an accurate representation of how many cases are positive for the pathogen of interest. Syndromic surveillance monitors disease patterns using syndromic indicators, which are primarily based upon clinically diagnosed (but not confirmed) episodes or symptom presentation. However, previous work has shown that despite these limitations, syndromic data can be extremely sensitive to community-based infections and act as potential early warning of imminent problems. This 'broad brush' approach of using non-specific indicators may capture patients who do not specifically meet the case definition, e.g. ILI. Experience from using the NHS Direct/HPA syndromic surveillance system has demonstrated that calls for 'fever' in children aged between five and 14 years can be used as an early warning indicator of influenza activity [28]. Fever calls in this age group are sensitive to increasing community-based influenza activity, thus demonstrating that using an indicator that is not based upon a range of presenting symptoms associated with influenza can be reliably used to monitor influenza activity [28].

Another potential disadvantage of syndromic surveillance is the impact of media reporting. In situations such as the outbreak of severe acute respiratory syndrome (SARS) in 2003, and the current H1N1 influenza pandemic, the mass media reporting on these events can cause anxiety amongst the population. This can prompt symptomatic patients, who would normally have self-treated their symptoms, to seek healthcare advice such as a GP consultation or a call to NHS Direct. It is therefore very difficult to disentangle the effects of media reporting from the true burden of infection in the community, and without laboratory reporting it is not possible to estimate the proportion of true positives.

Syndromic surveillance constitutes the use of data systems that do not rely on confirmatory laboratory testing of patient samples. In principle, the data used in syndromic surveillance are primarily collected for other purposes, e.g. clinical management of patients. The general advantage of these systems is the provision of data that are timelier than traditional laboratory reporting, i.e. 'real-time'. In most cases, fewer resources are required to maintain the systems. They also have the potential to cover a greater range of disease indicators and therefore can be used to monitor many different scenarios within public health protection. This also includes the surveillance of non-infectious public health issues such as bioterrorist threat, chemical incidents, natural phenomena such as heatwaves or flooding, and mass gathering events, for instance the Olympic Games.

In recent years, there have been moves to utilise the massive potential of the internet for surveillance purposes. The health information seeking behaviour of the population has now changed with the wealth of online help available: in response, Google.org has released *Google Flu Trends*, a system that monitors influenza-based search queries from the Google search engine. Analyses of data collected from the US were modelled using CDC sentinel GP surveillance data with remarkably high correlation between the two data series [29]. This work has now been transposed to a publicly accessible website that uses this system to monitor regional influenza activity in the US, and has more recently expanded to cover Australia, New Zealand, Mexico and Europe [30,31]. In this week's issue of Eurosurveillance, Wilson *et al*. present a rapid communication comparing results from *Google Flu Trends* with data from existing surveillance systems in New Zealand [32].

The continuing advancement of syndromic surveillance is providing further public health monitoring of infectious diseases, and in particular influenza. Novel systems such as internet-based search queries are providing a new aspect to the established systems and thus providing another piece of the syndromic surveillance jigsaw.

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Antibiotic resistance in Europe: the challenges ahead

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On 18 November 2009, the second European Antibiotic Awareness Day will be celebrated throughout Europe. This European public health initiative coordinated by the European Centre for Disease Prevention and Control (ECDC) aims to communicate about the importance of prudent use of antibiotics in order to turn the tide on antibiotic resistance. Last year's campaign focused on antibiotic awareness of the general public. Thirty-two European countries participated producing information materials and implementing activities ranging from press conferences to national media campaigns [1]. The main focus of this year's European Antibiotic Awareness Day campaign is to work with primary care prescribers to promote appropriate use of antibiotics, with particular attention to respiratory tract infections such as common colds and flu. Campaign materials, including factsheets and leaflets, have been prepared together with professional organisations representing primary care prescribers and a multi-lingual website has been developed (http://antibiotic.ecdc.europa.eu).

Prudent use of antibiotics is not the only strategy for fighting antibiotic resistance. Good infection control practices, including hand hygiene as well as the screening and isolation of infected patients are necessary to prevent the spread of resistant bacteria. Several European countries have or have had national or regional campaigns on hand hygiene [2], but improving hand hygiene practices remains a challenge in many countries. A European Union (EU) Council Recommendation on patient safety, including the prevention and control of healthcare-associated infections has been adopted by EU Health Ministers on 9 June 2009 and lists a series of actions in this area [3]. ECDC will provide support by developing guidance documents for prevention and control of these infections.

Developing and marketing of new antibiotics with novel mechanisms of action represents a further essential strategy against antibiotic resistance as resistance inevitably builds over time. A recent report from ECDC and the European Medicines Agency (EMEA) identified a gap between increasing prevalence of multidrug-resistant bacteria in the EU and the current state of the development pipeline for new antibiotics [4]. This topic is one of the priorities of the current Swedish Presidency of the EU and was discussed at the conference "Innovative Incentives for Effective Antibacterials" [5].

Primary care accounts for 80 to 90% of all antibiotic prescriptions in humans, which is why public awareness campaigns on the prudent use of antibiotics generally focus on primary care. In the United States (US), the Centers for Disease Control and Prevention (CDC) are coordinating the campaign "Get Smart: Know When Antibiotics Work" [6], which is also focusing on the general public and healthcare providers. At a recent summit on 3 November 2009, the US and the EU agreed to establish a transatlantic task force on urgent antimicrobial resistance issues [7]. ECDC and the CDC are already cooperating closely on their public awareness campaigns on the prudent use of antibiotics. While the CDC are already preparing a campaign to address hospital prescribers, European Antibiotic Awareness Day will in 2010 also focus on prudent use of antibiotics in hospitals. ECDC is also working closely with the World Health Organization Regional office Europe to promote participation in the campaign of European countries that are not members of the EU.

This issue of Eurosurveillance highlights two topics that relate to antibiotic resistance and infection control in hospitals.

This issue of Eurosurveillance highlights two topics that relate to antibiotic resistance and infection control in hospitals. The first one is *Clostridium difficile*. Hensgens *et al.* [8] report on a shift in the PCR ribotypes identified in the Netherlands with PCR ribotype 027 almost disappearing whereas Arvand *et al.* [9] report that this PCR ribotype is still prevalent within Hesse, one federal state of Germany. As of now, the only available pan-European data for this micro-organism are from the European *C. difficile* infection survey (ECDIS) that was performed in November 2008 [10]. This survey highlighted the need for increased capacity building for the detection, typing and surveillance of *C. difficile* infections in Europe and ECDC will provide support to these activities.

The second topic is the emergence of totally or almost totally resistant bacteria in Europe. Last year, Souli *et al.* published a review on this topic in Eurosurveillance [11]. In this issue, a survey among European intensive care physicians shows that about one half had seen at least one patient infected by such bacteria and about one fifth had seen three patients or more in the preceding six months [12]. Studies are now needed to assess the extent of the spread of totally or almost totally resistant bacteria in Europe and to determine the risk factors for colonization and infection. In the meantime, ECDC will prepare interim guidance documents for prevention and control of these bacteria.

Antibiotic resistance is also an issue in zoonotic infections, foods, food animals, pets and agriculture and a joint opinion on antimicrobial resistance in zoonoses from several EU agencies has recently been finalised [13].

Antibiotic resistance is a moving target. While Europe is obviously making progress towards increased awareness about prudent use of antibiotics and the prevention and control of antibiotic-resistant bacteria and healthcare-associated infections, all the issues highlighted in this editorial deserve our full attention. These are the challenges ahead.

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Approaching measles and rubella elimination in the European Region – need to sustain the gains

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While there is considerable focus in the World Health Organization (WHO) European Region on the introduction of new vaccines and promotion of underutilized vaccines, there are increasing challenges in sustaining the gains made with existing vaccines, where the estimated vaccine coverage rate for measles is 94% in the Region [1]. Analyses reveal that most children are not immunised on time according to national immunisation schedules and that there are pockets of low immunisation coverage at regional or local levels in the countries. These two factors set the stage for outbreaks of vaccine-preventable diseases, such as were seen with measles in the western part of the European Region [2].

In 2002, the WHO Regional Committee for Europe adopted a resolution to eliminate indigenous measles and rubella in the 53 Member States in the Region by 2010. Elimination is defined as a situation in which sustained virus transmission cannot occur and secondary spread from importation of disease will end naturally without intervention. Key strategies to achieve this goal are: achieving and sustaining high coverage (≥ 95%) with two doses of measles and at least one dose of rubella vaccine through high-quality routine immunisation services; providing a second opportunity for measles immunisation through supplemental immunisation activities (SIA) in susceptible populations; using the opportunity provided by measles SIA to target populations susceptible to rubella with combined measles and rubella-containing vaccine; and strengthening measles, rubella, and congenital rubella syndrome (CRS) surveillance through rigorous case investigation and laboratory confirmation of all suspected cases [3]. The regional strategy encourages rubella vaccination opportunities, including supplementary immunisation activities, for all rubella-susceptible children, adolescents and women of child-bearing age. All national SIA conducted in the eastern part of the WHO European Region have included rubella vaccine. In addition, rubella vaccination is part of the routine immunisation schedule in all member states.

Since 1998, measles incidence in the WHO European Region has declined from 110 cases per 1,000,000 population to historically low levels of ≤ 10 cases per 1,000,000 in 2007 and 2008. In 2008, 29 member states reported a measles incidence of less than one per 1,000,000 population, selected as one of the indicators for monitoring progress towards elimination. This progress is based on high immunisation coverage achieved through a routine two-dose schedule for measles-containing vaccine and SIA to reach susceptible populations. The estimated regional coverage for the first dose of measles vaccine increased from 88% in 1998 to 94% in 2008. Moreover, reported coverage for the second dose ranged from 62% to 99% in 2008. From 2000 to 2008, at least 17 countries conducted nationwide SIA, reaching approximately 54 million people. Surveillance has been strengthened by improving case investigation procedures, expanding case-based reporting and increasing laboratory testing.

In this issue of Eurosurveillance, articles by Richard *et al.* and Marinova *et al.* show that outbreaks in the Region are occurring primarily among children aged five to 14 years who have not been immunised or who have received only one dose of measles vaccine [4,5].

Since 1998, measles incidence in the WHO European Region has declined from 110 cases per 1,000,000 population to historically low levels of ≤ 10 cases per 1,000,000 in 2007 and 2008. In 2008

While measles incidence in the Region has declined to low levels, there has been a resurgence of measles cases in western European countries owing to suboptimal coverage of measles vaccine leading to pockets of susceptible people (Figure 1). In 2008, 92% of reported measles cases (n = 8,264) occurred in western European countries, primarily Austria, France, Germany, Italy, Spain, Switzerland and the United Kingdom. The majority of cases were not immunised (82.2%) [6]. This contrasts with the situation from 2004 to 2006, when more measles epidemics occurred in the eastern part of the Region, with six of the newly independent states of the former Soviet Union accounting for 75% of reported cases [6] (Figure 2).

With the decline in the number of measles cases, many national immunisation programmes in the Region are challenged by a combination of beliefs that lead to questioning the value of immunisation and the health threat posed by measles, and result in parents' hesitancy to vaccinate children.

The two articles in this edition of Eurosurveillance clearly show that measles can be a serious health threat and lead to complications (40.5% in Bulgaria) and hospitalisation (15% in Switzerland and 69.7% in Bulgaria; important to note that the percentage of hospitalised can be affected by national policies on treatment). Furthermore, Richards *et al.* report one measles-related death in a previously healthy child. In addition, deaths have been reported from France and the Netherlands in 2009 [10].

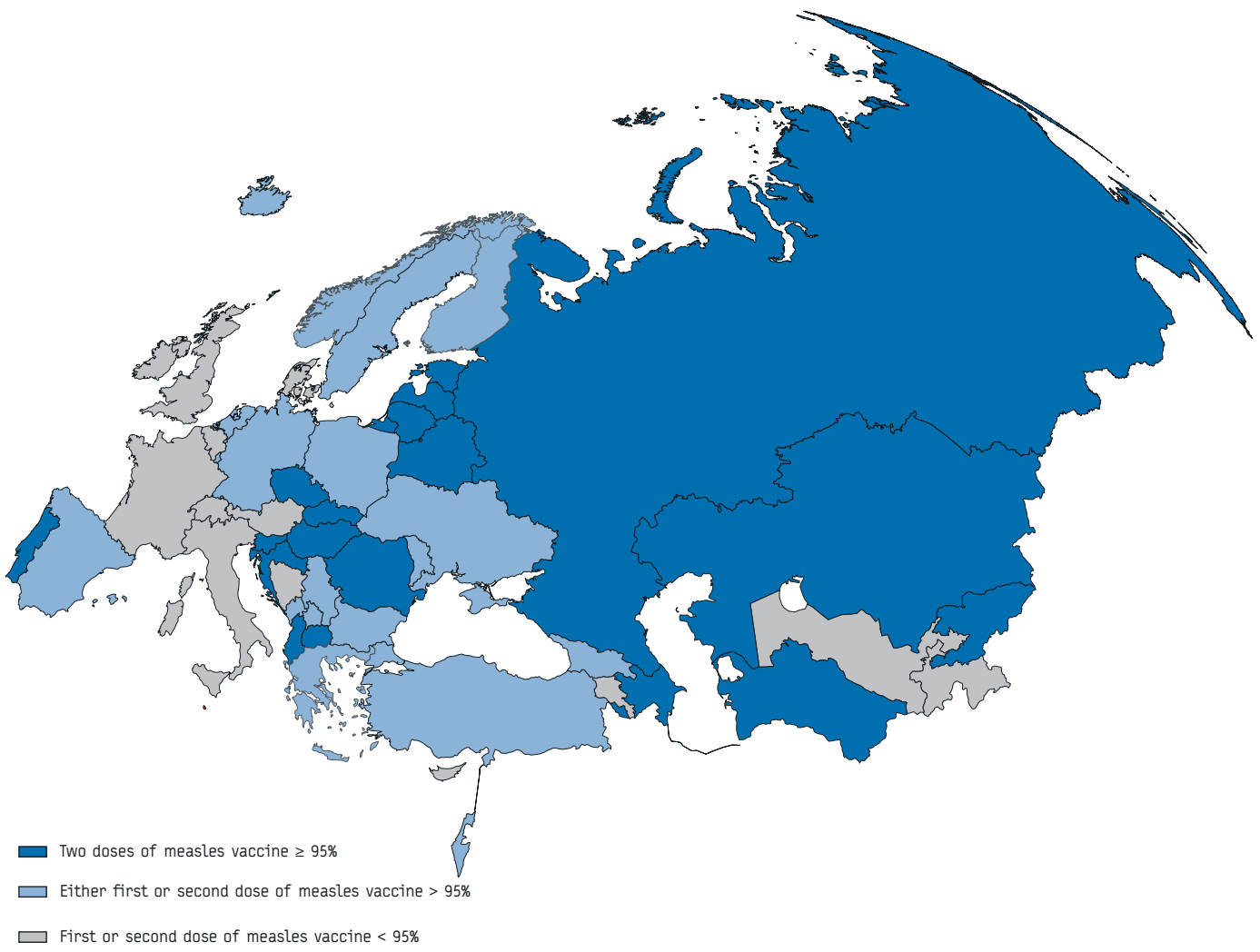
Genotyping data from both countries revealed that measles are exported to other countries in the European Region. Immunisation should be seen as a social responsibility in the European Region [11]. As demonstrated in this issue for Switzerland, the ongoing transmission in western Europe has in several cases led to exportation of measles to other WHO regions, including the Region of the Americas, where the disease was eliminated in 2002 [4,7,9]. The cost to society and health care systems of investigating and controlling measles outbreaks needs to be further analysed. The results should be used for high-level advocacy and to ensure political commitment from governments.

In addition to measles outbreaks, large, sustained mumps outbreaks have been reported in the Region. Stein-Zamir *et al.* report in this issue on a mumps outbreak in religious academies in Jerusalem with a high number of cases in fully vaccinated people [12]. While it is unclear how vaccination coverage was ascertained, the finding that outbreaks occur in individuals who have received two doses of mumps vaccine has been also reported in other countries, especially in universities, the military and other closed settings, such as in Ireland, Luxembourg, the Republic of Moldova, the former Yugoslav Republic of Macedonia and the United Kingdom [13,14,15,16,17,18]. Vaccine failure, waning immunity and programmatic documentation of vaccine histories have been given as explanations for these outbreaks and further studies are needed to understand and document the causes.

As the WHO European Region approaches measles and rubella elimination, there is a need to better monitor progress. The three agreed criteria for this purpose are disease incidence, quality surveillance and immunity profile. Surveillance needs to be strengthened through advocacy with member states and adoption of the recently revised WHO regional surveillance guidelines, which have been adapted to address lower measles incidence levels and

Figure 1

Coverage of measles containing vaccine (first and second dose), WHO European Region, 2008



Note: The designations employed and the presentation of this material do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers and boundaries.

Source: World Health Organization Regional Office Europe, 2009

to emphasize the importance of laboratory confirmation, case-based reporting and the use of standardised performance indicators [19]. In October 2009, a group of international experts from all continents met in Geneva to assess the current standardised surveillance performance indicators and the indicators for monitoring progress towards measles elimination. Interruption of indigenous measles transmission for 36 months is considered one of the criteria for elimination. Follow-up is needed at the global level to finalise the modifications based on the findings from WHO regions.

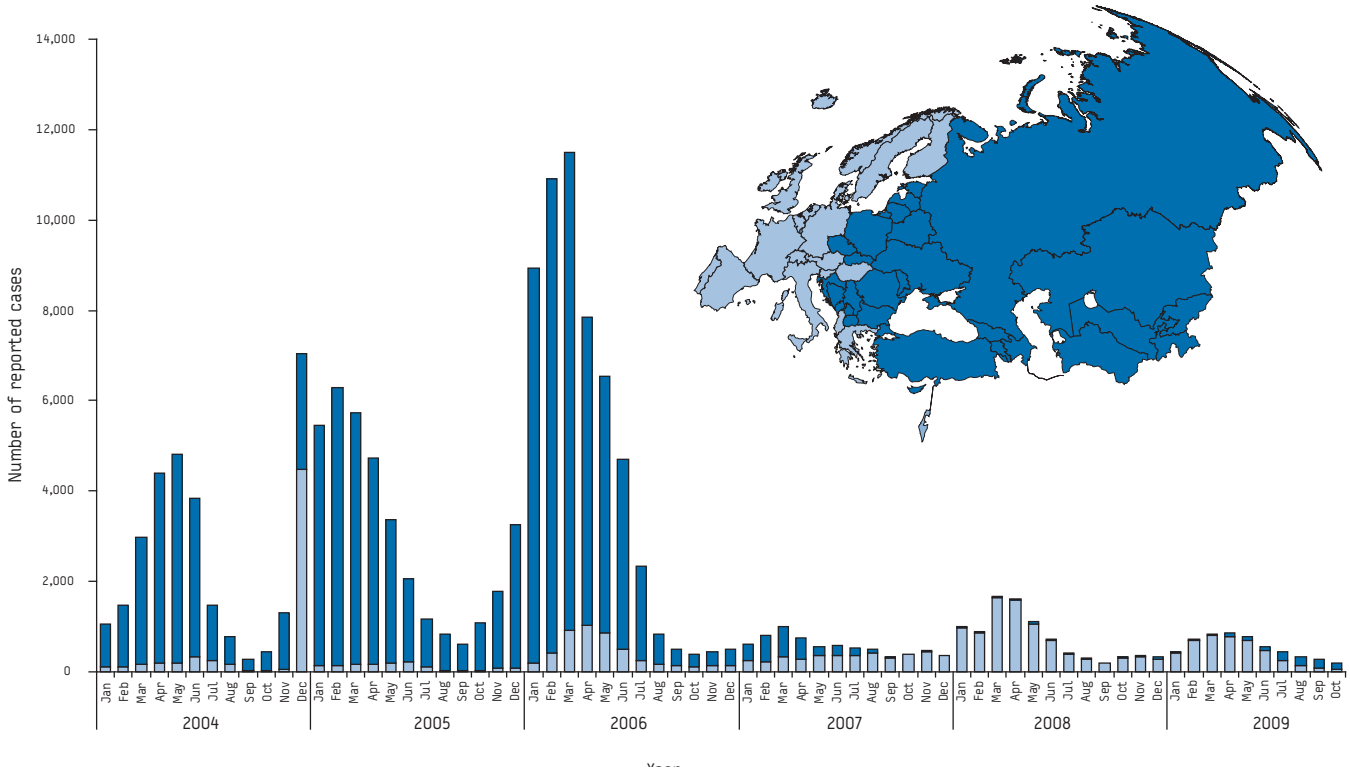
Kelly *et al*. from Australia report that many industrialised countries will not be able to meet the targets for the indicators, especially for the surveillance indicators. The annual process of certification of the European Region's polio-free status shows that many countries do not meet the targets for the surveillance performance indicators and not all countries conduct acute flaccid paralysis (AFP) surveillance. The national and regional certification commissions have therefore validated countries' documentation of polio-free status using other indicators related to their health systems, including the ability of the country to detect a wild poliovirus. For verifying measles and rubella elimination in member states, it is expected that once national and regional commissions for verifying elimination are formed, they will evaluate the available evidence with regard to the quality of the surveillance system of a country, with the indicators of incidence and immunity in order to verify if a country has eliminated measles and rubella. Similar criteria will also be used to document and verify elimination of rubella. As described by Aytac *et al*. [20], serosurveys are useful in determining rates of seropositivity but interpretation and generalisability of results should be carefully evaluated prior to developing immunisation policy in a country.

With 2010, the deadline for measles and rubella elimination, approaching, the WHO European Region faces serious threats to sustain the gains made and to reach the goal. The ongoing monitoring of performance measure indicators, disease incidence and coverage should be continued to guide the programme and verify that elimination has been achieved. To achieve elimination, enabling factors, including resources and societal support, will need to be strengthened while barriers to immunisation need to be removed. To this effect, high-level political and societal commitments are required to increase and sustain high level coverage (>95%) with two doses of measles vaccine in children. Improving immunisation coverage to ≥95% must be of primary importance to prevent transmission especially among hard-to-reach populations, which include cultural or ethnic minority groups, nomadic groups, and populations that are experiencing civil unrest and/or political instability, are geographically isolated or refusing vaccination owing to religious or philosophical beliefs.

The WHO Regional Office for Europe is working with member states to identify and target populations at risk and health care professionals to communicate the need for immunisation, as well as to trace children who have not received two doses of vaccine. The annual European Immunization Week held each April provides an

Figure 2

Reported measles cases, WHO European Region, 2004–2009



Source: World Health Organization Regional Office Europe, 2009

opportunity for member states to tailor their messages actively to communicate the benefits and risks of immunisation and strongly advocate the protection of children with political leaders, health care professionals and the general population [7].

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Progress in the surveillance of respiratory syncytial virus (RSV) in Europe: 2001-2008

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Respiratory syncytial virus (RSV) surveillance is important to get insight into the burden of disease and epidemic pattern of RSV infection. This information is useful for healthcare resource allocation as well as the timing of preventive messages and palivizumab prophylaxis. For influenza surveillance the European Influenza Surveillance Scheme (EISS) was established in 1996, but no surveillance platform is available for RSV. To improve surveillance an RSV Task Group was established in 2003 and recommendations for RSV surveillance were developed. By 2008, progress was made for four out of six recommendations: the number of European countries testing specimens for RSV increased from six to fourteen; nose and/or throat swabs were generally used for detection of influenza and RSV; a total of 25 laboratories performed molecular testing for diagnosis and participated in a quality control assessment for RSV with an overall good performance; four of the ten countries that joined EISS in 2004 started reporting RSV detections in addition to influenza in the period 2004-8. Limited progress was achieved for standardising methods and the development of a sentinel surveillance system of representative hospitals. Improving RSV surveillance is possible by further harmonising the data collection and increased reporting of RSV.

Introduction

Respiratory syncytial virus (RSV) is the most important viral agent causing severe respiratory disease in young children [1-3]. RSV is also being recognised as a significant pathogen in adults [2,4] causing moderately severe respiratory disease especially in the elderly [5,6]. Influenza is widely recognised as a major cause of morbidity and mortality in humans [7,8]. Since RSV and influenza virus infections are associated with similar clinical symptoms [9] and frequently co-circulate around the same time of the year, there is substantial potential for confusion regarding the cause of influenza-like illness [10].

Influenza and RSV account for similar numbers of deaths in children and their impact varies by winter and age group. RSV is associated with more deaths than influenza in children aged 1-12 months [11]. Excess deaths due to RSV and influenza virus infection have also been reported for the elderly population [5,8]. When comparing cause-specific mortality due to influenza virus and RSV infection in all ages, it has been estimated that most deaths were associated with influenza A(H3N2) viruses, followed by RSV, influenza B, and influenza A(H1N1) [8].

While influenza is on the list of communicable diseases that must be covered by the European Community network for surveillance, RSV is not on this list [12]. Nonetheless, RSV causes considerable burden of disease and RSV surveillance is important for determining the burden of illness in all age groups and in defining seasonality and epidemic pattern. This facilitates the preparation of hospital settings to receive more children and to define the timing of the start of palivizumab prophylaxis [13]. Palivizumab can be administered as passive immunoprophylaxis and is the only strategy that has been demonstrated to reduce RSV hospitalisations in high-risk children [14]. For real-time influenza surveillance the European Influenza Surveillance Scheme (EISS), a collaborative multinational project, was established in 1996 [15], but no such scheme was available for other respiratory viruses including RSV. Since RSV and influenza infections typically occur in the winter, EISS made it possible to report RSV detections into the EISS database, on a voluntary basis, from 1996 until September 2008.

In 2003 an RSV Task Group was established within EISS to explore the possibility to design a comprehensive RSV surveillance scheme within the EISS framework. This Task Group was composed of four epidemiologists and two virologists. Three meetings were organised between July 2003 and January 2006 and updates on the activities were presented to the EISS group during the EISS Annual Meetings. A retrospective analysis was carried out. Additionally, RSV surveillance recommendations were published in 2006 [16], and are presented below:

1. Specimens collected as part of an influenza surveillance programme should also be tested for RSV.
2. Both combined nose/throat swabs and nasal pharyngeal aspirates are acceptable for RSV diagnosis.
3. The application of molecular techniques such as real time PCR in the diagnosis of respiratory disease has been demonstrated and we advocate this technique for RSV detection.
4. Further developments are encouraged on the use of standardised methods and laboratory techniques.

5. The development of a sentinel approach of representative hospitals should be considered.
6. New countries joining EISS are encouraged to integrate RSV surveillance alongside influenza surveillance.

Our objective was to assess whether the RSV reporting within EISS in the period 2004-2008 complied with these surveillance recommendations, and to describe the detection and reporting of seasonal influenza and RSV infections in six selected countries in Europe.

Methods

Data collection in EISS

EISS was based on an integrated clinical and virological surveillance model. Sentinel primary care physicians reported weekly the number of new cases of influenza-like illness and/or acute respiratory infections and obtained respiratory specimens from a sample of patients for laboratory testing. The specimens were tested for influenza and in several countries for RSV as well. Weekly consultation rates and laboratory test results were entered by the national surveillance networks into the EISS database via an internet-based system [17]. Non-sentinel, mainly hospital-based data for influenza and RSV were also collected, but will not be presented in this paper.

Since September 2008, European influenza surveillance has been carried out by the European Centre for Disease Prevention and Control (ECDC) and involves all 27 European Union Member States and Norway. Three other countries Serbia, Switzerland and Ukraine are reporting data to World Health Organization (WHO) Regional Office for Europe.

This paper presents a descriptive study. Surveillance data for seven winter seasons (2001-2 to 2007-8; week 40-20) in the EISS database were screened for RSV detections by country. The database containing virological detections of RSV and influenza was downloaded by September 2008. An RSV reporting country was defined as a country that reported at least 10 sentinel specimens positive for RSV from 2001-2008. With this method the progress for recommendation 1 and 6 could be assessed. For the other

Table 1

Reporting of respiratory syncytial virus (RSV) and influenza data to the European Influenza Surveillance Scheme (EISS) in the period 2001-2008

Season	Number of countries reporting RSV*	Number of countries reporting influenza	Number of RSV detections	Number of influenza detections
2001-2	6	18	203	2276
2002-3	8	19	335	3787
2003-4	12	22	143	2732
2004-5	12	23	557	5483
2005-6	14	28	803	3171
2006-7	14	30	888	5077
2007-8	13	31	929	5076

*Countries reporting RSV:
2001-2: CZ, FR, DE, SI, CH, UK-E, UK-S.
2002-3: CZ, FR, DE, NL, SK, SI, CH, UK-E, UK-S.
2003-4: CZ, FR, DE, NL, SK, SI, CH, UK-E, UK-S.
2004-5: AT, CZ, DK, FR, DE, IT, LU, PL, RO, SI, CH, UK-E, UK-S.
2005-6: AT, CZ, DK, EE, FI, FR, DE, IT, LU, NL, PL, RO, SI, UK-E, UK-S.
2006-7: AT, CZ, DK, EE, FI, FR, DE, IT, LU, NL, PL, RO, SI, UK-E, UK-S.
2007-8: AT, HR, CZ, DM, EE, FI, FR, DE, LU, NL, PL, SI, UK-E, UK-S.

Abbreviations: Austria (AT), Croatia (HR), Czech Republic (CZ), Denmark (DK), Estonia (EE), Finland (FI), France (FR), Germany (DE), Italy (IT), Luxembourg (LU), the Netherlands (NL), Poland (PL), Romania (RO), Slovenia (SI), Slovakia (SK), Switzerland (CH), UK-England (UK-E), UK-Scotland (UK-S).

Table 2

Number of sentinel influenza and respiratory syncytial virus (RSV) detections by country in the period 2001-2008

Country	Number of RSV detections per season mean (range)	Number of influenza detections per season mean (range)	Total number of RSV and influenza detections mean (range)	Percentage of RSV cases (%)* (range)
Czech Republic	18 (5-30)	206 (83-311)	223 (102-327)	8 (3-19)
France	145 (47-227)	1053 (824-1374)	1198 (947-1601)	12 (4-18)
Germany	43 (12-138)	1129 (553-2145)	1172 (568-2172)	4 (1-10)
The Netherlands**	12 (1-19)	121 (15-142)	133 (16-153)	4 (0-16)
Slovenia	6 (1-12)	101 (69-132)	106 (77-135)	5 (1-12)
UK-England	44 (14-125)	231 (82-432)	275 (107-477)	16 (8-56)
UK-Scotland	23 (14-35)	101 (31-193)	123 (50-220)	18 (11-38)

* The percentage of RSV cases in relation to the total number of samples that tested positive for either influenza or RSV.
** No RSV detections were reported for the Netherlands in the winters of 2001-2 and 2004-5.

recommendations the progress was summarised by collecting relevant data from inventories and a quality control assessment.

RSV detections: six countries

Country selection

Data from the Czech Republic, France, Germany, Netherlands, Slovenia and the United Kingdom (UK) (represented by England and Scotland) were assessed to describe the RSV surveillance in these countries. All had reported data for at least five winter seasons. Sentinel primary care physicians included general practitioners (GPs) in the United Kingdom and the Netherlands, and GPs and paediatricians in the Czech Republic, France, and Germany, and GPs, paediatricians and specialists in Slovenia. The sentinel doctors represented 1-5% of all physicians working in the country.

Case definition

Data on new cases were based on reporting of consultations for influenza-like illness (ILI) in the Netherlands, Slovenia and United Kingdom. Consultations for acute respiratory infections (ARI) were collected in France and Germany. From 2001-2 to 2004-5 the Czech Republic reported the number of new cases of ARI, and from 2005-6 onwards they reported cases of ILI in addition to ARI [18]. Case definitions for ARI and ILI differed slightly between countries [19]. The type of specimen that was collected (nose and/or throat swab) as well as transport conditions were similar [20]. Samples were generally collected within five days after onset of symptoms and systematically tested for both influenza virus and RSV in all countries. In Germany, only specimens of children aged 0-3 years were tested for RSV. Cases were defined positive for RSV or influenza when at least one laboratory test yielded a positive result. Between-country comparisons will not be made due to methodological differences.

Results

Recommendation 1

Specimens collected as part of an influenza surveillance programme should also be tested for RSV.

Seventeen countries had reported RSV detections in the period 2001-2008: Austria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Luxembourg, Netherlands, Poland, Romania, Slovenia, Slovakia, Switzerland, UK- England and Scotland. Since England and Scotland have their own sentinel surveillance systems, these are presented separately in this paper. The number of countries reporting influenza data increased from 18 in 2001-2 to 31 in the winter of 2007-8 (Table 1).

In 2001-2 only six countries reported RSV detections in addition to influenza, but their number gradually increased, particularly around 2003-4, among both countries that had participated since 2001 and new members (see also results for recommendation 6). From 2005-6 no further increase in the number of countries reporting RSV was observed (Table 1).

Recommendation 2

Both combined nose/throat swabs and nasal pharyngeal aspirates are acceptable for RSV diagnosis.

Different types of specimens are used for detection of influenza and RSV [21]. Generally the nasopharyngeal aspirates have a high sensitivity, and are often used in a hospital setting. Easier to use and less painful are nasal/nasopharyngeal swabs [22]. An inventory carried out in 2002 indicated that in sentinel surveillance systems in Europe nose and/or throat swabs were taken [20]. Twelve out of 20 national networks collected combined nose/throat swabs. The remaining networks collected either nasopharyngeal, nasal, or throat swabs. In addition, three networks took blood samples and one network obtained nasal aspirates [20]. Since all countries had already used the recommended type of respiratory sample and fulfilled the recommendation, no progress was assessed after 2002.

Recommendation 3

The application of molecular techniques such as real time polymerase chain reaction (PCR) in the diagnosis of respiratory disease has been demonstrated and this technique is advocated for RSV detection.

In 2006, laboratories were invited to participate in a quality control study for molecular methods. Of the 33 laboratories participating in EISS, 25 performed this technique with an overall performance of 88% correct results [23]. The majority (22 out of 25) of laboratories used an in-house molecular assay. In particular, real time PCR and nested PCR assays provided the highest performance scores (93% correct score; range 70-100) and were used in 19 laboratories. Three laboratories used commercial assays and the percentage of correct results ranged from 50% to 80% [23].

Recommendation 4

Further developments in the use of standardised methods and laboratory techniques are encouraged.

Limited progress was made in standardising methods. Only for influenza, not RSV, laboratory protocols were shared and standardised reagents were made available via the EISS website. However, with the application of molecular methods, as indicated in recommendation 3, and quality control assessment of this method, the quality of laboratory testing of RSV is ascertained.

Recommendation 5

The development of a sentinel system of representative hospitals should be considered.

No efforts were made to develop a European sentinel surveillance system consisting of representative hospitals, though national initiatives may have been undertaken. For example, a laboratory-based surveillance for RSV involving different hospital laboratories in Slovenia was implemented in 2006 [24].

Recommendation 6

We recommend the new networks joining EISS to integrate RSV surveillance alongside influenza.

Ten new countries became members of EISS between 2004 and 2008: Austria, Bulgaria, Croatia, Estonia, Finland, Cyprus, Greece, Hungary, Ukraine and Serbia [25]. Of these, four countries followed the recommendation and started reporting RSV data (Table 1).

RSV detections: six countries

To illustrate the data that were collected by EISS, we present the results of RSV detections for six countries. All countries reported at least five seasons of data, which provided insight in the occurrence of RSV in these countries. RSV and influenza detections are

presented in Table 2. The percentage of RSV-positive specimens largely differed by season, e.g. from 3% to 19% in the Czech Republic (Table 2). For all seasons and countries together the percentage of RSV-positive specimens varied from 4% in Germany and the Netherlands to 16-18% in the United Kingdom. RSV activity usually started a few weeks before the onset of influenza activity (data not shown). The data collected are useful to describe the seasonality of RSV and show that RSV is detected in patients with ILI and/or ARI.

Discussion and conclusion

Progress in RSV surveillance was made in the period 2001-2008, with the most obvious increase in the number of reporting countries during the time the RSV Task Group was active, between 2003-2006. Progress was made particularly in terms of the number of countries testing specimens for RSV and the use of molecular techniques. The results for the six countries that had reported at least five years of data showed that RSV surveillance and reporting is feasible in Europe. The overall percentage of RSV-positive specimens for the Czech Republic, France, Germany, Netherlands, Slovenia and the UK amounted to 4-18% indicating that a substantial number of patients who consulted their sentinel physician with influenza-like illness or acute respiratory infection actually had an RSV infection. The EISS surveillance is real time and therefore can be relevant for timing of the influenza and RSV peak and providing insight into the morbidity and seasonality of these respiratory illnesses.

Limited progress was made for recommendation 4 on the use of standardised laboratory methods. With the use of mainly in-house developed methods that perform well [23], the standardising of methods was not further explored. The rationale was that standardising methods is important and is encouraged by sharing protocols, but more important is the ability of the laboratory test to correctly identify RSV. Furthermore, limited progress was made for recommendation 5 on the development of a sentinel approach of hospitals. This recommendation was ranked as a lower priority because non-sentinel data from hospitals are currently being collected. The non-sentinel data could be used for the future establishment of a sentinel laboratory monitoring system and would then need to be assessed for representativeness and quality of data collection.

In this paper we presented data on sentinel RSV and influenza detections. Relatively low numbers of positive RSV tests were reported and this is therefore a limitation. In addition to sentinel data, RSV reports from non-sentinel sources, mainly derived from hospitalised infants are also available and these can provide insight into the epidemic peak of RSV during wintertime. We think that both sources of data are important and complement each other. Sentinel data highlights the occurrence of RSV in the community, where it is an important confounder in influenza surveillance. And hospital-based data present the circulation of RSV in more severe cases and high-risk groups.

The limitations of the sentinel influenza surveillance carried out by EISS are related to differences in case definitions [19], sampling guidelines and laboratory techniques among the different countries [20]. Some difficulty in obtaining swabs from all age groups has been reported, especially for young children in the Netherlands and the elderly in the Netherlands and France [16]. Another limitation is that we could not further investigate other possible causes of respiratory infections such as rhinovirus, adenovirus and coronavirus [26,27] and human metapneumovirus [28]. Country resources however may limit the extension of testing for other viruses in addition to influenza and RSV. Furthermore, no comparison regarding the occurrence of RSV and influenza between the different countries could be made because of differences in data collection procedures and laboratory methods. Additionally, differences in healthcare seeking behaviour may influence the findings between countries.

Currently diagnostic specimens are collected from patients presenting with ILI or ARI. Although ILI and/or ARI case definitions have been used for the detection of influenza for many years, this may not be the optimal clinical indicator for RSV. To investigate the clinical impact and determine the burden of illness of RSV one should extend the diagnostic categories to include acute bronchitis and otitis media [29]. This may become feasible with the movement towards sentinel networks based on electronic data.

We presented the progress in RSV surveillance based on an influenza surveillance network and data collected for six countries. This illustrated the feasibility of reporting RSV data and showed that a proportion of about 4-18% of the patients were infected with RSV. Sentinel monitoring of RSV and influenza virus is important and may even be extended to other respiratory viruses as the development of multiplex PCR [30] facilitates the detection of other causative agents of respiratory illness. All countries are encouraged to test their specimens for RSV and improvements can be made as less than half of the countries participating in EISS had reported these data. Furthermore, swabbing procedures should be further harmonised and regular quality control of laboratory methods should be performed. When these criteria are met, surveillance of RSV and influenza virus will contribute to a better insight into the burden of respiratory diseases and may be used by healthcare organisations to decide on the timing of palivizumab prophylaxis for RSV in Europe. Overall, this paper illustrated that an existing influenza surveillance system can be relatively easily broadened to include the surveillance of RSV and may be extended to other viruses in the future.

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Legionnaires' disease cluster linked to a metal product aqueous pre-treatment process, Staffordshire, England, May 2008

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In May 2008, a report of two workers from the same construction equipment manufacturing plant who were admitted to hospital with Legionnaires' disease confirmed by urine antigen prompted an outbreak investigation. Both cases were middle aged men, smokers, and with no travel, leisure or other common community exposure to *Legionella* sources. There were no wet cooling towers at the plant or in the surrounding area. No increase in respiratory disease or worker absenteeism occurred at the plant during the preceding month. Wider case ascertainment including alerts to hospitals and medical practitioners yielded no further cases. The environmental investigation (and sampling of water systems for *Legionella*) identified a *Legionella pneumophila* serogroup 1 (Mab 2b) count of >3.0x10^4cfu/l in water samples from an aqueous metal pre-treatment tunnel, which generates profuse water aerosol. Drainage, cleaning and biocide treatment using thiazalone eliminated *Legionella* from the system.

Introduction

Legionnaires' disease is an atypical pneumonic illness caused by the inhalation of aerosolised *Legionella* bacteria. These bacteria are found naturally in environmental water sources usually in low numbers. Multiplication of this organism is favoured when water is stagnant and warm. Poorly maintained aerosol-generating devices and water systems such as wet cooling towers, and spa pools are well documented sources of Legionnaires' disease [1]. Aside from travel exposure, the majority of cases and clusters of Legionnaires' disease in Europe are associated with community sources, mainly cooling towers and spa pools. Direct links with industrial manufacturing processes are less common [2,3].

On 15 May 2008, public health authorities in the West Midlands, England, were notified of two confirmed cases of Legionnaires' disease, admitted to the same hospital on the previous day. Both cases worked on the production line at the same construction and agricultural equipment manufacturing plant (plant X). The local health protection unit declared this a presumptive Legionnaires' disease cluster and led an outbreak control team to investigate common infection sources at work and in the community. This paper describes the disease cluster, the environmental investigation and the control measures implemented.

Methods

A confirmed case of Legionnaires' disease was defined as a person working at plant X who had clinical symptoms of pneumonia, was confirmed radiologically and by laboratory evidence of infection with *Legionella pneumophila* serogroup 1 (Lp-1), with onset of symptoms after 22 April 2008. Laboratory confirmation consisted of detection of Lp-1 antigen in urine.

Searching for additional cases included a review of worker sickness absenteeism and reports of respiratory illness at plant X during the preceding month. The occupational health service at the plant informed the work force of potential risks and advised early reporting of respiratory symptoms. All workers with onset of respiratory symptoms after 22 April 2008 were urgently investigated and offered a urine antigen test. In addition, clinicians and microbiologists at local medical referral centres and hospitals, as well as neighbouring health protection units were alerted.

The cases and their close family members were interviewed in hospital shortly after admission using a standardised questionnaire to elicit demographic details, clinical history, risk factors for Legionnaires' disease, and sources of potential *Legionella* exposure during the previous 14 days. Details were obtained regarding travel (abroad and locally), recreational activities (water exposure, spa pool exposure), hospital admissions, domestic risk factors, and occupational activities.

Environmental health and safety officials undertook an environmental investigation and risk assessment including a review of local wet cooling towers, and a description of water systems at the plant with collection of water samples for *Legionella* culture and isolation.

Laboratory confirmation of clinical cases used *Legionella* urine antigen Binax NOW rapid immunochromatographic assay for the qualitative detection of *L. pneumophila* serogroup 1 antigen in urine samples [4]. Isolation and typing of environmental *Legionella* consisted of concentrating 1 litre water samples by membrane filtration and elution of the deposit. The deposit was heat- and acid-treated to reduce unwanted bacterial growth. Treated and untreated

portions of the deposit were inoculated onto selective buffered charcoal yeast extract agar containing cysteine and iron [5].

Results

Two confirmed cases (cases A and B) were admitted to hospital on 14 May 2008 with clinical pneumonia. Symptom onset had been on 6 and 8 May 2008, respectively. Both cases were 40-50 year-old men with a history of heavy cigarette smoking. They responded well to standard treatment, did not require mechanical ventilation, and were discharged from hospital after eight days. Attempts at sputum sample collection were unsuccessful and clinical Lp-1 isolation was therefore not possible.

The cases lived in different towns (9 miles apart) and drove to work using different routes. Both had not travelled locally, within the country or abroad in the preceding two months, and had no exposure to common domestic, leisure and community aerosolised water sources. Both were full-time production line workers at plant X but were not close friends and had no contact outside of work. They reported working on different stages of the production line approximately 20 metres apart.

Plant X has a workforce of 642 people and is situated in a semi-rural town in a district of approximately 500,000 residents. Case searching at the plant did not yield any further cases. No increase in absenteeism was detected at the plant during the six months prior to identification of the two cases. Fourteen workers were identified who had been absent from work in the previous four weeks, of which 11 reported respiratory symptoms. None of these had clinical pneumonia or were admitted to hospital, and all tested urine antigen-negative for Lp-1. The two confirmed Legionnaires' disease cases did not represent an increase in notifications above the average of two cases (range: 0-9) per year that occurred in the prior 14 years in this district. A review of all industry-linked Legionnaires' disease reports in this district since 1994 identified only two cases but their exact exposure could not be identified.

The plant has a basic rectangular floor plan, housing a comprehensive production line and small administrative section. No wet cooling or air conditioning systems are used at the plant. In addition there are no cooling towers in the town or in the immediate vicinity of the plant with no adjacent industries or office buildings.

The plant used four water systems:

1. Two independent domestic type hot and cold water systems supplying the restroom and changing facilities. These systems had been drained in April 2008, were regularly monitored, and had no stagnant water sections.
2. A paint mist trap in an unheated spray paint booth. Here a below ground-water jet traps paint mist under negative pressure to an extraction stack. The water is at ambient temperature.
3. An aqueous metal pre-treatment tunnel. Steel parts on a monorail move through a degreasing and rinsing tunnel in preparation (pre-treatment) for painting. The system has a complex network of pipelines and tanks providing jet spraying of parts with solutions (including alkaline degreaser and an acidic phosphate solution) and water (which has a pH neutralising effect) at successive stages inside a tunnel.

Different solutions and water are drawn from their respective tanks by pumps and fed to spray nozzles inside the tunnel. There are six pre-treatment stages: a cleaning stage followed by two water rinses, then a 'keying chemical' stage with a further two water rinses. Each stage has its respective supply and collection tank. The chemical tanks were heated to 55-60 ºC. The water for rinsing is mains-fed and supplies four unheated water tanks (volume of each tank: 8,000 to 15,000 litres) at 25-38 ºC. The brushes covering the conveying railing were missing and there was no local extraction for the tunnel. Aerosols were visibly leaking from the gap of the conveying railing and the large openings at the entrance and exit of the tunnel.

Prior to this incident, the aqueous pre-treatment process had not been risk-assessed as a source of *Legionella* organisms and potential human exposure. No management system (protocol) for monitoring (including *Legionella* sampling), disinfecting and cleaning the water systems was in place.

Case A worked on the assembly production line, and Case B worked at the aqueous pre-treatment and powder coating section. Case A walked past the pre-treatment plant a number of times daily to an adjoining factory exit where he smoked.

Baseline sampling and culture of all water systems (a, b, and c) was undertaken on 16 May 2008. No *Legionella* was isolated from the domestic hot and cold water system (a) or the paint mist water trap system (b). Water samples from the aqueous pre-treatment system (c) contained *L. pneumophila* serogroup1 (Mab 2b) at a count of $>3.0 \times 10^4$ colony-forming units (cfu)/l.

Drainage and cleaning of the aqueous pre-treatment system (c) and the domestic-type hot and cold water system (a) were undertaken during the initial two weeks following the detection of the two cases, followed by chlorine dioxide shock treatment of the pre-treatment system. For maintenance, biocide treatment with thiazalone was preferred over chlorine and other halogen-based products, as these may interact with degreasing chemicals, causing corrosion and affecting product quality. The subsequent dosing regime was reviewed regularly and modified until a suitable balance was achieved, taking into account the short half life of thiazalone. During plant shut down at each weekend, all tanks were completely drained and cleaned.

Subsequent water samples from the water tanks supplying the metal pre-treatment process (c) yielded *L. pneumophila* serogroup 1 (Mab2b) in diminishing numbers over a four week period, leading to eradication on 20 June 2008.

Discussion and conclusions

We report on two epidemiologically linked Legionnaires' disease cases with likely occupational exposure to an aqueous pre-treatment system in a construction equipment manufacturing plant. The aqueous pre-treatment system carried the highest risk as a probable source of infection because of the isolation of *L. pneumophila* serogroup 1 from the water and associated aerosolisation. Because clinical samples were not available for further typing and matching to Lp-1 isolated from the water samples, definitive causality could not be established. Future investigations should therefore prioritise obtaining clinical isolates to confirm the aqueous pre-treatment system as the source of infection. The domestic systems (a) were reasonably controlled, and the paint-mists water trap system (b)

had a *Legionella*-inhibitory temperature (below 15 °C) with water aerosols under suction. Therefore, the risk of human exposure from those systems is low.

No prior risk assessment of the aqueous pre-treatment system had been undertaken at the plant. Immediate and medium-term control measures (water sampling, biociding, cleaning/drainage) were effective in controlling *Legionella* growth and preventing further cases of Legionnaires' disease.

Legionnaires' disease clusters have been reported from industrial settings with workers exposed to sources of aerosolised water, including from biological treatment plants in the pulp and paper industry [6], contaminated metal-working fluids in the automotive industry [7], factories that use water to cool moulded plastics [8], and waste water treatment facilities [9]. Aqueous cleaners are generally believed to present a low risk to workers' health and gained popularity in industry as degreasing of metal parts by organic solvents was gradually phased out [10]. To the best of our knowledge this is the first report implicating an aqueous metal pre-treatment plant as a possible source of *Legionella* linked to a cluster of Legionnaires' disease.

Aqueous pre-treatment systems are prone to *Legionella* growth due to favourable water temperature, the presence of nutrients such as rusts and dirt from metal parts, convoluted surfaces that favour biofilm development, and recirculation of the water. Since the report of these two cases, five similar aqueous pre-treatment systems have been inspected by the United Kingdom's Health and Safety Executive, and *Legionella* has been isolated in four. A cleaning and disinfection regime similar to the one reported here was implemented and has prevented further growth of *Legionella*. The findings of this subsequent investigation are being submitted for publication.

Significantly, aqueous pre-treatment systems generate profuse water aerosol, and preventing escape may prove complex. Assessing the risks for Legionnaires' disease in similar systems, common in the metal manufacturing industry, is recommended.

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A foodborne outbreak of norovirus gastroenteritis associated with a Christmas dinner in Porto, Portugal, December 2008

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An outbreak of acute norovirus gastroenteritis was detected and epidemiologically linked to a Christmas dinner reunion of 22 recent graduate students in a restaurant in Porto, Portugal, in December 2008. A retrospective cohort study was carried out using online standardised questionnaires. Sixteen primary and three secondary cases were identified and the risk ratios with 95% confidence intervals for each food item were calculated. The response rate to the online questionnaires was 96%. The outbreak met all four Kaplan's criteria and the attack rate was 73%. Norovirus GII.4 2006b was detected in stools and emesis samples of two primary cases. The ingestion of soup and lettuce salad was considered a risk factor for this norovirus outbreak, as determined by statistical analysis. Our investigation demonstrated two routes of transmission of norovirus starting with foodborne exposure followed by secondary person-to-person spread. To our knowledge this is the first study identifying norovirus as the causative agent of a foodborne outbreak in Portugal.

Background

Noroviruses are the leading cause of foodborne outbreaks of acute gastroenteritis and the most common cause of sporadic infectious gastroenteritis among persons of all ages [1-6]. In the present study we describe the investigation by statistical and virological methods of what we think to be the first report of a foodborne norovirus outbreak in Portugal. On 27 December 2008, a group of 22 former students of the University of Porto, now living in different regions of Portugal and abroad, gathered at a Christmas dinner party. This meeting was the only person-to-person contact that this group had had in months. They sat at two different tables (with 4 and 18 individuals, respectively) and were served separately without any contact between the two tables during the meal. Symptoms of loose stools and vomiting appeared 24 hours after the dinner in a 28-year-old couple from the group. This couple had not shared any other meal since they had spent Christmas holidays away from each other. The dehydration was so severe that they required hospitalisation. They received intravenous fluid therapy and oral loperamide in order to recover fluid balance, oral metoclopramide for nausea and emesis and oral omeprazol for gastric and duodenal protection. Both developed fever (39.0ºC - 39.5ºC) and received intravenous paracetamol and antibiotic therapy with oral ciprofloxacin, which was maintained for seven days. At that time no laboratory diagnosis was made for gastroenteritis pathogens. The two patients spent the night in the hospital for observation and received further intravenous fluids now with acetylsalicylic acid for the fever. At that time and based on the symptoms the possibility of a foodborne outbreak was considered. Preliminary investigations of the couple led to the Christmas dinner served to another 20 persons as the most probable origin of infection. A retrospective study was initiated in order to find the full extent of the outbreak and its probable source.

Methods

Epidemiological investigation

A list of people who attended the Christmas dinner was retrieved from the index cases, the 28-year-old couple who presented with vomiting, diarrhoea, abdominal pain, nausea and fever. A structured questionnaire was developed and emailed to the 22 participants of the dinner to obtain information about sex, age, food intake,

Table 1

Comparison of Kaplan's criteria with the primary cases of an outbreak of gastroenteritis in Porto, Portugal, December 2008 (n=16)

Kaplan's criteria	Outbreak in Porto
1) Vomiting in > 50% cases	Vomiting in 94% of the cases
2) Duration of illness 12-60 hours	81% of cases had duration of illness between 12-60 hours*
3) Incubation period of 15-36 hours	94% of cases had incubation period of 15-36 hours
4) Bacterial pathogens not present	Stool samples found negative for bacteria

*This study questionnaire asked for the duration of illness in terms of days and not in hours. 81% of the cases presented duration of illness between 12 and 60 hours and 19% had duration of illness between 60 and 72 hours.

Figure 1

Geographic distribution of the primary and secondary cases of an outbreak of gastroenteritis in Portugal, December 2008

Figure 2

Cases associated with an outbreak of gastroenteritis in Porto, Portugal, December 2008 - January 2009, by date of onset of symptoms (n=19)

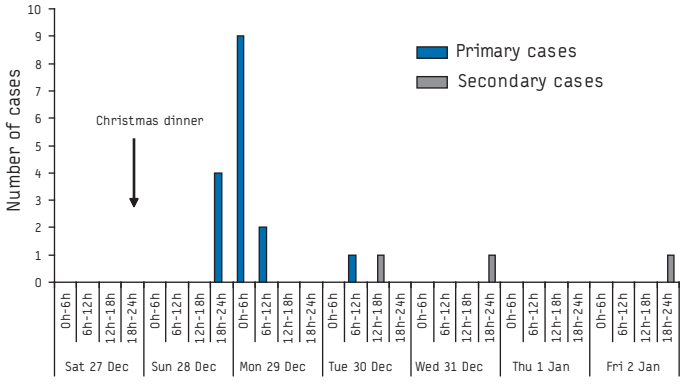

Table 2

Univariate analysis of risk attributed to specific food items consumed during a dinner party associated with an outbreak of gastroenteritis in Porto, Portugal, December 2008

Food item	Univariate analysis		
	RD	RR	95% CI (RR)
Lettuce salad	0.197	1.31	0.74-2.32
Iced cake	0.197	0.76	0.43-1.35
French fries	0.06	0.92	0.47-1.79
Soup	0.385	1.63	1.06-2.50
Cheese	0.058	0.92	0.47-1.81
Bread	0.047	1.07	0.45-2.55

RD: risk difference; RR: risk ratio; CI: confidence interval;

onset and nature of symptoms and duration of illness. They were also asked to report similar cases in their households and close environment during the same or the following week in order to obtain details about possible secondary cases caused by person-to-person transmission.

Primary case was defined as a person who ate at the restaurant on the night of 27 December 2008 and experienced diarrhoea (alone) or a vomiting episode plus one or more of the following symptoms: abdominal pain, nausea, and fever within 72 hours after the restaurant meal. Secondary case was defined as a close contact (household member) of a primary case who did not participate in the dinner of 27 December and experienced diarrhoea (alone) or a vomiting episode plus one or more of the following symptoms: abdominal pain, nausea, and fever within a two week period after the meal.

The primary attack rate (AR) was calculated as the number of primary cases divided by the total number of people dining at the restaurant on 27 December and therefore possibly exposed to the causative agent.

To measure the association between eating specific food items served at the Christmas dinner and developing illness, Mantel-Haenszel estimates of the risk ratio (RR) with 95% confidence intervals for each food item were calculated.

Laboratory investigation

Two stool samples and one emesis sample were collected from the couple 36 hours after the Christmas dinner and tested for bacterial, parasitic and viral enteric pathogens. Routine bacterial culture for *Salmonella* and *Shigella* was performed according to standard procedures and microscopic methods were used to screen for protozoa and helminths. Stool specimens were examined for rotavirus and adenovirus by a commercial immunochromatographic test. All samples were examined for the presence of norovirus by reverse-transcription polymerase chain reaction (RT-PCR) using JV12y/JV13i oligonucleotide primers [7] followed by nucleotide sequencing of the RT-PCR products.

Results

Epidemiological and clinical characteristics of cases

Of the 22 dinner participants, 21 completed the questionnaire (response rate 96%) and 16 met the primary case definition yielding an overall attack rate of 73%. All cases (nine female and seven male) reported symptoms in compliance with Kaplan's criteria [8,9] (Table 1).

Based on the answers to the questionnaires three further persons were identified who met the definition of secondary case, two of these were parents of two primary cases living in Porto, the third was identified in Lisbon and was a close contact of an asymptomatic person who had participated in the dinner (Figure 1).

The 16 primary cases reported the following clinical symptoms: diarrhoea (n=12, 75%), vomiting (n=15, 94%), abdominal pain (n=8, 50%), nausea (n=7, 44%), fever (n=5, 31%), fainting (n=1, 6%) and asthenia (n=7, 44%). Two persons (the 28-year-old couple) had to be hospitalised because of the severity of dehydration and received intravenous fluids. Among the five dinner participants who did not fully meet the case definition criteria, two had abdominal pain, two reported nausea and three reported asthenia.

Clinical symptoms in the primary cases started abruptly 24-36 hours after the Christmas dinner, on Sunday and Monday, 28-29 December 2008. The mean incubation period was 28 hours (Figure 2). The duration of illness ranged from 12 to 76 hours (mean 45 hours). The last case associated with this outbreak was a secondary case in Lisbon who had onset of symptoms on Friday 2 January 2009, six days after the dinner. This person had contact with one of the asymptomatic guests of the dinner who traveled from Porto to Lisbon on 1 January.

Food risk assessment

From the data obtained through the questionnaires on food items consumed at the dinner soup was identified as the most likely source of the outbreak with a RR of 1.63 (95% CI: 1.06-2.50), followed by lettuce salad with a RR of 1.31 (95% CI: 0.74-2.32) (Table 2).

Laboratory investigation

Macroscopic analysis of one stool sample revealed live blood. This was confirmed by the presence of erythrocytes by optical microscopy. Both stool samples tested negative for *Salmonella* and *Shigella* and for rotavirus and adenovirus. The two stool samples and the emesis sample tested positive for norovirus. Nucleotide sequencing of the RT-PCR products demonstrated that all three isolates were identical and belonged to genotype GII.4 2006b.

Discussion

In the present study we describe a foodborne outbreak associated with a dinner in a restaurant in Porto, Portugal. Our combined epidemiological data and virological findings suggested that the causative pathogen was norovirus which was detected from the faecal and vomit specimens obtained from the couple who required hospitalisation. This strain was identified as a GII.4 2006b which has been predominant at a global scale for the past three years [10,11]. The involvement of other enteric pathogens in this outbreak cannot be ruled out with the exception of *Salmonella, Shigella,* enteric protozoa, helminths, rotavirus and adenovirus for which the faecal samples tested negative. The treatment of the hospitalised couple with loperamide is questionable since the use of antimotility agents in severe gastroenteritis may be harmful [12]. Normally, except the rehydration therapy, no further drugs are necessary in viral gastroenteritis treatment. The clinical and epidemiological characteristics of this outbreak including an attack rate of 73%, a mean incubation period of 28 hours, and a mean duration of illness of 45 hours as well as the occurrence of secondary cases are in accordance with a norovirus outbreak. Moreover, this cluster of cases met all four epidemiological criteria for a norovirus outbreak [8,9].

No definitive conclusion on the source of this outbreak could be reached, since food samples were not available for norovirus detection. However a foodborne origin was supported by the analysis performed with the web-based tool developed by the Foodborne Viruses in Europe (FBVE) network for the investigation of norovirus food-related outbreaks [13]. Risk associated with individual food item revealed, unexpectedly, that soup, despite being a warm product, was the most likely source of the outbreak based on its highest RR (1.63, 95% CI: 1.06-2-50). Lettuce salad has been frequently associated with norovirus outbreaks [14] and in the present study was also associated with a high RR (1.31, 95% CI: 0.74-2.32). French fries, cheese and bread were not considered a risk factor given their RR (~1). Whether the food was contaminated before arriving at the restaurant or infection was due to poor food handling practices could not be determined since information on hygiene conditions, food handling practices and health status of the restaurant staff were not available.

Our data indicated that there were two routes of transmission in this outbreak. The origin was a foodborne transmission which caused infection in the primary cases who, subsequently, through person-to-person transmission, infected secondary cases among household and close contacts. The last case associated with this outbreak was detected six days after the dinner in a person resident in Lisbon who had contact with one of the participants of the Christmas dinner group. Although no laboratory confirmation was performed, the Lisbon case met in full the definition of secondary case, but the possibility that this patient was not associated with the outbreak cannot be ruled out.

To our knowledge this is the first study identifying norovirus as the causative agent of a foodborne outbreak in Portugal.

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An outbreak of hospital-acquired Staphylococcus aureus skin infection among newborns, Nan Province, Thailand, January 2008

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In January 2008, we investigated a cluster of neonates with bullous impetigo in a hospital of northern Thailand in order to control the outbreak and identify a potential source of the infection. We reviewed medical records and working timetables of healthcare workers (HCWs) and conducted a case-control study. We performed an environmental study and took bacteriological samples from HCWs and equipments. According to our case definitions, we identified 16 confirmed cases and 14 probable cases. The attack rate was 42%. Most cases had skin blisters (28 cases) followed by pustules (five cases) and exfoliation (three cases). The location of the lesion was the trunk (17 cases), neck (14 cases) or armpits (nine cases). Nineteen cases had symptoms onset after discharge from hospital. Median age at onset was 4 days. The strain isolated from an infected newborn shared the same phage type as the contaminated equipment. Insufficient hand hygiene was an observed risk behaviour of HCWs and visitors. Exposure to a nasal carrier of *Staphylococcus aureus* (adjusted OR: 80.3, 95% CI: 4.8 – 1350.3) and ward sharing with a symptomatic case (adjusted OR: 35.6, 95% CI: 1.9 – 654.7) increased the risk of acquiring the infection. The outbreak ended abruptly after implementation of hand hygiene practices and equipment cleaning.

Introduction

Bullous impetigo is a superficial bacterial skin infection, mainly affecting infants and small children, usually caused by *Staphylococcus aureus* which can lead to severe illness in the form of staphylococcal scalded skin syndrome (SSSS), septicaemia, or pneumonia [1,2]. Newborn infants are prone to skin infection due to the vulnerability of their skin [3]. Healthy carriers of *S. aureus* such as healthcare workers (HCWs) [4,5] can transmit the bacteria to others [6,7]. Thai Ministry of Public Health included nosocomial infections in mandatory reporting in 1982 [8]. The prevalence of nosocomial infections in Thailand was 11.7% in 1988, it diminished to 7.4% in 1992, to 6.4% in 2001 and slightly increased to 6.5% in 2006 [9]. Most hospitals in Thailand have targeted surveillance systems in place for high risk population such as intensive care patients, post-surgery patients and patients with invasive devices. However, staff shortage and high workload are the main problems in tackling nosocomial infections in Thailand [10-12].

Hospital A is a district hospital with 90 beds and 50-60 births take place here on average, every month. This hospital takes care of seemingly uncomplicated pregnancies. If the woman is considered at high risk, she is transferred to the provincial hospital, which offers better facilities for critical care.

A pregnant woman close to delivery stays in the pre-delivery room until delivery is imminent, when she is transferred to the delivery room. If caesarean section becomes necessary, she is transferred to the operating room. After delivery, mother and newborn stay in the same room and bed at the postpartum ward. There are two postpartum wards, ward A and ward B. Ward A is the first priority for hospital stay after the delivery because it is located in the same building with the delivery room. Ward B is usually empty and the room is used as the alternative ward if ward A is full. Newborns delivered by caesarean section stay in the nursery for approximately one hour for close observation of vital functions. If their condition is stable, they are sent to the postpartum ward immediately. After uncomplicated deliveries, mother and child may be discharged from hospital even after 48 hours.

Methods

On 25 January 2008, a medical officer at hospital A notified the Bureau of Epidemiology, Department of Disease Control in the capital, of an increasing number of neonates with bullous impetigo and requested assistance for an outbreak investigation. In this report we describe an outbreak of the staphylococcal bullous impetigo occurring in a district hospital in northern Thailand between 11 and 27 January 2008. Our objectives were to control the outbreak, to identify potential sources of infection and to investigate risk factors for illness.

During the outbreak, hospital A had 34 HCWs of whom 19 were exposed to newborns (eight nurses, five student nurses, four nurses' aids and two doctors). These 19 HCWs worked in all the units of maternal and newborn care. Following the rules and policies of Hospital Accreditation, there was one infection control nurse (ICN) responsible for hospital infection control activities which included surveillance for hospital-acquired infections, supervision of infection control practices for healthcare workers, and evaluation of medical products that could increase the risk for infection. Due

to shortage of staff, this nurse was also involved in direct patient care.

Descriptive epidemiology

We started our study by reviewing medical records of the cases occurring in hospital to identify the first case of the cluster. We determined the investigation period by counting backward ten days from the onset of the first case [13]; thus the observation period began on 1 January 2008. A probable case was defined as a newborn infant (age ≤30 days) with skin pustule, blister or exfoliation on any part the body who was born between 1 January and 25 January 2008. A confirmed case had in addition methicillin-sensitive *Staphylococcus aureus* isolated from the skin lesion. We contacted the parents of all 71 neonates who were born during 1-25 January 2008. Sixty of them responded. The paediatrician was asked to collect date of onset of each case and to describe the skin lesion by anatomical location. In addition, all parents of cases were interviewed about potential community infection risk factors.

Environmental and laboratory investigation

We interviewed eight HCWs who worked in the delivery room and post partum wards and observed their routine neonatal care practice. We inspected the delivery room, the neonates' room and the disinfection unit where we observed the adherence to standard infection control procedures. We enquired about schedules for room cleaning and requested disinfection protocols from the ward's chief nurse. A laboratory technician collected samples from the most frequently used neonatal care equipments, such as radiant warmer, weight scale, baby-crib and stethoscopes. Environmental samples, 37 specimens, from the bathing counter, soap and washing water for instance were also collected for bacterial culture. Hand swab and nasal swab samples were collected from all HCWs. We took swabs on the first web space between the thumb and index finger and in the right nostril. In order to confirm the epidemiological links between positive culture samples from cases and environmental samples, we performed limited phage typing.

Analytic epidemiology

We conducted a case-control study by comparing 16 laboratory-confirmed cases with 30 healthy neonates (no skin lesion) that were born in the same hospital during the same period. Type of birth, room location for neonates, exposure to neonatal equipment and exposure to each HCW were tested for statistical association with case status by calculating odds ratio (OR) and 95% confidence interval (CI). We used the working timetable of each HCW as a proxy of newborn exposure by matching their schedule to the first 24 hours after birth of each neonate. We used multiple logistic regression technique to diminish the effect of possible confounding factors. The variables with significant p-value, less than 0.05, from the univariate analysis were put in the model. We used Excel 2003 and STATA 10.0 programmes for data analysis.

Results

Descriptive results

The onset date of the index case was on 11 January 2008. Sixty (84.5%) out of 71 neonates were physically examined again from 25 January to 27 January 2008, of which we identified a total of 30 cases (attack rate = 42%): 16 confirmed and 14 probable cases. Skin blister was the most common symptom (28 cases), followed by skin pustule (five cases) and skin exfoliation (three cases). Skin lesions were located at the trunk (17 cases), neck (14 cases), armpits (9 cases), groins (seven cases), upper extremities (seven cases) and lower extremities (five cases).

No serious case or complication has been recorded during this outbreak. The age of illness onset ranged from 1 to 12 days; median age was 4 days. Eleven of the 30 cases had symptoms during hospitalisation and 19 showed symptoms only after discharge from hospital. From the interviews with the parents, we found out that no other family members had skin infections during that time. The sex specific attack rate was 46% (16/35) for male and 56% (14/25) for females. The attack rate by room location was highest in ward A (61%) followed by the nursery (44%) and zero in ward B.

The epidemic curve (Figure) illustrated a gradually increasing number of cases at the beginning of the outbreak, a sharp increase

Table 1

Phage typing from one case, from neonatal care equipment and from carriers among healthcare workers, hospital A, Nan Province, Thailand, January 2008

Sample	Result
Case 1	MSSA- phage type 29/52/80/3C/55/95/81/94/96
Radiant warmer in the delivery room	MSSA- phage type 29/52/80/3C/55/95/81/94/96
Weighting scale in the delivery room	MSSA- phage type 29/52/80/3C/55/95/81/94/96
Baby crib in ward A	MSSA- phage type 29/52/80/3C/55/95/81/94/96
Bathing counter in ward A	MSSA- phage type 29/52/80/3C/55/95/81/94/96
Nurses' aid A4 (nasal swab)	MSSA- phage type 29/52/80/3A/3C/55/6/47/53/54/75/77/83A/94/96
Nurse R5 (hand swab)	MSSA- phage type 29/52/52A/80/3A/71
Student nurse S5 (nasal swab)	Non-typable

Table 2

Univariate analysis of potential exposures of neonates with bullous impetigo, hospital A, Nan Province, Thailand, January 2008 (n=46)

Exposures	Crude OR (95% confidence interval)	p-value
Admission in ward A	11.3 (1.3 – 512.2)	0.011
Ward sharing with symptomatic cases	5.4 (0.9 – 54.9)	0.034
Exposure to nurses' aid A4 (carrier)	12.1 (2.0 – 122.0)	0.001
Exposure student nurse S2 (non carrier)	7.0 (1.5 – 36.6)	0.004
Exposure student nurse S4 (non carrier)	4.6 (1.1 – 20.5)	0.018

Table 3

The association between neonates with bullous impetigo and five exposures, significant p-value (p<0.05) from univariate analysis, by multiple logistic regression, hospital A, Nan Province, Thailand, January 2008 (n=44)

Exposures	Adjusted OR (95% confidence interval)	p-value
Admission in ward A	14.5 (0.4 – 578.2)	0.156
Ward sharing with symptomatic cases	35.6 (1.9 – 654.7)	0.016
Exposure to nurses' aid A4 (carrier)	80.3 (4.8 – 1350.3)	0.002
Exposure to student nurse S2 (non carrier)	0.8 (0.08 – 7.9)	0.860
Exposure to student nurse S4 (non carrier)	6.2 (0.6 – 60.5)	0.116

in the second week, and a peak on 25 January. The outbreak ended rapidly after ward closure for two days during 26 and 27 January. A week before the outbreak started, five student nurses had arrived at the maternal and neonatal care unit for nursing practice and they left in February 2008. When an increasing number of bullous impetigo cases was noticed, the ward nurses began to strengthen hand washing. However, they did not report the cases to the hospital infection control nurse until 25 January, because previously, newborn skin infections had not been included in the hospital infection surveillance protocol.

Environmental investigation and laboratory results

Our investigation revealed that the delivery room was cleaned with household detergent three times per week. We found that some equipment such as radiant warmers and the weight scale were cleaned only on superficial surfaces after utilisation. Postpartum wards, where the newborns stayed, were usually crowded with many visitors, who could easily touch and play with newborns without having properly washed hands.

Laboratory results

Methicillin-sensitive *Staphylococcus aureus* (MSSA) from all 16 confirmed cases had the same antibiotic sensitivity pattern and all were resistant to penicillin. Only one isolate was phage typed because the other isolates had already been discarded. Among 37 samples from neonatal care equipments, four specimens were positive for *S. aureus*. Two positive items, a radiant warmer and a weight scale, were found in the delivery room and three, a bathing counter, a baby-crib and a bed sheet of a case, were found in ward A. Three out of 34 healthy HCWs had positive cultures for *S. aureus*. Nurses' aid A4 and student nurse S5 had nasal carriage of *S. aureus* and nurse R5's hand swab was positive for *S. aureus*. None of the three carriers had a skin lesion.

MSSA phage type 29/52/80/3C/55/95/81/94/96 was identified from all four samples of contaminated neonatal care equipment. In addition, we identified phage type 29/52/52A/80/3A/71 and 29/52/80/3A/3C/55/6/47/53/54/75/77/83A/94/96 from nurse R5 and nurses' aid A4 respectively while phage type of Student nurse S5 was non-typable due to the limitations of laboratory technique (Table 1). The phage type of the newborn case was 29/52/80/3C/55/95/81/94/96, the same as the contaminated equipments and shared the same group as the carriers.

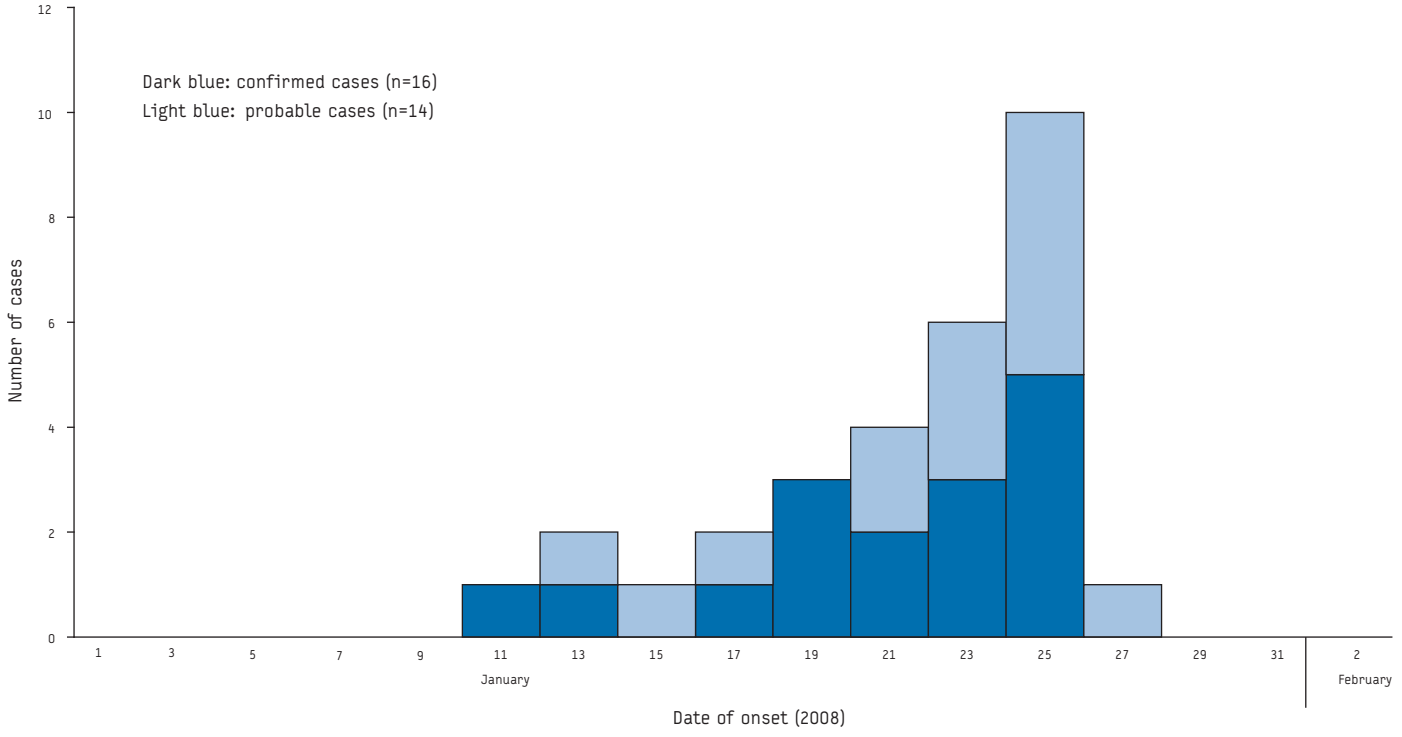
Analytic results

In the case-control study, neonates exposed to nurses' aid A4, who was a nasal carrier of *S. aureus*, had the highest risk of illness [crude OR: 12.1 (95% CI: 2.0 – 122.0), p=0.001]. In the analytic study, 36 potential exposures were tested for association; among these, only five variables as displayed in Table 2 had p value less than 0.05. Univariate analysis (Table 2) also indicated an association between illness and four other variables: staying in ward A [crude OR: 11.3 (95% CI: 1.3 – 512.2), p=0.011], exposure to non-carrier student nurse S2 [crude OR: 7.0 (95% CI: 1.5 - 36.6), p=0.004], sharing ward with the symptomatic case during hospitalisation [crude OR: 5.4 (95% CI: 0.9 - 54.9), p=0.034] and exposure to non-carrier student nurse S4 [crude OR: 4.6 (95% CI: 1.1 - 20.5), p=0.018].

In the multiple logistic regression model shown in Table 3, we found that both exposure to nurses' aid A4 and sharing ward with a symptomatic case remained significantly associated with illness

Figure

Epidemic curve of staphylococcal bullous impetigo cases by date of onset in a district hospital, Nan Province, Thailand, January 2008 (n=30)



in our model with adjusted OR equal to 80.3 [(95% CI: 4.8 – 1350.3), p=0.002] and 35.6 [(95% CI: 1.9 – 654.7), p=0.016] respectively.

Control action and outbreak response

After confirmation of the outbreak, the following measures were taken:

- Cases were treated and isolated in ward B;
- Delivery room and ward A were closed between 26-27 January 2008 for cleaning and disinfection;
- Medical devices such as the radiant warmer and newborn weight scale were cleaned with detergent and disinfected with 70% alcohol;
- HCWs carriers of *S. aureus* were treated with the topical antibiotic Muropicin, and required to abstain from nursing until nasal swabs were negative, i.e. seven days;
- Adherence to infection control measures was enforced such as hand hygiene, wearing masks and hair caps during routine nursing care;
- Alcohol hand rub was provided at each bed in postpartum wards.

Furthermore, we also recommended strengthening the hospital infection surveillance system with competency building for ward nurses to detect outbreaks and early report them to the hospital infection control practitioners.

On the last day of our investigation we joined the hospital meeting, presented the investigation results and discussed the infection control breaches such as insufficient hand hygiene and personal protective equipment. This meeting led to cleaning of the delivery room on a daily basis and cleaning neonatal care equipments after every use with detergent and 70% alcohol. Moreover, the chief ward nurse decided to implement new strategies such as limiting the number of visitors permitted to stay in the postpartum wards. Surveillance of newborns' skin infection was included in the infection control policy.

Discussion and conclusion

This outbreak of staphylococcal skin infections in newborns was detected late because most of the cases developed symptoms only after discharge. We implicated the environmental equipment as possible source of infection because it had the same phage type as the one from a case. Contact with a HCW who was a staphylococcal carrier was an important risk factor in our study, as has been seen in previous studies (7,14,15). With our limited resources it was impossible to determine if, and if so, which HCW could have been the source of the outbreak, although two of them were suspected. The high attack rate may be due to the circumstance that all newborns were exposed to the same equipment, such as the radiant warmer, weight scale and baby crib.

In a review by Williams [4] nose was the most frequent body site yielding staphylococci (40 to 44%) and the carrier rate among nurses in hospital ranged between 21 to 70%. Our study suggested a low prevalence (9%) of carrier status. However, our carrier rate may be underestimated because of a different technique of specimen collection and the limited laboratory capacity in a Thai district hospital.

Our investigation demonstrates that deficient infection control procedures may lead to outbreaks of staphylococcal infections among newborns. However, implementation of recommended infection control methods, such as proper hand washing and thorough cleaning of equipment, can quickly control an epidemic outbreak as demonstrated in this case and other similar cases [14,15]. The insufficient budget allocation for infection control is however a major problem in Thai medical system.

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Influenza-like illness surveillance using a deputising medical service corresponds to surveillance from sentinel general practices

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Standard sources of data for influenza surveillance include notifications of laboratory-confirmed cases and notifications from sentinel general practices. These data are not always available in a timely fashion, leading to proposals to use more immediate data sources such as over-the-counter drug sales, ambulance call-outs and web searches to monitor influenza-like illness (ILI). We aimed to assess data from a deputising medical service as another source of data for timely syndromic influenza surveillance. We measured the extent of agreement between the weekly percentage of patients with ILI reported from sentinel general practices and the corresponding weekly percentage reported from a deputising medical service in Victoria, Australia over ten years, from 1999 to 2008. There was good agreement between the two data sources, with suitably narrow limits of agreement. The deputising medical service did not use a standardised definition of ILI and is not supplemented by laboratory confirmation of suspected cases. Nevertheless, the results of this study show that such data can provide low cost and timely ILI surveillance.

Introduction

In temperate southern Australia, the influenza season occurs between May (late autumn) and October (early spring). Sentinel general-practitioner (GP) surveillance, operational in Victoria during the influenza season, reports weekly on the number of patients fulfilling the Australian nationally agreed case definition of influenza-like illness (ILI): cough, fever and fatigue. Respiratory specimens taken from a proportion of cases permit diagnosis of laboratory-confirmed influenza [1]. Not all ILI cases are confirmed as influenza. In Victoria, Australia, the proportion of confirmed cases between 2003-2007 varied from 18-47%, annually [2].

Besides notifications from sentinel GPs, another standard method of influenza surveillance is to count the number of laboratory-confirmed cases notified to a public health authority [1]. Both these standard data sources, which involve laboratory testing, are associated with a reporting lag due to the time taken for specimen testing and reporting. For instance, the median interval between symptom onset and registration for a laboratory test was three days for a patient recruited through sentinel GPs in Victoria in 2007 and 2008.

To overcome the problem of delay, surveillance using more immediate data sources without laboratory confirmation, referred to as syndromic surveillance, have been implemented. These include over-the-counter drug sales [3], telephone calls to health information lines such as nurse on call [4], ambulance call-outs [5], school or workforce absenteeism [6,7], and web searches [8-10].

One surveillance source, previously described by Turner and Kelly [11] but not formally assessed, is a deputising medical service, that is, an out-of-hours service for GP consultations. Many deputising services record the reason for the call-out and the final diagnosis in an electronic database, such as the GP house call surveillance system in Bordeaux, France [12]. The aim of this study was to measure the extent of agreement between ILI surveillance data from the deputising service and data from the sentinel GP system in Melbourne, Australia, in order to assess whether the former could be used for routine influenza surveillance.

Methods

The Melbourne Medical Deputising Service (MMDS) is a deputising, out-of-hours general practice service. Deputising doctors attend patients in their homes within a 45 km radius of the Melbourne Central Business District. Demographic (e.g. age, sex) and clinical data (e.g. diagnosis) are entered by the deputising doctor into a customised database, usually within 24 hours of the consultation. Access to the data is available on a password-protected page of the MMDS website. The data are available for use in a surveillance system as soon as they are entered, i.e. within 24 hours of the consultation.

We routinely obtain the proportion of ILI call-outs from the MMDS once a week, although they could be obtained daily with a 24-hour lag. The weekly data extraction uses a validated search algorithm that identifies the number of call-outs for ILI. This is divided by the total number of call-outs for that week and expressed as a percentage per 100 call-outs. MMDS data are available throughout the year. The search algorithm has been validated by manual confirmation of the diagnosis of all patients identified by the search algorithm for week 34 in the years 2002 to 2007, a week of high activity for all years in that period. The search algorithm successfully identified ILI call-outs searching for the terms 'flu' and 'influ' and excluding terms such as 'reflux'

and 'fluid' that included the letters 'flu'. New exclusion terms, 'fluvax', 'at risk' and 'immunisation', were added to the algorithm in 2009 to exclude pandemic H1N1 influenza contacts who received prophylactic antiviral treatment.

For the sentinel GP system, we used the number of consultations that met the nationally agreed definition of ILI expressed as a percentage of total visits as the comparator. We then assessed the degree of consensus between this measure and that from the MMDS, using a standard statistical method developed by Bland and Altman [13,14]. This method is based on reporting the difference between the two measures, and the 95% limits of agreement, which provide an interval in which 95% of the differences between the two measurements are expected to lie. If the limits of agreement describe differences that are not of material importance, the data sources can be used interchangeably.

As described by Bland and Altman [14], it is not unusual for the difference between two measures and the standard deviation to increase with increasing values of the two measures being assessed, and this should be accounted for in the statistical analysis, otherwise the limits of agreement will be too wide for low values of weekly ILI proportions and too narrow for high values. Accordingly, we regressed the difference of the weekly ILI percentages on their average, using absolute residuals to estimate the standard deviation.

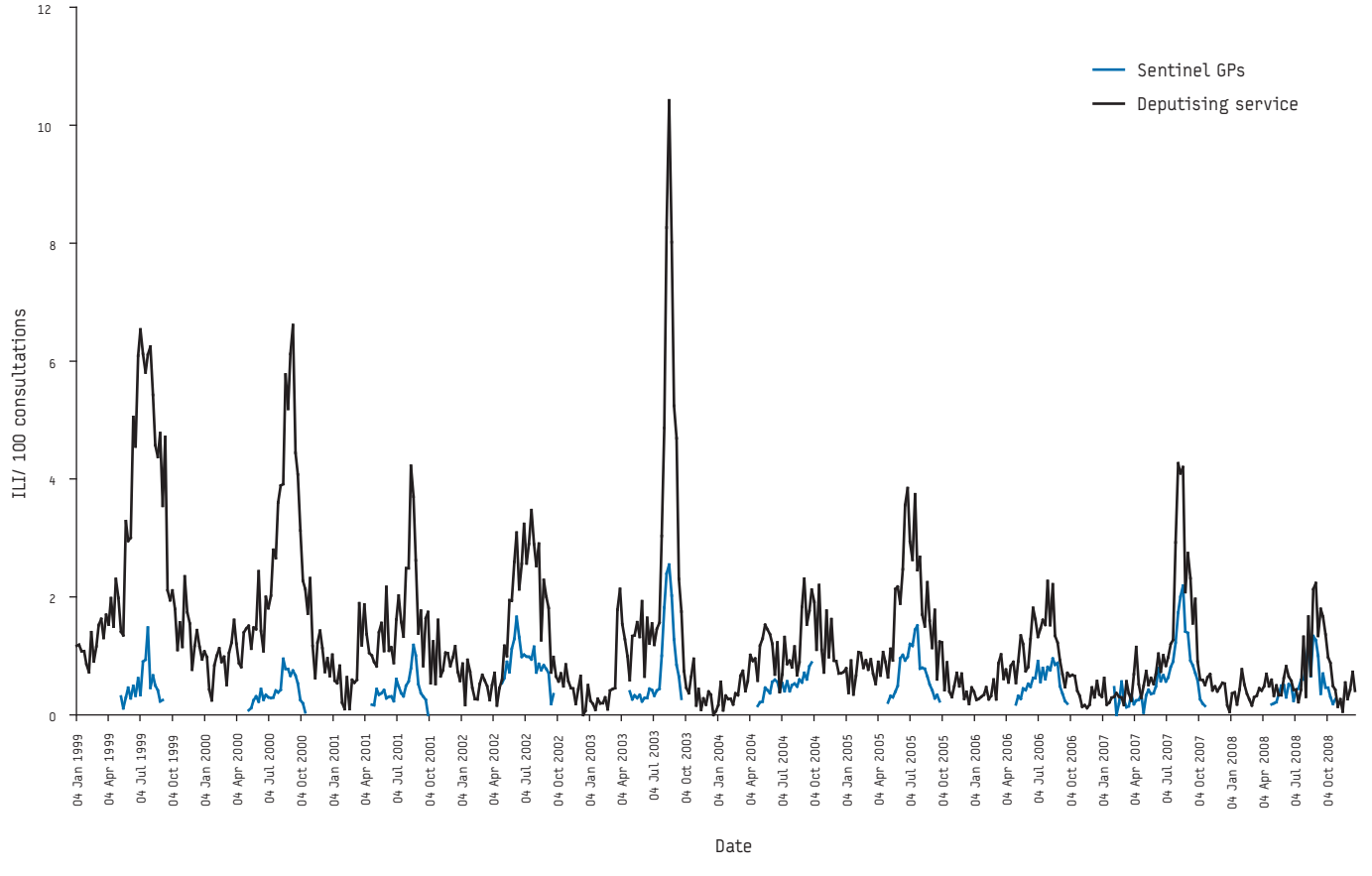
To further assess the comparability of the two surveillance systems, we calculated the area under the receiver operator characteristic (ROC) curve for the 10 years of data from the deputising service against the weeks with *higher than expected* seasonal activity as currently defined by a sentinel GP weekly ILI percentage of 1.5%, described by Watts *et al.* [15]. In the context of this study, as described by Bland and Altman [16], an area under the ROC curve of 0.5 would mean that the deputising service was no better than chance in detecting the influenza season, while a value of 1.0 would mean that it was a perfect measure. Confidence intervals for the area under the ROC curve were obtained using the algorithm of DeLong *et al.* [17].

Results

From 1999 to 2008, the weekly percentages of ILI reported through the deputising service were similar to the percentages seen in the sentinel GP system during periods of low seasonal activity, but were larger in periods of higher activity, although this was less evident in later years (Figure 1). The difference between the two

Figure 1

Weekly percentage of ILI reported through the deputising service versus the sentinel GP system, Melbourne, Australia, 1999-2008



GP: general practitioner; ILI: influenza-like illness

ILI data sources was small, but increased during the peak of the season, with data from the deputising service recording higher values than data from the sentinel GPs (Table).

The 95% limits of agreement increased with increasing ILI activity, the importance of which, as noted by Bland and Altman [13], is a matter of judgement, rather than a statistical issue. Our judgement is that the limits of agreement are appropriately small

Table

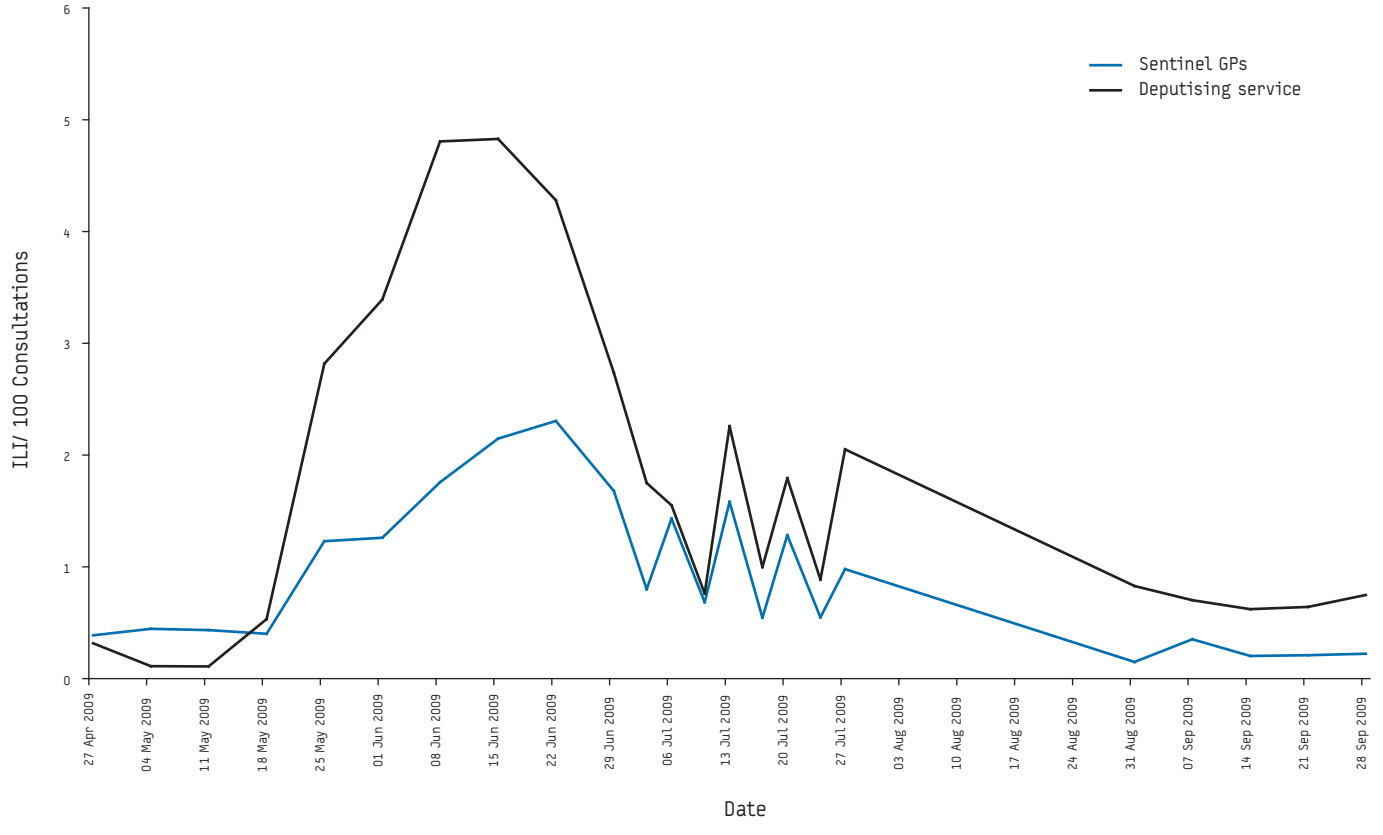
95% limits of agreement for deciles of the average* of deputising service and sentinel general practitioner data

(Cumulative percentage of observations) Average of ILI from deputising service and sentinel GP per 100 consultations	Difference between deputising service and sentinel GP ILI per 100 consultations	95% limits of agreement
(10%) 0.42%	0.0%	-0.6%, 0.3%
(20%) 0.58%	0.2%	-0.4%, 0.8%
(30%) 0.72%	0.4%	-0.3%, 1.1%
(40%) 0.86%	0.6%	-0.2%, 1.4%
(50%) 1.00%	0.8%	-0.0%, 1.6%
(60%) 1.19%	1.0%	0.1%, 1.9%
(70%) 1.45%	1.3%	0.2%, 2.3%
(80%) 1.87%	1.6%	0.5%, 2.8%
(90%) 2.53%	3.1%	0.8%, 3.6%

* Assessing 95% limits of agreement against the average is the preferred method of assessing whether one set of measurements can substitute for (is equivalent to) another [19].
GP: general practitioner; ILI: influenza-like illness.

Figure 2

Weekly percentage of influenza-like illness reported through the deputising service versus the sentinel general practitioner system, Melbourne, Australia, Victoria, Australia, influenza season 2009



GP: general practitioner; ILI: influenza-like illness

during periods of normal seasonal ILI activity as well as at the start and end of the season, and that the wider limits at the peak of the season, or in seasons of higher activity, are of no material importance.

The area under the ROC curve was 0.91 (95% confidence interval (CI) 0.83, 0.98), confirming very close agreement between the systems when dichotomised around ILI activity describing normal and *higher than expected* seasonal activity.

Having both surveillance systems in place has been very useful in the H1N1 influenza pandemic of 2009 as the two surveillance systems provided complementary and confirmatory surveillance data when influenza A(H1N1)v was the dominant circulating strain [20]. As with previous years, however, ILI proportions from the two surveillance systems were more similar for lower values (Figure 2).

Discussion

There was good agreement between the weekly percentages of ILI in the deputising service and sentinel GP system, although the agreement for high ILI values was not as close as for lower values. This is probably because the deputising service is an out-of-hours service, which is likely to have a higher percentage of call-outs for acute illnesses, such as influenza. The deputising service is also less likely to see non-acute illnesses, effectively increasing the ILI percentage relative to sentinel GPs who would continue to see patients for chronic diseases during the peak of the influenza season. Moreover all ILI consultations are captured by the deputing service database, whereas GP data are recorded on paper forms which makes complete capture of all ILI patients is unlikely. This would reduce the reported ILI percentage from sentinel GPs compared with the deputising service.

We did not use the correlation coefficient to assess whether the deputising service data were equivalent to the sentinel GP data as some authors have done [8], because this approach has been questioned in a series of much cited papers by Bland and Altman [13,14,18,19]. There are two reasons for not using the correlation coefficient to assess equivalence of two data sources: First, if the values of the data vary across a wide range, as is the case for ILI data from both deputising service and sentinel GPs, the correlation coefficient will be close to 1.0 even if one measure is not a good substitute for the other. Second, correlation ignores any systematic bias between the two measures. To overcome these problems, Bland and Altman recommended reporting the difference, or bias, between the two measures and the 95% limits of agreement and we have followed their advice in this study.

We did not examine agreement for different age groups. However, for the most recent five-year period included in the analysis (2004-2008), the percentage of ILI cases under the age of 15 years was similar in the two systems (19.5% in the deputising service versus 18.8% in the sentinel GP system), while the ILI cases from the deputising service were slightly older than those from sentinel general practice (mean 40.7 years versus 39.9 years) and showed more variation (standard deviation 25.6 versus 20.3). This was because of the growing number of out-of-hours consultations by the deputising medical service at care facilities for the elderly in the latter years of surveillance; 8.6% of ILI cases identified by the deputising service were 80 years or older while the corresponding percentage for sentinel general practice was only 2.2%.

Deputising medical service surveillance does not use a standardised definition of ILI and is not supplemented by laboratory confirmation of suspected influenza cases. Nevertheless we have shown that data from a deputising medical service can provide low cost and timely ILI surveillance throughout the year, equivalent to ILI surveillance provided by sentinel GPs. Further confirming its utility, surveillance data from the deputising service confirmed the onset and peak of ILI activity during the 2009 pandemic in Victoria.

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Clostridium difficile ribotypes 001, 017, and 027 are associated with lethal C. difficile infection in Hesse, Germany

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From January 2008 to April 2009, 72 cases of severe *Clostridium difficile* infection were reported from 18 different districts in the state of Hesse, Germany. A total of 41 *C. difficile* isolates from 41 patients were subjected to PCR ribotyping. PCR ribotype (RT) 027 was the most prevalent strain accounting for 24 of 41 (59%) of typed isolates, followed by RT 001 (eight isolates, 20%), RT 017 and 042 (two isolates each), and RT 003, 066, 078, 081, and RKI-034 (one isolate each). Eighteen patients had died within 30 days after admission. *C. difficile* was reported as underlying cause of or contributing to death in 14 patients, indicating a case fatality rate of 19%. The patients with lethal outcome attributable to *C. difficile* were 59-89 years old (median 78 years). Ribotyping results were available for seven isolates associated with lethal outcome, which were identified as RT 027 in three and as RT 001 and 017 in two cases each. Our data suggest that *C. difficile* RT 027 is prevalent in some hospitals in Hesse and that, in addition to the possibly more virulent RT 027, other toxigenic *C. difficile* strains like RT 001 and 017 are associated with lethal *C. difficile* infections in this region.

Introduction

Clostridium difficile infection (CDI) is a major cause of morbidity and mortality from healthcare-associated infections in economically developed countries. CDI is primarily linked with hospital admission and prior antimicrobial treatment. The symptoms can range from mild diarrhoea to serious manifestations such as pseudomembranous colitis, toxic megacolon or perforation of colon [1]. In recent years, a hypervirulent strain, which has been characterised by pulsed field gel-electrophoresis as North American pulsed-field gel electrophoresis type 1 (NAP1) and by PCR as ribotype (RT) 027, has emerged in North America, Canada, and several European countries [2-6]. This strain has primarily been described in association with hospital outbreaks but may also cause community-acquired infection. RT 027 is characterised by production of *C. difficile* toxins A and B and a third toxin (binary toxin), deletions in the regulatory gene *tcd*C that potentially allow increased toxin A and B production, and resistance to new fluoroquinolones such as moxifloxacin [7,8].

In Germany, a hospital associated outbreak of the *C. difficile* RT 027 strain was reported in 2007 from Rheinland-Palatina in south-western Germany [9]. Since then, RT 027 has sporadically been isolated in other geographic regions of Germany [10]. A recent study found a high prevalence (55%) of *C. difficile* RT 001 in patients with *C. difficile*-associated diarrhoea (CDAD) in southern Germany [11]. Isolates corresponding to RT 001 did not contain the binary toxin genes *cdt*A and *cdt*B and displayed resistance to moxifloxacin and erythromycin [11].

In December 2007, a requirement for mandatory notification of severe CDI was introduced in Germany [12]. According to this requirement, severe CDI was defined as pseudomembranous colitis confirmed by endoscopy or histology, or CDAD or toxic megacolon with positive laboratory results for *C. difficile* associated with one of the following conditions:

- readmission to the hospital because of recurrent CDI,
- admission to intensive care unit because of CDAD or its complications,
- abdominal surgery because of toxic megacolon, perforation or refractory colitis,
- death within 30 day after CDAD, with CDI as underlying cause or contributing to death,
- detection of RT 027.

The Hesse State Health Office (HSHO) receives notifications on severe CDI from local health authorities of the state of Hesse, which is located in western Germany and has approximately six million inhabitants. Following the introduction of the federal notification requirement, we initiated a pilot study to characterise *C. difficile* isolates associated with severe CDI in Hesse by offering for free a complete microbiological diagnostic service including culture, toxin detection, antimicrobial resistance testing and ribotyping to those healthcare facilities in Hesse that do not have access to these analyses. In this report, we present the results of our study during the first 16 months after introduction of these measures.

Patients and methods

Study population

From January 2008 to April 2009, 60 patients with notifiable CDI were reported by local health authorities via electronic notification system (SurvNet) to the HSHO. A total of 24 *C. difficile* isolates from 24 of these patients had been submitted by the microbiological laboratories of the respective hospitals to a

national reference laboratory for *C. difficile* (Institute for Medical Microbiology, University of Mainz, or Robert Koch Institute (RKI), Wernigerode, Germany) for ribotyping. The ribotyping results of these isolates were reported to HSHO along with the case reports and corresponded in 23 of 24 cases to RT 027.

In addition, we received 22 stool samples from 17 patients with severe CDI that were sent to the microbiological laboratory of HSHO for detection and molecular typing of *C. difficile* during the study period. Comparison of the electronic notification reports with the data of these 17 patients revealed that 12 of them had not been reported by the electronic notification system. These cases were additionally enrolled in this study. The 17 patients were hospitalised in 13 different hospitals. Seventeen isolates (one isolate per patient) were forwarded to the national reference laboratory at the RKI for PCR ribotyping.

C. difficile culture, toxin analysis, and antimicrobial susceptibility testing

Faecal culture for *C. difficile* was performed on *C. difficile*-selective agar containing cycloserine, cefoxitin, and amphotericin B (Bio Mérieux) under anaerobic conditions. Identification of *C. difficile* was performed by routine microbiologic techniques and a rapid confirmatory latex agglutination test for *C. difficile* (Microgen Bioproducts). Twelve of 17 *C. difficile* isolates that were isolated in the HSHO laboratories were tested for in vitro toxin production with an ELISA detecting toxin A and/or B (Biopharm). Of the remaining five cases, four had been tested positive for toxin A/B directly from the stool specimen and were therefore considered to be toxin-positive. One isolate was lost because of fungal contamination and could not be used for ELISA or antimicrobial susceptibility testing. Sixteen isolates were subjected to susceptibility testing for erythromycin and moxifloxacin by E-test (AB-Biodisc).

PCR ribotyping

PCR ribotyping was performed at the RKI according the protocol of Bidet *et al.* [13], except that PCR Products were run on 1.5% agarose gels in 1× TBE at 85 volts for 4 h. Through cooperation with the reference laboratory for *C. difficile* at the Leiden University Medical Centre in the Netherlands and the German reference laboratory for gastrointestinal infections in Freiburg, the RKI accumulated a reference strain collection of 76 different *C. difficile* ribotypes, including 25 reference strains from the Cardiff Anaerobe Reference Laboratory in Wales, United Kingdom [14]. PCR ribotypes that differed from reference patterns by at least one band were assigned novel PCR ribotypes and marked with the prefix RKI [15]. Ribotyping at the University of Mainz was performed as described by Brazier *et al.* [6] by using the 25 reference strains from the Cardiff Anaerobe Reference Laboratory.

Results

Study population

From January 2008 to April 2009, a total of 72 severe CDI cases were reported to the HSHO by local health authorities or by clinicians in Hesse (Figure 1).

Thirty-eight patients (53%) were male and 34 (47%) were female. The patients age ranged from 30 to 94 years with a median age of 80 years (Figure 2).

The clinical symptoms included diarrhoea (72 cases), recurrent infection leading to hospital admission (19 cases), pseudomembranous colitis (nine cases), sepsis (five cases), colitis (two cases), and colon perforation, peritonitis and pancreatitis (one case each). Twenty-three of the cases were reported because

Figure 1

Cases of severe *C. difficile* infection reported from January 2008 to April 2009 in Hesse, Germany (n=72)

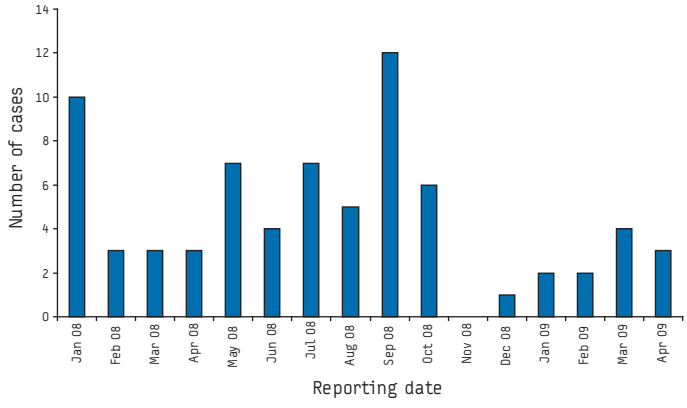


Figure 2

Age distribution of patients with severe *C. difficile* infection in Hesse, Germany (n=72)

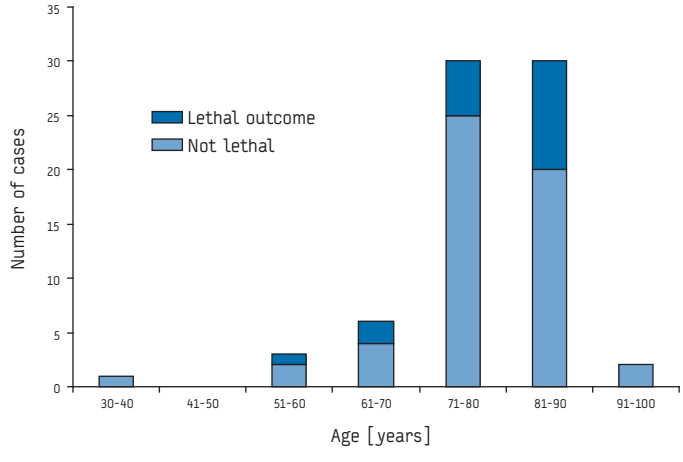
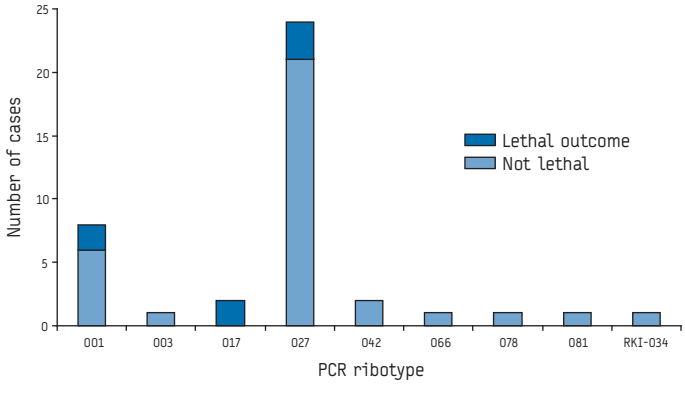


Figure 3

Assignment of *C. difficile* isolates collected from patients with severe CDI to PCR ribotypes, Hesse, Germany (n=41)



of detection of RT 027. The clinical outcome was disclosed in 60 cases (86%). The infection was lethal within 30 days after diagnosis in 18 cases (25%). Infection by *C. difficile* was reported as underlying cause of or contributing to death in 13 cases, and in one case as the most probable cause of death. The patients with lethal outcome that could be attributed to CDI were between 59 and 89 years-old, with a median age of 78 years.

PCR ribotypes, toxin production, antimicrobial susceptibility

Ribotyping results were available for 41 isolates obtained from 41 of the 72 patients with severe CDI. Twenty-four ribotyping results were reported to our institution via electronic notification system, while 17 isolates were isolated in the microbiological laboratory of our institution and forwarded for ribotyping to the national reference laboratory at the RKI. A total of 24 isolates were identified as RT 027, eight isolates as RT 001, two isolates each as RT 017 and 042, and one isolate each as RT 003, 066, 078 and 081. One isolate could not be assigned to any known RT and was designated as RKI-034 (Figure 3).

Production of toxin A and/or B was assessed in culture supernatants of the 12 *C. difficile* isolates cultured in our institution from patients with severe CDI. All isolates were tested positive for toxin A and/or B production. Interestingly, direct toxin detection in stool samples was negative in four of these 12 cases, confirming the higher sensitivity of culture compared to direct toxin detection in stool samples. Antimicrobial susceptibility results were available for 16 isolates. Six of the eight RT 001 isolates were tested and displayed resistance to moxifloxacin and erythromycin. Both RT 017 isolates, one of the two RT 042 isolates and the RT 078 isolate were resistant to moxifloxacin. Six isolates were susceptible to moxifloxacin. These results suggest that resistance to moxifloxacin is not a specific marker for RT 027.

Characterisation of *C. difficile* isolates associated with lethal infection

Eighteen (25%) patients had died during the hospitalisation period associated with severe CDI. Ribotyping results were available for seven of the cases with lethal outcome and identified RT 027 in three cases and RT 001 and 017 in two cases each (Figure 3). The clinical symptoms, previous antimicrobial therapy, and antimicrobial susceptibility results of these seven cases are summarised in the Table 1.

Discussion

In this study, we present the first results on surveillance of severe CDI in the state of Hesse with approximately six million inhabitants. A total of 72 cases of severe CDI were included in this study. Sixty cases were reported through the federal notification system, whereas 12 additional cases were enrolled because of our offer to analyse samples from patients with severe CDI in our diagnostic laboratory at no charge. Taking into account possible underreporting and the restricted use of microbiological diagnostic tools such as culture and ribotyping because of economic considerations, it can be hypothesised that the real incidence of severe CDI might be markedly higher in our region.

Sixty-nine (96%) of 72 patients included in this study were older than 60 years. The median age was 80 years. We observed a high rate (19%) of disease-related fatality in our study. Eleven of 14 patients with lethal outcome that was attributable to CDAD were older than 70 years. This finding is in accordance with the results of a recent study that identified advanced age (over 70 years) as a significant risk factor for illness and death among patients with CDAD [16]. However, it can not be ruled out that the emergence and circulation of epidemic and highly virulent *C. difficile* strain(s) may have contributed to an increased case fatality rate in our study.

Nine different *C. difficile* ribotypes were associated with severe CDI in our study. Ribotypes 027 and 001 were the most prevalent strains, while all other ribotypes were encountered only once or twice. Twenty-four of 41 typed isolates (59%) were RT 027. Since detection of RT 027 represents a case definition criterion for severe CDI in Germany, the high proportion of RT 027 may at least partially be attributed to a sampling bias. However, since the majority of RT 027 isolates were reported from a distinct district, a local outbreak in a particular hospital in that region can not be excluded. Further studies are required to evaluate this hypothesis. Taken together, our data show unequivocally that *C. difficile* 027 has emerged and is prevalent in Hesse.

Eight isolates (20%) were identified as RT 001 in this study. The high prevalence of RT 001 in our study is in accordance with

Table

Clinical data of patients with lethal *C. difficile* infection for whom isolates were available for analysis and ribotyping (n=7)

Patient, age, sex	Date of reporting	Hospital department	Clinical symptoms	Previous antimicrobial therapy	Erythro-mycin	Moxi-floxacin	PCR ribotype
Patient 1, 83, f	9 Mar 2008	medicine	CDAD, dialysis, hemi-colectomy,	ceftriaxon, clarithromycin, imipenem	n.d.	n.d.	027
Patient 2, 62, f	20 Mar 2008	medicine	CDAD, colitis, peritonitis	ceftriaxon, vancomycin, metronidazole	S	R	017
Patient 3, 86, m	22 Jul 2008	medicine	fracture, intracranial bleeding, dialysis, CDAD	ceftriaxon	n.d.	n.d.	027
Patient 4, 83, m	31 Jul 2008	medicine	urinary tract infection, CDAD, colitis	ampicillin-sulbactam	R	R	001
Patient 5, 73, f	9 Sept 2008	geriatrics	cystitis, CDAD, readmission	levofloxacin, vancomycin	n.d.	R	027
Patient 6, 72, m	10 Oct 2008	urology	gastroenteritis, CDAD	unknown, metronidazole	R	R	017
Patient 7, 59, m	11 Dec 2008	medicine	pseudomembranous colitis, sepsis	clarithromycin, amoxicillin, ampicillin-sulbactam	R	R	001

CDAD: *Clostridium difficile*-associated diarrhoea; n.d.: not defined; R: resistant; S: sensitive.

the results of Borgmann *et al*. who found a high prevalence (55%) of RT 001 in patients with CDAD in southern Germany in 2008 [11]. Thus, RT 001 appears to be a common *C. difficile* genotype in western and southern Germany. It is noteworthy that RT 001 used to be the most prevalent strain associated with hospital outbreaks in English hospitals in 2005, but its prevalence has declined to 7.8% of isolates in 2007-2008 [6]. Future studies are necessary to follow up the distribution of this ribotype in Germany.

One of the isolates in our study was identified as RT 078. An increased prevalence of CDI due to this ribotype in the Netherlands has been reported by Goorhuis *et al*. [17]. In the latter study, CDI due to both RT 078 and RT 027 presented with similar severity, but CDI associated with RT 078 affected a younger population and was more frequently community-associated. In our study, the patient suffering from severe CDI due to RT 078 was 60 years-old and therefore younger than the average. Our results indicate that RT 078 is prevalent in hospitals in Hesse. They are in agreement with the data by Rupnik *et al*. [18] who found RT 078 in 7.5% of *C. difficile* isolates collected from hospitals in Göttingen and the surrounding regions in the Lower Saxonia, Germany in 2006.

Ribotyping results were available for seven isolates associated with lethal CDI; three isolates were identified as RT 027, and two isolates each as RT 001 and 017. Our data suggest that, along with the hypervirulent RT 027, other toxigenic *C. difficile* strains such as RT 001 and 017 are associated with severe and lethal CDI in Hesse. It is noteworthy that ribotyping results were not available for half of the lethal cases of CDI in this study. Therefore, it is possible that also other ribotypes may be involved in severe CDI with lethal outcome. Our experience shows that offering the possibility to submit samples from patients with severe CDI to a specialised laboratory at no charge may help to collect more complete information.

In conclusion, the results presented here suggest that severe CDI is prevalent among hospitalised patients in Hesse. Severe CDI was associated with a high case fatality rate, especially in patients over 70 years of age. Nine different *C. difficile* ribotypes were associated with severe CDI. Lethal infections were observed in association with RT 001, 017, and 027. This study underlines the need for further studies on molecular epidemiology of *C. difficile*.

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"RAISIN" – a national programme for early warning, investigation and surveillance of healthcare-associated infection in France

The RAISIN Working Group[1]
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Surveillance is a key component of the French plan for prevention of healthcare-associated infection (HAI) and has progressively evolved in the past decades. We describe the development and current organisation of surveillance of HAI in France and summarise key achievements and results. Surveillance of HAI is under the auspice of the national institute for public health surveillance through a central coordinating structure, the Réseau d'alerte, d'investigation et de surveillance des infections nosocomiales (RAISIN), which consists of five regional coordinating structures, two national advisory committees of the Ministry of Health and public health agencies. Surveillance includes the performance of national prevalence surveys every five years (latest in 2006), specific surveillance networks to follow trends and characterise HAI that are national priority, and mandatory reporting of HAI that meet specific criteria for alert purposes. RAISIN prioritises activities, defines technical specifications of surveillance systems, coordinates their implementation, and supports response to alerts, emergences or outbreaks of HAI. We demonstrate that the French surveillance program of HAI has become comprehensive and contributes to evaluating the impact of control and prevention of HAI. Data from RAISIN indicate a general decrease in the risk of HAI in acute care in France. They show a decrease in HAI during recent years, particularly of those related to methicillin-resistant *Staphylococcus aureus* (MRSA) for which a drop of 38% was documented between 2001 and 2006. RAISIN is also integrated into European surveillance of HAI coordinated by the European Centre for Disease Prevention and Control.

Background

Healthcare-associated infections (HAI) are leading causes of morbidity and mortality among hospitalised patients [1]. Five to 10 % of patients admitted to acute care hospitals acquire during their stay one or more infections according to recent European prevalence surveys [2-4].This proportion is greater in immuno-compromised patients and patients with underlying diseases, undergoing invasive procedures, admitted to an intensive care unit (ICU) and the elderly. In a multicenter study of tertiary-care hospitals, HAI contributed to the death of 2.8% of patients that died 48 hours after admission. Extrapolated nationwide this indicates that HAI may account for about 4,200 deaths per year in France [5]. Outbreaks of HAI are frequent and may spread between HCF through patient transfers [6]. Also HAI cause disability, reduce quality of life and create emotional stress [7, 8]. Effective infection control measures may prevent 20 to 30% HAI [9-11]. Surveillance is a key element of the control and prevention of HAI because it provides data relevant for appropriate intervention methods [10-13]. HAI have a growing social and political impact in many western countries with aging populations because the elderly are more susceptible to infections and require increasingly intensive healthcare [14,15]. In France, surveillance of HAI is integrated in the national HAI control and prevention program which was implemented more than two decades ago [16]. In this paper, we describe the organisation of HAI surveillance in France and its main outcomes.

Organisation of HAI control and prevention in France

The control, prevention and surveillance of HAI are based on interacting local, regional and national structures with complementary roles. Their organisation and coverage have developed progressively since 1988 and have been reinforced on several occasions. All public HCF (since 1988) and private HCF

Figure

Nosocomial infection surveillance coordination structures and locations, France

(since 1999) are legally obliged to set up an infection control committee to define an HAI control program that is implemented by a control team. French authorities recommend one infection control nurse for 400 beds and one infection control practitioner for 800 beds; smaller HCF share infection control personal through networks. Five interregional infection control coordinating centers, Centre de coordination de la lutte contre les infections nosocomiales (CClin), were created in 1992 to coordinate control, prevention, counseling, surveillance and training activities and support hospitals in implementing the national program (Figure). Each CClin coordinate a network of regional antenna (n = 23), legally instituted in 2006. At the national level, two committees advise the Ministry of Health: one on strategic orientations, the other one is an expert committee that produces recommendations for the prevention of adverse health care events, including HAI.

Surveillance of HAI in France

A first survey of HAI was conducted in 46 hospitals in 1990 and after this, the first large scale surveillance activity was a national prevalence survey in 1996 which was repeated in 2001 and 2006 [18-21]. Surveillance HCF, participating on voluntary basis (hereafter referred to as voluntary HCF), targeting high priority HAI were developed by the CClin from 1993 onward. The system was completed in 2001 by a mandatory notification of HAI events, described in the section *Notification of HAI, alert and response to outbreaks*, to provide timely assistance to HCF for control purpose [22]. Surveillance of HAI was initially implemented through an interregional coordination level under the Ministry of Health. With the creation of a national institute for public health surveillance, Institut de Veille Sanitaire (InVS) in 1998, the coordination for HAI surveillance moved to the InVS. A coordinating structure that gathers in a contractual way the InVS, the five CClin, the Ministry of Health and its advisory committees and other public health agencies and bodies involved in HAI prevention was therefore set up: the Réseau d'Alerte, d'Investigation et de Surveillance des Infections Nosocomiales (RAISIN, nosocomial infection early warning, investigation and surveillance network). It prioritises surveillance activities, defines technical specifications of HAI surveillance, coordinates implementation of surveillance programs and studies and assists in investigating outbreaks [23].

Definitions for nosocomial infections

The definitions used for surveillance were adapted from the United States' Centers for Disease Control and Prevention (CDC) in 1992 [24,25] and further updated in 1999 to take into account long-term care patients [26] and surgical site infections (Table 1) [27,28]. In 2007, definitions for HAI were updated and expanded to outpatients care structures [29].

Surveillance activities

Prevalence surveys

Three national HAI prevalence surveys were performed in 1996, 2001 and 2006, to advocate and train HCF for HAI surveillance and control, to estimate the burden from HAI describe their characteristics and assess trends over time [19-21]. All public and private HCF were invited to participate. Participating HCF enrolled on a given day in June all inpatients present that day. Standardised questionnaires were used by trained investigators to collect data from medical records, microbiological laboratories, temperature charts and interviews with physicians or nurses. Data included characteristics of the participating HCF and patients: age, sex, admission date, individual risk factors including immunosupression, the Mac Cabe Score [30], extrinsic risk factors such as presence of a urinary or a vascular catheter and surgery within 30 days prior to the time of the survey. Up to three HAI were recorded for each patient. For each HAI, date of onset, infection site, microorganism and source were recorded. Each HCF entered data using dedicated software for validation, analysis and standardised reporting for feedback. Data were then transferred to CClin for aggregation and analysis at regional level, and to InVS, which managed the national database, analysis and report.

The number of HCF and patients included increased overtime. However, the number of patients per HCF decreased due to the smaller size of newly recruited hospitals (Table 2). Results were relatively stable for most parameters in all three surveys, however, the prevalence of HAI, infected patients and methicillin-resistant *Staphylococcus aureus* (MRSA) decreased from 1996 to 2006, especially after 2001 (Table 2). Comparisons between 2001 and 2006 were restricted to 1,351 HCF that participated in both surveys, used similar case definitions and were adjusted for all available confounding variables to account for changes in methods in 2006 (exclusion of asymptomatic bacteriuria) and the inclusion of smaller hospitals in most recent survey. The multivariate analysis indicated a 12% decrease in the prevalence of infected patients and of 38% for infection with MRSA [21].

Incidence surveillance networks

Since 1993, five incidence surveillance networks of voluntary HCF were set up: surgical site infections (SSI), intensive care units (ICU), blood and body fluids exposure (BBFE), bloodstream infections (BSI) and multidrug-resistant bacteria (MDRB) infections. The first two networks use the methodology proposed by the United States National Nosocomial Infections Surveillance System (NNIS) system and produce standardised indicators [72]. Denominator data collection is, however, patient-based and not aggregated by unit of care which allows adjustment on individual risk factors. Surveillance of BBFE uses the method proposed by the American National Surveillance System for Healthcare Workers (NaSH) [73]. The BSI and MDRB networks are laboratory-based. For each surveillance network, data are collected, entered and analysed by participating HCF using dedicated software. Data are sent to CClin for validation and aggregation into a regional database for analysis. Surveillance methods that were implemented through the five CClin were standardised nationwide between 1999 and 2003, and regional data are now aggregated into national databases [31]. Annual national HAI surveillance reports are available on the Raisin website [23]. Current efforts focus on facilitating data collection and on developing new indicators such as the standardised incidence ratio [32].

Surveillance of surgical site infections (SSI): the ISO-Raisin network

Since 1999, regional SSI surveillance data are aggregated into a national database. Each year, CClin include voluntary surgery wards for a two or three months survey of at least 200 surgical patients each (excluding re-interventions) with a post-operative 30 day-follow-up. Data include risk factors (age, sex, score of the American Society of Anesthesiologists, [33] pre- and post-operative hospital stay, type and duration of procedure, emergency/elective procedure, video-endoscopy and Altemeier wound class) and SSI, if any [34, 35]. Participation increased from 1999 to 2006, from 230 (8.2%) to 568 (20%) of the 2,804 public and private HCF (Table 3). The annual number of procedures rose from 79,803 in

Table 1

Definitions for Hospital-acquired infection (HAI) and Surgical site infections (SSI) in France

Definitions for Hospital-acquired infection (HAI) and Surgical site infections (SSI) in France	
Hospital-acquired infection (HAI)	Infections occurring at least 48 hours after the patient's admission.
Surgical site infections (SSI)	Infections occurring within 30 days after an operative procedure if no implant is left in place or within one year if an implant is in place and the infection appears to be related to the operative procedure.

Table 2

Participation and main results of nosocomial infection point prevalence surveys, France, 1996 to 2006

Year	Hospitals (n, % of all French hospitals beds)	Patients included (n)	Prevalence of HAI (%) all HAI [acquired only]	Prevalence of infected patients (%) all HAI [acquired only]	Proportion of MRSA among S. aureus (%)
1996	830 (77%*)	236,334	n.a [7.6]	n.a. [6.7]	57%
2001	1,533 (77%†)	305,656	7.5 [6.4]	6.9 [5.9]	64%
2006	2,337 (94%¶)	358,467	5.38 [4.34]	4.97 [4.01]	52%

HAI: healthcare-associated infections; MRSA: methicillin-resistant Staphylococccus aureus; n.a: not available
* for public hospitals only;
† 55% for private hospitals and 91% for public hospitals
¶ 84% for private hospitals and 99% for public hospitals
Note: the 1996 survey only collected data on HAI acquired in the reporting facility; the 2001 and 2006 surveys included HAI acquired in the reporting facility AND imported from another facility; both types of rates are given when available

Table 3

Annual participation and trends in healthcare-associated infections incidence through RAISIN (Réseau d'alerte, d'investigation et de surveillance des infections nosocomiales) incidence surveillance networks, France, 1999 – 2006

Surveillance Network	Year of Surveillance							
	1999	2000	2001	2002	2003	2004	2005	2006
ISO-Raisin (surgical site infections)								
Healthcare Facilities (n)	230	248	292	303	271	340	425	568
Surgical wards (n)						811	1,027	1,331
Procedures (n)	79,803	82,348	109,419	114,579	107,576	126,451	150,006	193,946
Overall SSI incidence (%) ¶	2.0	1.8	1.7	1.5	1.5	1.6 [1.59]	1.37 [1.24]	1.26 [1.26]
Overall SSI incidence (%) (NNIS-0) ¶	1.1	0.9	0.9	0.8	0.9	0.9 [0.93]	0.78 [0.73]	0.74 [0.58]
REA-Raisin (infections in intensive care units)								
Intensive care unit wards (n)						116	141	158
PNE per 1,000 intubation-days						17.1	17.4	16.1
COL per 1,000 catheter-days						5.86	5.56	4.87
BSI per 1,000 patient days						3.32	3.35	3.27
UTI per 1,000 urinary catheter-days						8.44	7.94	7.94
AES-Raisin (blood and body fluids exposures)								
Healthcare facilities (n)				228	228	371	385	518
BBFE per 100 beds ‡				6.9	7.5	8.9 [7.9]	8.8 [7.6]	8.0 [7.2]
BN-Raisin (bloodstream infections)								
Healthcare facilities (n)					268	137	286	
BSI per 1,000 patient days					0.60	0.62	0.45	
BMR-Raisin (multidrug-resistant bacteria)								
Healthcare facilities (n)					478	488	527	589	675
MRSA cases per 1,000 patient days *					0.63	0.68 [0.71]	0.62 [0.68]	0.58 [0.63]	0.55 [0.60]
ESBL cases per 1,000 patient days †					0.13	0.14 [0.17]	0.15 [0.18]	0.16 [0.20]	0.17 [0.19]

BBFE: blood and body fluids exposures; BSI: bloodstream infections; COL: central venous catheter colonisation with or without catheter-related infection/bacteraemia (CRI/CRB);
ESBL: extended-spectrum beta-lactamase, MRSA: methicillin-resistant Staphylococcus aureus; NNIS: National Nosocomial Infections Surveillance System [REF]; PNE: ventilator-associated pneumonia;
SSI: surgical site infections; UTI: urinary tract infections (UTI) associated with indwelling urinary catheter.
¶ Results within brackets calculated for cohort of 374 surgical wards participating in the SSI survey from 2004 to 2006.
‡ Results within brackets calculated for cohort of 173 healthcare facilities participating in the BBFE survey from 2004 to 2006.
* Results within brackets calculated for cohort of 255 healthcare facilities participating in the MRSA survey from 2003 to 2006.
† Results within brackets calculated for cohort of 228 healthcare facilities participating in the ESBL survey from 2003 to 2006.

1999 to 193,946 in 2006. Incidence of SSI varied according to NNIS score from 0.85% for the lowest risk patients (NNIS-0) to 12.92% for the highest risk patients (NNIS-3). In this group, SSI incidence decreased over time (Table 3). Among NISS-0 patients, SSI icidence significantly decreased for herniorraphy (-70%), cholecystectomy (-55%), appendicectomy (-53%), colon surgery (-33%), caesarean section (-56%), and breast surgery (-39%) [36-38-]. Surveillance of SSI is well accepted and provides standardised indicators to evaluate prevention. It suggests a positive impact of the French national HAI control program, at least in lower risk patients.

Surveillance of HAI in intensive care units (ICUs): the REA-Raisin network

The REA (Réanimation)-Raisin targets device related-infections in ICUs: ventilator-associated pneumonia (PNE), central venous catheter colonisation (COL) with or without catheter-related infection/bacteraemia (CRI/CRB), urinary tract infections (UTI) associated with indwelling urinary catheter and BSI. Six months per year, voluntary ICU collect for data for patients hospitalised more than two days in the ICU on patients' characteristics (age, sex, admission date), risk factors (trauma, antibiotic treatment, diagnosis category, immunosupression, new simplified acute physiology score -SAPS II [39], invasive devices) and infections. Incidence rates are adjusted per 1,000 device-days [40]. In 2006, 158 ICUs (accounting for about 25% of French ICU) included 22,090 patients, of whom 3,113 (14.1%) had at least one infection (5,284 nosocomial events). The most frequent micro-organisms were *Pseudomonas aeruginosa* (15.0%), *E. coli* (14.8%), *S. aureus* (14.0%), *Candida albicans* (5.7%) and *S. epidermidis* (5.5%) ; 39,5% of *S. aureus* strains were resistant to methicillin in 2006 (2004: 48.7%). Incidence rates decreased from 2004 to 2006 for PNE (-5.9%), COL (-16.9%), BSI (-1.5%) and UTI (5.9%) [40-42] which suggest an improvement for HAI in ICU (Table 2).

Surveillance of blood and body fluids (BBFE) exposure: the AES-Raisin network

The AES (Accident d'Exposition au Sang)-Raisin network monitors the incidence of reported occupational BBFE in French healthcare workers. Since 2002, a prospective national follow-up of healthcare workers has been set up in tertiary hospitals, local medical centers and specialised psychiatric centers [43]. All reported BBFE are documented by the occupational physician using an anonymous standardised questionnaire [44]. In 2006, 518 HCF, accounting for 18% of 2,804 French HCF and 43% of hospital beds, recorded 14,876 BBFE; the majority of these (72%) were needle-stick injuries. Around half (48.6%) of 12,123 percutaneous injuries were avoidable through adherence to standard precautions. The BBFE incidence rate was 8.0 per 100 hospital beds (Table 3), 1.5 per 100 full-time equivalent physicians, 6.5 per 100 full-time equivalent nurses and 1.8 per 100 full-time equivalent nurses' aides. Human immunodeficiency virus (HIV) serology was unknown in 3,353 (22.5%) patients that were the source of a BBFE.

Extrapolating results nationwide, it was estimated that 35,418 BBFE occurred in 2006 in France. In 173 HCF that participated over all years, compliance to glove use increased from 60.6% in 2004 to 66.1% in 2006 and sharps disposal containers accessibility increased from 65.2% to 68.6%, while BBFE incidence decreased slightly (Table 3) [45].

Surveillance of bloodstream infections (BSI): the BN-Raisin network

Surveillance of BSI was conducted from 2002 to 2004 through the BN-Raisin network. It provided a reference for the incidence, microbial ecology and origin of acute invasive HAI to assess the impact of control measures for specific routes of infection [46]. The laboratory-based network included all wards of voluntary HCF for three months each year. In 2004, 286 HCF (10% of public and private HCF) participated. For each nosocomial BSI a standardised questionnaire documented patients' characteristics (age, sex, type of hospital and medical specialty), source of the bacteraemia, organisms and antibiotic susceptibility and follow-up for seven days after onset of bacteraemia. Incidence was calculated per 1,000 patient days (pd) [47]. In 2004, overall incidence was 0.45 (Table 3). Among identified sources, venous catheters and urinary tracts catheters were the most common (24.9 and 24.8% respectively). The main microorganisms isolated were *E. coli* (20.5% of isolated pathogens, 2.8% of which produced extended-spectrum beta-lactamase - ESBL), *S. aureus* (24.9%, 41.4% of which were MRSA) and coagulase-negative *Staphylococci* (24.8%). Death occurred in 11.8% patients with BSI and was more frequent in patients infected with *P. aeruginosa* (21.5%) than patients with BSI caused by other bacteria (11.22%). These results indicate that venous and urinary tract catheter-related bacteraemia should be targeted for prevention with priority.

Surveillance of hospital-acquired multidrug-resistant bacteria (MDRB): the BMR-Raisin network

France is one of the European countries mostly affected by MDRB, particularly MRSA [48]. The BMR (Bactériémie Multirésistante)-Raisin network assesses the impact of national efforts on the incidence of MDRB HAI. Data on MRSA and ESBL-producing *Enterobacteriaceae* are collected prospectively three months a year from all diagnostic specimens other than screening isolates; duplicates, strains with the same susceptibility profile per patient, are excluded and incidence rates per 1,000 pd are calculated and stratified by type of ward [49].

In 2006, 675 HCF participated (24% of the 2,804 public and private HCF) a 41% increase since 2002. The MRSA incidence was 0.55 per 1,000 pd and greater in acute (0.65) and in intensive

Table 4

Mandatory notification criteria and cumulative number, France, 2001 – 2006

Notification criteria for healthcare-associated infections	N	%
1. Rare or noticeable HAI, due to …	2,644	63.8
1a. microorganism characteristics, including resistance	1,806	43.5
1b. infection site	746	18.0
1c. associated medical devices	353	8.5
1d. medical practices	167	4.0
2. Patient's death linked to HAI	823	19.8
3. Airborne or waterborne HAI	622	15.0
4. Otherwise mandatory notification (e.g., legionellosis)	466	11.2
5. Other (none of the above)	566	13.6
Total number of notifications	4,147	100.0

HAI: healthcare-associated infections.
Note: sum of all notification criteria is >100% as healthcare facilities can use one or more criteria
Source: Bulletin épidémiologique hebdomaire 51-52/2006 and 30-31/2008.

care (1.91) than in rehabilitation and long term care facilities (0.37). In 255 HCF that participated from 2003 to 2006, MRSA incidence decreased by 15% (Table 3). The ESBL incidence was 0.17 per 1,000 pd in 2006; it was twice higher in acute care (0.20) compared to rehabilitation and long term care facilities (0.11). Among the 228 HCF that participated from 2003 to 2006 incidence of ESBL increased from 0.17 to 0.19 (+12%, Table 3) in line with a growing proportion of *Escherichia coli* among *Enterobacteriaceae* species (2003:25%; 2006: 43%). These results suggest a positive impact of the HAI national program on hospital-acquired MRSA [50]. In contrast, the emergence of ESBL, especially for *E. coli,* is of concern [50,51]. Similar trends have been observed by the National Observatory for the Study of Antimicrobial Resistance (Observatoire National de l'Etude de la Résistance Bactérienne aux Antibiotiques - Onerba), [52], an independent organisation that promotes standardisation of methodologies, conducts descriptive studies on antimicrobial resistance and contributes to the European Antimicrobial Resistance Surveillance System (EARSS) since 2001 [48,53].

Notification of HAI, alert and response to outbreaks

Prevalence or incidence surveys do not cover all hospitals and HAI and do not allow prompt detection of emerging HAI or outbreaks. Therefore, a national HAI infection notification system was implemented in 2001 to detect unusual events, promote early outbreak investigation and control and identify emerging problems. HCF have to notify HAI to CClin and the district health authority, which in turn inform the InVS. Notification criteria are:

- rare or severe infections, concerning microorganism characteristics (i.e. resistance), the infection site, a contaminated device/product or practice failure;
- infections leading to death;
- airborne or waterborne infection (e.g. legionellosis);
- otherwise reportable diseases (e.g. tuberculosis etc.).

As the system is designed to detect unusual events, there is no restrictive list of events to notify. The reporting form includes the nature of the event and main characteristics, investigations and control measures performed, and allows to request assistance [22,54,55]. At the national level, InVS provides support for outbreak investigation and analyses data to detect unusual trends.

From 8 January 2001 to 12 December 2006, the InVS received 4,117 notifications from 918 HCF (33% of all HCF in France), accounting for 12,561 HAI and 1,482 deaths (13%). Twenty-six percent notifications (1,059 out of 4,117) were related to clusters (ranging from 2 to 178 cases) and external assistance was requested for 8% (319). The average monthly notifications increased from 30 in 2001 to 80 in 2006. The median time between an event and notification to InVS decreased from 62 days in 2001 to 9 days in 2006. The most frequently used notification criteria were related to microorganisms (33%), deaths associated with HAI (15%), infection sites (13%), airborne/waterborne HAI (11%), contaminated devices (6%), or practice failures (3%). The most frequently notified microorganisms were *S. aureus* (15%, 47% of which were MRSA), *Enterobacteriaceae* (11%, 72% of which produced ESBL), *Acinetobacter* (9%, 28% of which were imipenem-resistant), *P. aeruginosa* (8%, 37% of which were imipenem-resistant and 27% ceftazidime-resistant), or *Legionella* (7%). *Enterococcus faecalis* or *E. faecium* accounted for 3% of all notifications, 91% of which were vancomycin-resistant (VRE) [55].

Today, the system is well accepted; it provides daily assistance in outbreak investigation and control to HCF, and allowed the early detection and control of outbreaks or emerging pathogens at local, regional or national level, such as an outbreak of hepatitis C in a hæmodialysis unit in 2001 [56], an outbreak of VEB-1 ESBL-producing *Acinetobacter baumannii* in northern France in 2003 [6], an outbreak of *Enterobacter sakazakii* associated with a contaminated powdered infant formula in 2004 [57], the national emergence of VRE in 2005 [58] or of 027/NAP1 *Clostridium difficile* in 2006) [59]. Following the detection and extensive investigation and follow-up of these major events, national recommendations were updated accordingly or issued where not available.

Specific studies through the RAISIN network

Specific studies are performed through Raisin to assess the impact of a particular threat or document and characterise a specific HAI issue. We illustrate the benefits of three such nation-wide public health oriented studies.

Survey to estimate the presence of glycopeptide intermediate S. aureus *(GISA)*

In 1999, following reports of clinical isolates of *S. aureus* with reduced susceptibility to glycopeptides (Glycopeptide intermediate *S. aureus* – GISA, being intermediately resistant to teicoplanin and susceptible to vancomycin) a survey was carried out in 2000 and 2001 to estimate the incidence of GISA and their proportion within MRSA strains. An optional GISA module was proposed to hospital laboratories participating in MDRB surveillance. During one month, each first MRSA strain isolated from a clinical sample was documented with a standardised questionnaire and then screened for GISA using recommendations from the French Society for Microbiology. One hundred and sixty-five volunteer hospitals included 2,066 patients with a clinical MRSA isolate, 254 (12%) of which were suspected to be GISA, however, only 45 (2.2%) were confirmed GISA, an incidence of GISA of 2.3 per 100,000 pd. Analysis of the antibiotic susceptibility profiles suggested that most strains were closely related to the gentamicin-resistant MRSA clone that was responsible for the MRSA epidemic in French hospitals until 1995 [60]. Although this study confirmed the presence of GISA strains in French hospitals in 2000-2001, such strains were rarely identified by French hospitals.

Survey on risk of bacterial pneumonia from defective bronchoscopes

In 2002, flexible bronchoscopes of the same brand were recalled after a defect (a loose biopsy-port cap in the bronchoscopes) that reduced the efficacy of disinfection procedures and might be responsible of transmitting infections from patients to patients was identified by the French Health products safety agency (Agence Française de Sécurité Sanitaire des Produits de Santé Afssaps). InVS and CClin assessed the risk of bacterial pneumonia among patients exposed to these medical devices in a retrospective study including the last 30 patients in each participating HCF exposed to the bronchoscopes before they were recalled. Of 347 HCF contacted, 211 (67%) participated in the survey and traced 4,112 patients for exposure to 97 (85%) of 114 defective bronchoscopes. One bacterial pneumonia (0.07%) was documented among exposed patients within 2 to 10 days after exposure. In addition we found that 16 (1.3%) patients were colonised or infected with a *Mycobacterium* on the day of bronchoscopy, in nine cases *Mycobacterium tuberculosis*. This demonstrated that tracing patients exposed to specific bronchoscopes was possible in French

hospitals, suggested that the risk of bacterial pneumonia associated with the defective bronchoscopes was low but that exposure of patients to transmission of mycobacterial infection was possible if the bronchoscopes were not adequately reprocessed after use [61].

National survey to assess the prevalence of hepatitis C virus and hygiene practices in dialysis units

Following a large outbreak of hepatitis C virus (HCV) infection in a dialysis unit in 2001 [56] a national survey was undertaken to assess the prevalence of HCV and of hygiene practices in dialysis units. Two complementary studies were carried out: one through Raisin and the French Nephrology Society who sent a standard mail questionnaire to all hæmodialysis units between October and December 2004 and a second was an observational audit of infection control practices on a 10% random sample of dialysis units. Of 873 hæmodialysis units, 477 (55 %) participated, 200 dialysis centers and 277 autodialysis units. HCV prevalence was 6.6 % in hæmodialysis centers and 5.9 % in autodialysis units. The audit of practices survey indicated a high level of compliance with infection control recommendations but identified breaches for which corrective actions were needed [62].

Laboratory support to surveillance

In France, laboratory support to surveillance (detection, typing and molecular epidemiology) is performed through a network of 47 national reference centers (NRC) funded by InVS and designated every four years through a call for tender. The list of NRC is revised regularly by a national committee and their specific missions and tasks are defined according to surveillance needs [63]. Several NRC provide an important contribution to surveillance and outbreak investigation of HAI caused by pathogens such as MRSA, *P. aeruginosa, Legionella*, hepatitis C virus, or glycopeptide-resistant *Enterococcus*. Following *C. difficile* 027 introduction in 2006 in France, a network of five regional laboratories (one in each CClin area) coordinated by a specific NRC was created to enhance the national capacity of typing of *C. difficile* strains isolated from patients suffering severe disease or outbreaks identified through the mandatory notification system. This close institutional interaction between routine surveillance activities, detection of new emerging infectious threats and the planning of reference laboratory resources greatly facilitated the response to 027 *C. difficile* spread in French hospitals [59]. A prospective surveillance of *C. difficile* infections has been implemented in 2009.

Discussion

The surveillance of HAI in France has gradually evolved over two decades to become comprehensive finally. It has documented encouraging results in recent years which probably reflect the positive impact of control and prevention efforts. The collegial management of a comprehensive system through Raisin allows standardisation of protocols and a close interaction between private and public hospitals, regional structures and national public health agencies. The very high level of participation of hospitals in the 2006 national prevalence survey illustrates the effectiveness of this three level - national, inter-regional and local- approach.

The surveillance activities in which Raisin is involved include planned surveys, surveillance networks and assistance to investigation of and response to unusual HAI events. These complementary activities allow each participating structure a comprehensive understanding and knowledge of the HAI epidemiology, which facilitate response and public health actions and finally promote the prevention of HAI. The generic and flexible early warning system for HAI has clearly and repeatedly shown a strong added value to prevalence studies and surveillance networks. It supports HCF in the control of outbreaks that may spread to other hospitals regionally or even nationally. Besides regional or national alerts described previously, it also allowed responding to recurrent outbreaks such as several outbreaks of hepatitis C transmission in health care settings [64,65].

Efficient surveillance is resource intensive. Because of reporting delays, often required complex analysis (including risk-adjustments), and the voluntary participation of HCF, HAI surveillance has been criticised and sometimes felt not linked enough with day-to-day action by consumers and policy planners. Pushed by a strong social demand, the French Ministry of Health has implemented a national program of mandatory patient care performance indicators in all HCF. The first published indicators are scores related to the HCF efforts to control and prevent nosocomial infection and of appropriate use of antibiotics [66,67]. Additional indicators are under consideration and include the rate of MRSA infection in HCF. The Raisin database on hospital-acquired multidrug-resistant bacteria (BMR-Raisin) was extensively used to help define and construct this last indicator. However, publicly reported performance data cannot replace surveillance because HAI, surveillance has a unique value in the evaluation of efforts to reduce the incidence and prevalence of HAI.

On a European level, Raisin, through its coordinating structure and its institutional integration with the InVS, has permitted to interact efficiently with European surveillance and early warning schemes, which since 2005 are part of the European Centre for Disease Prevention and Control (ECDC) mandate. French SSI surveillance data are included from 2004 to 2006 in the Hospitals in Europe Link for Infection Control through Surveillance (HELICS) database, representing 86,434 (17%) of the 521,186 procedures included in HELICS-SSI database [38] and for 57,963 (41%) of the 142,558 patients included in the HELICS-ICU database [42]. France collaborates actively to the European Early Warning and Response System (EWRS) for HAI threats that may spread to other European Member States [68]. The link between the EWRS and the HAI notification system is made by InVS as part of its risk assessment of alerts. If an HAI event is severe and may spread to other Member States, the EWRS is used to inform all EU partners and ECDC about the nature of the event, its potential risk of spread and the measures taken to limit its spread [69]. This was done for several severe outbreaks such as the VEB-1-producing *A. baumannii* outbreak in hospitals in northern France [6], an international outbreak of *Klebsiella pneumoniae* infections in patients of an hepatic surgery centre [70], and the 027 *C. difficile* outbreak in 2006 [59]. The timely share of authoritative information between national public health authorities before it has been published and communicated via the media is extremely useful to national and EU public health authorities in order to anticipate and plan and coordinate response.

A European HAI surveillance scheme implies some adjustment of national systems with the commonly agreed European methodology. When this will be done in all Member States, the comparison of rates and of trends overtime by countries will become legitimate and may yield interesting insights regarding quality and structure of care across Europe. However, comparison of rates needs to be done carefully, as differences in healthcare systems, methodologies, and sample sizes may have a huge influence on rates and their significance [71]. In Europe, the methods, case definitions and data collected on HAI are not harmonized, which preclude

comparison of results and burden of HAI between EU Member States. European harmonisation of surveillance schemes for HAI such as prevalence surveys, SSI and ICU surveillance need further European consideration.

As France is now in its 2009-2012 plan for the prevention and control of HAI, surveillance will continue to be adjusted to new developments and challenges. Foreseen evolutions include the evaluation and adjustment of current surveillance networks, the move of the HAI notification system which is still done through paper forms to a fully electronic scheme and the extension of surveillance to HAI that occur in health care settings other than hospitals.

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Oseltamivir-resistant influenza A(H1N1) viruses detected in Europe during season 2007-8 had epidemiologic and clinical characteristics similar to co-circulating susceptible A(H1N1) viruses

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During the 2007-08 influenza season, high levels of oseltamivir resistance were detected among influenza A(H1N1) viruses in a number of European countries. We used surveillance data to describe influenza A(H1N1) cases for whom antiviral resistance testing was performed. We pooled data from national studies to identify possible risk factors for infection with a resistant virus and to ascertain whether such infections led to influenza illness of different severity. Information on demographic and clinical variables was obtained from patients or their physicians. Odds ratios for infection with an oseltamivir resistant virus and relative risks for developing certain clinical outcomes were computed and adjusted through multivariable analysis. Overall, 727 (24.3%) of 2,992 tested influenza A(H1N1) viruses from 22 of 30 European countries were oseltamivir-resistant. Levels of resistance ranged from 1% in Italy to 67% in Norway. Five countries provided detailed case-based data on 373 oseltamivir resistant and 796 susceptible cases. By multivariable analysis, none of the analysed factors was significantly associated with an increased risk of infection with an oseltamivir-resistant virus. Similarly, infection with an oseltamivir-resistant virus was not significantly associated with a different risk of pneumonia, hospitalisation or any clinical complication. The large-scale emergence of oseltamivir-resistant viruses in Europe calls for a review of guidelines for influenza treatment.

Introduction

In Europe, virological surveillance of antiviral susceptibility of influenza viruses has been performed since 2004 through the European Union (EU)-funded European Surveillance Network for Vigilance against Viral Resistance (VIRGIL), in collaboration with the European Influenza Surveillance Scheme (EISS), the World Health Organization (WHO) and national influenza centres (NICs) [1]. In January 2008 this surveillance system started to detect significant proportions of oseltamivir-resistant viruses among influenza A(H1N1) specimens collected in several European countries from November 2007 onwards [2]. This was associated with a histidine to tyrosine mutation at residue 275 of the neuraminidase protein (H275Y or H274Y in N2 numbering), which is known to confer high level resistance to the neuraminidase inhibitor oseltamivir [3]. Oseltamivir resistance was confirmed in most EU countries as more influenza A(H1N1) viruses were isolated and tested, although at very different levels ranging from under 2% of all influenza A(H1N1) viruses tested in Italy and Spain to over 40% in Belgium, Estonia, France and Norway by the end of the 2007-8 influenza season [4,5]. These differences, however, were also influenced by the time during the season when specimens were collected and the number of influenza A(H1N1) viruses tested for oseltamivir susceptibility in each country [6]. The wide circulation as well as outbreaks of oseltamivir-resistant viruses, together with a rise in resistance proportions throughout the season indicated that influenza A(H1N1) H275Y-mutated strains were fit and transmissible [6]. This was supported by the absence of correlation between oseltamivir resistance and exposure to oseltamivir at population level [7]. However, it was unclear whether there were any factors favouring infection with an oseltamivir-resistant virus and whether such an infection would affect the clinical course of influenza illness with or without treatment.

In order to obtain additional data on the characteristics of patients infected with influenza A(H1N1) viruses, the EISS and VIRGIL coordination centres rapidly set up an enhanced

surveillance system requesting the European NICs to report for confirmed influenza A(H1N1)-infected patients additional information (such as clinical outcome and exposure to antivirals) to that already routinely collected. Furthermore, a number of countries in the EU and European Economic Area (EEA) conducted specific epidemiological investigations based on a general protocol developed by the European Centre for Disease Prevention and Control (ECDC) in collaboration with some EU countries with the following objectives:

- To identify risk factors for infection with an oseltamivir-resistant versus an oseltamivir-susceptible influenza A(H1N1) virus during the 2007-8 influenza season.
- To assess whether patients infected by an oseltamivir-resistant influenza A(H1N1) virus had a different risk of a severe clinical outcome than patients infected by an oseltamivir-susceptible influenza A(H1N1) virus.

The study hypothesis was that oseltamivir-resistant influenza A(H1N1) viruses emerged during the 2007-8 season were different from co-circulating oseltamivir-susceptible influenza A(H1N1) viruses in terms of risk factors for infection and severity of illness.

This article reports on the descriptive analysis of data from the enhanced surveillance and on the analysis of the pooled data from the national epidemiological studies.

Methods

Surveillance data

The descriptive analysis of influenza surveillance data concerns information collected during the season 2007-8 from week 40/2007 to week 20/2008 in countries participating in EISS. National surveillance systems collect standard case-based epidemiological information for all patients undergoing clinical sampling for laboratory confirmation. However, this information is not routinely reported to EISS. Laboratory confirmation is carried out for surveillance purposes on a subset of individuals presenting with influenza-like illness (ILI) and/or symptoms of acute respiratory infection (ARI) to one of the sentinel physicians participating in the national influenza surveillance. The selection of patients with ILI or ARI undergoing virological testing can be either random/systematic, as recommended by EISS, or left to the physician's clinical judgement [8]. Virological testing is usually performed at the NICs, which are WHO-recognised laboratories for influenza and in Europe collaborate within the Community Network of Reference Laboratories (CNRL) for human influenza [9]. The sentinel physicians are part of national networks that intend to cover a representative sample of the general population. Moreover, case-based information is collected nationally on patients tested for influenza as part of the individual clinical management (non-sentinel samples). Such samples cover a heterogeneous group of individuals including hospitalised patients who are likely to have experienced a more severe influenza illness. In Norway, however, both non-sentinel and sentinel specimens are collected mainly from patients presenting to the primary healthcare system. Additional information on the organisation and functioning of virological influenza surveillance in Europe can be found elsewhere [10].

During the season 2007-8, when higher than expected levels of oseltamivir resistance were detected in influenza A(H1N1) viruses in many European countries, the data routinely collected by EISS and VIRGIL was expanded to include the following additional information: oseltamivir susceptibility, age, gender, geographic location, hospital or community-based, date of specimen collection, date of disease onset, exposure to antivirals of the patient or household contact (in the 14 days preceding onset of illness), influenza vaccination status, and whether complications, hospitalisations or death occurred in the 14 days following onset of illness. Oseltamivir susceptibility was determined phenotypically or by sequencing or by both, as described elsewhere [6]. Data were uploaded during the season and were downloaded on 19 August 2008. The descriptive virological surveillance data presented in this paper might differ slightly from those presented previously [6], as data for the present paper were downloaded one month later and countries could have updated the database since then. In addition, the weeks included in reference [6] (weeks 40-19) differed by one week from the data presented in this paper (weeks 40-20). A descriptive analysis was carried out and individual characteristics were assessed.

Some European countries experiencing high levels of oseltamivir resistance collected additional information on influenza A(H1N1) cases by retrospectively interviewing patients and/or their physicians. The ECDC supported and coordinated such studies by providing a study protocol and organising three meetings as well as regular teleconferences with the study group. To increase the efficiency and timeliness of a European study, only those countries were invited to participate in which at least 50 virus isolates had been tested for antiviral resistance and some level of oseltamivir resistance had been detected as of February 2008. Of the six countries that met this criterion for inclusion, five (Germany, Luxembourg, the Netherlands, Norway and the United Kingdom (UK)) agreed to participate and to provide their databases for a pooled analysis by ECDC.

Epidemiological studies

Questionnaires and study procedures developed by each of the five participating countries were submitted to the ECDC in order to identify common variables for the joint analysis. In all participating countries, the study population included all individuals diagnosed with an influenza A(H1N1) virus infection between week 40/2007 and week 20/2008 for whom antiviral susceptibility testing was performed and for whom it was clear whether the specimens came from sentinel or non-sentinel sources.

Analysis of risk factors for infection with resistant virus

To identify risk factors for infection with an oseltamivir-resistant influenza A(H1N1) virus, a nested case control approach was chosen within the cohort of subjects with laboratory-confirmed influenza A(H1N1) infection. Cases were defined as individuals with laboratory-confirmed influenza A(H1N1) infection whose isolates showed phenotypic (IC50 level) or genetic (H275Y mutation) markers of oseltamivir resistance, and controls were defined as individuals with laboratory-confirmed influenza A(H1N1) infection whose isolates were susceptible to oseltamivir by either phenotypic or genetic analysis. Information was collected for cases and controls on age, sex, country of residence, location of initial sampling (sentinel versus non-sentinel), pre-existing medical conditions, influenza vaccination status, antiviral exposure (i.e. prophylaxis or treatment in the 14 days preceding symptom onset) and travel history within 10 days before symptom onset.

Analysis of outcomes of infection with resistant virus

To assess whether patients infected by oseltamivir-resistant influenza A(H1N1) virus were at higher risk of a severe clinical outcome than patients infected by oseltamivir-susceptible influenza

A(H1N1) virus, a cohort approach was chosen, with cases and controls as the exposed and the unexposed subjects, respectively. The outcomes investigated were symptoms at presentation, hospitalisation for any cause related to influenza, pneumonia, death, and any other clinical complication attributable to influenza virus infection.

Data collection

Retrospective data for the case control analysis and follow-up information for the cohort analysis were collected using slightly different methods and data sources in the different countries. In Germany a subset and in Luxembourg all patients with a confirmed influenza A(H1N1) infection were contacted by local or national public health offices and administered a questionnaire by telephone (Germany) or mail (Luxembourg) in addition to the information already retrieved from the routine surveillance datasets. In the Netherlands, all sentinel physicians and virologists (and subsequently the treating clinicians in the hospitals) who had provided specimens positive for influenza A(H1N1) were contacted by the national public health institute and sent a questionnaire by mail. Those not responding were contacted by telephone. In Norway, general practitioners (GPs) and clinicians in hospitals who had reported an influenza A(H1N1) case to the NIC were contacted by the national public health institute and administered

Table 1

Influenza detections and oseltamivir resistance of influenza A(H1N1) viruses in countries reporting data to EISS and VIRGIL during the 2007-8 influenza season (surveillance database)

Country	Specimens tested positive for influenza virus	Influenza A detections; (% in brackets)	Influenza A(H1) virus detections[a] / subtyped viruses	Influenza A(H1N1) viruses tested for oseltamivir resistance[b]	InfluenzaA(H1N1) viruses resistant to oseltamivir[b]; (% in brackets)	Proportion of resistant viruses detected by sentinel sources	Case-based clinical data available in surveillance database (yes/no)
Austria	531	457 (86)	262/262	164	12 (7.3)	100	Yes
Belgium	918	596 (65)	312/318	32	17 (53.1)	100	Yes
Bulgaria	21	16 (76)	16/16	9	0	n.a.	n.a.
Croatia	176	113 (64)	91/91	6	0	n.a.	n.a.
Czech Republic	262	176 (67)	135/135	24	0	n.a.	n.a.
Denmark	306	203 (66)	182/196	45	2 (4.4)	n.a.	Yes
Estonia	244	207 (58)	137/198	7	3 (42.9)	100	Yes
Finland	209	165 (79)	69/138	13	3 (23.1)	n.a.	No
France	2,887	1,820 (63)	255/267	496	231 (46.6)	n.a.	No
Germany	2,199	1,098 (50)	1,002/1,042	505	66 (13.1)	79	Yes
Greece	213	140 (66)	136/136	65	7 (10.8)	80	Yes
Hungary	212	173 (82)	154/154	11	0	n.a.	n.a.
Ireland	211	110 (52)	74/81	63	7 (11.1)	100	Yes
Italy	210	111 (53)	49/62	106	1 (0.9)	0	Yes
Latvia	608	586 (96)	340/343	15	0	n.a.	n.a.
Luxembourg	463	264 (57)	18/18	227	59 (26.0)	78	Yes
Netherlands	443	232 (52)	165/191	171	46 (26.9)	30	Yes
Norway	856	466 (54)	296/313	273	184 (67.4)	20	Yes
Poland	88	53 (60)	24/24	10	1 (10.0)	n.a.	No
Portugal	118	52 (44)	52/52	29	6 (20.7)	n.a.	No
Romania	482	372 (77)	361/372	49	4 (8.2)	100	Yes
Serbia	63	60 (95)	60/60	18	0	n.a.	n.a.
Slovakia	198	159 (80)	119/120	14	0	n.a.	n.a.
Slovenia	269	252 (94)	173/174	28	1 (3.6)	n.a.	No
Spain	1,738	805 (46)	539/564	106	2 (1.9)	100	Yes
Sweden	1,318	487 (37)	71/82	36	4 (11.1)	0	Yes
Switzerland	620	394 (64)	128/135	53	10 (18.9)	90	Yes
Turkey	n.a.	n.a.	n.a.	3	0	n.a.	n.a.
Ukraine	128	85 (66)	35/35	67	23 (34.3)	n.a.	No
United Kingdom	1,887	1,044 (55)	475/545	347	38 (11.0)	29	Yes
Total	**17,878**	**10,471 (59)**	**5,765/6,003**	**2,992**	**727 (24.3)**		

Countries marked in bold were included in the analytical study.
EEA: European economic area; EFTA: European Free Trade Association; EU: European Union; n.a.: not available.
[a] Data available in EISS database on 8 July 2008.
[b] Data extracted 27 August 2008 from the EISS-VIRGIL. A number of countries tested all influenza A(H1N1) and influenza A viruses for oseltamivir resistance by pyro-sequencing. Some samples were not definitely proven to be H1 subtype, therefore the number of H1 virus detections can be lower than the number of tests for resistance.

Table 2

Risk factors for being infected with an oseltamivir-resistant virus, data from five EU and EEA/EFTA countries, 2007-8 influenza season (n=1,169)

Factor	Categories	% oseltamivir-resistant virus[a,b] N: 373 (1,169)	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)[c,d]
Age in years	0-17	28 (572)	1	1
	18-64	43 (439)	1.93 (1.49-2.51)	1.39 (1.01-1.91)
	>65	60 (10)	3.76 (1.05-13.51)	2.33 (0.52-10.47)
Sex	Female	36 (536)	1	n.i.
	Male	32 (527)	0.82 (0.63-1.05)	
Sample source	Non-sentinel	45 (517)	1	1
	Sentinel	21 (652)	0.32 (0.25-0.42)	0.81 (0.55-1.20)
Seasonal influenza vaccination	No	35 (781)	1	n.i.
	Yes	25 (24)	0.61 (0.24-1.55)	
Any chronic underlying disease	No	48 (435)	1	n.i.
	Yes	69 (55)	2.42 (1.32-4.41)	
Diabetes	No	56 (362)	1	n.i.
	Yes	90 (11)	7.83 (0.99-61.82)	
Immunosuppression	No	49 (465)	1	n.i.
	Yes	78 (18)	3.61 (1.17-11.12)	
Cardiovascular disease	No	57 (366)	1	n.i.
	Yes	57 (7)	1.02 (0.23-4.64)	
Respiratory disease	No	72 (228)	1	n.i.
	Yes	80 (15)	1.53 (0.42-5.59)	

CI: confidence interval; EEA: European economic area; EFTA: European Free Trade Association; EU: European Union; n.i.: not included in the final model.
[a] Numbers in parentheses represent denominators for each category.
[b] Totals per each variable may be smaller than the total number of cases due to missing values.
[c] The final model included age, source of the sample and reporting country.
[d] P-value from likelihood ratio test comparing the model with and without age was <0.08.

Table 3

Effect of oseltamivir resistance on clinical outcomes, data from five EU and EEA/EFTA countries, 2007-8 influenza season, sentinel networks (n=790)

Outcome		% oseltamivir-resistant virus[a] N: 138	% oseltamivir-susceptible virus[a] N: 652	Crude risk ratios (95% CI)	Adjusted risk ratios (95% CI)[b]
Symptoms at presentation [c]	Sudden onset	97 (99)	96 (459)	1.01 (0.81-1.26)	n.i.
	Fever	97 (99)	96 (381)	1.01 (0.81-1.26)	n.i.
	Headache	82 (82)	65 (165)	1.25 (0.92-1.69)	n.i.
	Myalgia	85 (130)	83 (456)	1.01 (0.82-1.25)	n.i.
	Dry cough	92 (130)	90 (471)	1.03 (0.84-1.26)	n.i.
	Sore throat	66 (79)	53 (163)	1.23 (0.87-1.74)	n.i.
	Runny nose	56 (78)	59 (164)	0.95 (0.67-1.36)	n.i.
Complications [c]	Hospitalisation[d]	2 (123)	1 (247)	1.34 (0.22-8.01)	1.25 (0.21-7.58)
	Any clinical complication	8 (120)	5 (244)	1.69 (0.73-3.92)	1.59 (0.68-3.71)
	Pneumonia	2 (85)	1 (148)	3.48 (0.31-38.40)	3.98 (0.35-45.42)
	Otitis	3 (86)	4 (149)	0.87 (0.22-3.46)	0.94 (0.23-3.84)
	Death	0 (123)	0 (248)		n.i.

CI: confidence interval; EEA: European economic area; EFTA: European Free Trade Association; EU: European Union; n.i.: not included in the final model.
[a] Numbers in parentheses represent denominators for each category.
[b] Adjusted for age but not for the presence of chronic medical condition because of the high proportion of missing values for this variable.
[c] Each case may have presented multiple symptoms and developed multiple complications.
[d] Hospitalisation is included here for practical reasons but may have occurred for reasons other than clinical complications.

a questionnaire by mail or telephone. In the UK, information was collected only on oseltamivir-resistant cases and there were no controls. GPs and hospital clinicians who had reported a case were contacted by national or local public health staff by telephone, and details were collected using a structured interview. In cases where clinicians were unable to provide the information, the patients were contacted directly.

Data management and analysis

Country-specific databases were shared with the ECDC for the final analysis. The databases were first analysed separately to detect differences in the results that would have to be considered in the pooled analysis. This was not possible for the UK data, which only included information on oseltamivir-resistant cases; however, these contributed to the pooled dataset. For each country, the prevalence of the various exposures in cases and controls was compared using contingency tables and the chi-squared test to check for statistical significance. Crude odds ratios were also computed. For the cohort approach, the prevalence (risk) of any of the considered clinical outcomes was calculated in exposed and unexposed individuals and the chi-squared test was used to check for statistical significance. Crude risk ratios were also computed. In order to allow for a pooled analysis of the five databases, they were merged into a unique database converting data from Access and Excel into STATA 10 format. Only variables collected by at least four of the five countries were retained in the final database.

The univariable analysis of the pooled database was conducted by using the procedures described above for the country-specific databases. The analysis of risk factors for severe influenza disease (cohort approach) was restricted to the population reported by sentinel surveillance systems. This was because individuals identified through non-sentinel sources are generally more likely to represent cases with more severe influenza and are thus already selected for the outcome of interest. By contrast, the analysis of risk factors for oseltamivir resistance was conducted first separately by source of the sample and then by combining the two populations. Multivariable analyses were conducted by using logistic regression to obtain adjusted odds ratios for the risk of being a case, and Poisson regression to obtain adjusted risk ratios for developing the outcomes of interest in the cohort analysis. Variables significant in univariable analyses ($p<0.05$) were included in the initial multivariable models. The presence of effect modification between study country and each variable was checked, and in the absence of a significant interaction, country was treated as a potential confounder. A backward elimination procedure was used to build the final models. Despite the common protocol, covariates were not uniformly collected in the different studies. In order to determine the possible confounding effects of these variables, a sensitivity analysis was therefore conducted excluding studies one by one from the univariable analysis and the final multivariable models and comparing the results with those of all studies included.

Evaluation of resistance to neuraminidase inhibitors was carried out either at country level (when laboratory capacity was available) or by the Health Protection Agency (HPA) in London in collaboration with the WHO Collaborating Centre for Reference and Research on Influenza (WHO-CC). Assessment of resistance was through phenotypic analysis (IC50) or genotypic analysis (sequencing) for detection of the mutation H275Y. A subset of viruses tested for antiviral susceptibility both at HPA and NICs yielded 100% concordant results with respect to resistance status. IC50 and genetic testing performed on a subset of viruses were also 100% concordant [6].

Results

Surveillance data

The 2007-8 influenza season in Europe was initially dominated by type A influenza viruses, and 96% of subtyped type A influenza viruses were A(H1) [6]. Type B influenza viruses became dominant in week 8/2008. For 30 countries in EISS, data on susceptibility of influenza A(H1N1) viruses to oseltamivir were reported (Table 1). From week 40/2007 to 27 August 2008, a total of 2,992 influenza A(H1N1) viruses were tested for oseltamivir resistance. Of these, 727 (24.3%) were resistant to oseltamivir (Table 1). Resistance was reported in 22 countries and ranged from 1% (n=106) in Italy to 67% (n=274) in Norway (Table 1). No resistance was found in eight countries, most of which were located in the central and eastern part of Europe (Bulgaria, Croatia, Czech Republic, Hungary, Latvia, Serbia, Slovakia and Turkey). However the period of testing and numbers of viruses tested were not representative and might have resulted in an underestimation of the real proportion of resistant viruses [6]. Oseltamivir-resistant viruses were detected in sentinel and non-sentinel patients, and the distribution varied by country (e.g. 20-30% were reported from sentinel sources in the UK, the Netherlands and Norway, and around 80% in Germany and Luxembourg). Sixteen countries also reported case-based clinical information through the enhanced surveillance (Table 1) system as described in the methods section. However, the level of completeness of data was low in countries not conducting ad hoc epidemiological studies and therefore the analytical part of this article is based on the data provided by the five countries conducting such studies.

Epidemiological studies

Analysis by country

None of the main variables collected (age, sex, travel history, influenza vaccination, chronic medical condition) was significantly associated with an increased risk of infection with an oseltamivir-resistant virus. Some of the variables analysed showed some effects that, although not statistically significant, deserve to be mentioned: In the Netherlands, individuals suffering from any kind of immunosuppression were more likely to be infected with an oseltamivir-resistant virus (odds ratio (OR): 5.5, 95% confidence interval (CI): 0.95 to 32; p=0.056). In addition, individuals reported through the sentinel system were less likely to be infected with a resistant virus (OR: 0.51, 95% CI: 0.25 to 1.04; p=0.065). In Norway, individuals aged between 18 and 64 years were more likely to be infected with a resistant virus than those younger than 18 years (OR: 1.84, 95% CI: 1.09 to 3.11; p=0.022).

Infection with a resistant virus was not significantly associated with an increased risk of pneumonia, hospitalisation or clinical complication in any of the five countries. In Luxembourg, the mean duration of influenza illness was longer in cases infected with oseltamivir-resistant virus than in oseltamivir-susceptible infections (10 and seven days, respectively; p-value=0.025 by T test for the hypothesis of no difference between the two groups). There was no difference between the two groups with regards to the maximum temperature of fever (39.3 versus 39.3 °C). In Norway, resistant cases were at higher risk of developing pneumonia (RR 3.15, 95% CI: 0.72 to 13.89); however, this association was not statistically significant. The results of the Norwegian study have recently been published as a separate article [11]. In the UK, the epidemiological information was only collected from the 36 cases with oseltamivir-resistant infection, and bronchitis and pneumonia were the most commonly reported complications affecting six (17%) and eight (22%) cases, respectively.

Results of the pooled data analysis

Following merging of the five national databases, information was available on 1,169 individuals with an influenza A(H1N1) infection, of which 373 (32%) were oseltamivir-resistant. Information was incomplete for key variables such as presence of a chronic medical condition (58% missing values) and hospitalisations (45% missing values). The distribution of missing values was not substantially different between data coming from sentinel networks and data from non-sentinel sources. The proportion of missing information can be calculated by summing up the denominators of each variable reported in Tables 2 and 3 and comparing this with the total number of subjects reported in the Tables.

The analysis of risk factors for oseltamivir resistance was first undertaken separately by reporting source (sentinel and non-sentinel) and subsequently, since there were no relevant differences between the two sources, data from sentinel and non-sentinel sources were analysed together. By univariable analysis (Table 2), individuals aged between 18 and 64 years were almost twice as likely to have an infection with a resistant virus than those younger than 18 years (OR:1.93, 95% CI: 1.49 to 2.51). Only 10 individuals over the age of 64 years were reported and an association of resistance with older age could therefore not be ascertained. Those suffering from a chronic medical condition were 2.4 times more likely to be infected with a resistant virus than healthy individuals (OR:2.42, 95% CI: 1.32 to 4.41). Individuals identified through the sentinel network were less likely to be infected with a resistant virus than those identified through non-sentinel sources (OR:0.32, 95% CI: 0.25 to 0.42).

Following multivariable analysis, none of these factors remained statistically significant. After adjusting for reporting country and source of the sample, the age-group of 18-64 year-olds was associated with a higher risk of being infected with an oseltamivir-resistant virus than the younger age group (OR:1.39, 95% CI: 1.01 to 1.91), however the p value from the likelihood ratio test comparing the models with and without the variable age was <0.08 (Table 2).

The cohort analysis to investigate the effect of oseltamivir resistance on disease severity and complications was restricted to subjects reported by the sentinel networks. There were no significant differences in symptoms at the time of sampling between exposed (oseltamivir-resistant) and non-exposed (oseltamivir-susceptible) patients (Table 3). The risk of influenza disease complications (hospitalisation, pneumonia, otitis media or death) was low for all subjects and did not significantly differ between exposed and non-exposed cases (Table 3).

The sensitivity analysis conducted on both univariable and multivariable models did not reveal substantial differences between countries. Where differences were detected, these only concerned the magnitude but not the direction of the effect. Tables with data of the full sensitivity analyses can be provided by the corresponding author upon request.

Four influenza-related deaths were reported among oseltamivir-resistant cases detected through non-sentinel sources, of which three occurred in the UK and one in the Netherlands and none among oseltamivir-susceptible cases. These were two children (one newborn and one two year-old), one young adult and one person older than 65 years. With the exception of the newborn, all had a chronic medical condition that put them at higher risk of severe influenza and none had received influenza vaccination. None of these cases received oseltamivir treatment.

Discussion

This article provides a comprehensive analysis of the epidemiological information that was collected in Europe during the influenza season 2007-8 on individuals infected with an oseltamivir-susceptible or -resistant influenza A(H1N1) virus. Through the analysis of surveillance data and by combining the results of five national observational studies, we have provided evidence that infection with an oseltamivir-resistant A(H1N1) influenza virus was not related to any of the risk factors analysed. In particular, we did not identify any association between having a chronic medical condition and infection with an oseltamivir-resistant virus. This finding is in contrast with previous observations where higher levels of oseltamivir resistance were mainly reported in vulnerable groups such as children and immunosuppressed individuals and in association with oseltamivir treatment [12-14], and is consistent with the results of a similar investigation conducted in the United States (US) [15] and Norway [11] during the same influenza season. A possible explanation for this finding could be that the oseltamivir-resistant influenza A(H1N1) viruses analysed in this study had become resistant by a process other than the selective pressure of oseltamivir treatment.

We observed a slightly higher risk of being infected with an oseltamivir-resistant virus among adults (18-64 years-old) compared with those younger than 18 years. We think that the most likely explanation for this finding is the confounding effect of different attitudes in different countries on when to consult a GP, and the fact that countries had a very different prevalence of oseltamivir-resistant viruses. This hypothesis was supported by the reduction of the odds ratio towards unity that we observed when adjusting the effect of age for country reporting. Residual confounding that we were not able to adjust for may explain the borderline effect of age observed in the multivariable analysis.

Prior to the 2007-8 influenza season, studies conducted in animal models found that amino acid mutations in the neuraminidase protein causing oseltamivir drug resistance reduced the pathogenicity of the virus because of their effects on the neuraminidase enzyme function [16-20]. Our study found that individuals infected with an oseltamivir-resistant A(H1N1) virus experienced similar symptoms and risk of clinical complications as individuals infected with the same virus subtype susceptible to oseltamivir. Hence there was no clinical evidence that the resistant viruses differed from the susceptible viruses in terms of pathogenicity in humans. The four deaths reported in the UK and the Netherlands seem consistent with the incidence of influenza-associated mortality in risk groups and it is unlikely that oseltamivir resistance played a role. However, it should be noted that the relatively small sample size might have prevented detection of significant differences in rare outcomes such as deaths.

All the viruses that were analysed genetically showed the same drug resistance mutation, the substitution of histidine by tyrosine at residue 275 (H275Y) in the neuraminidase gene, which is known to confer high levels of resistance to oseltamivir *in vitro* [3], but has a reduced transmissibility [17]. However, the rare isolation of viruses carrying the H275Y mutation from ill patients without known exposure to neuraminidase inhibitors [21] may indicate that some compensatory mutations within the neuraminidase, the haemagglutinin or other genes may be influencing virus

transmissibility. Such compensatory mutations are likely to have determined the widespread circulation of fully transmissible and pathogenic oseltamivir-resistant influenza A(H1N1) viruses in Europe, although this still has to be ascertained. Limited variations in the susceptibility to neuraminidase inhibitors that occurred naturally over time (from 1997 to 2005) have been described for influenza A(H5N1) viruses, but do not seem to have clinical relevance so far [22].

The strength of our study is the consistency of results between countries and various sources of data (sentinel and non-sentinel), which validates the results of the pooled analysis. However, there are also important limitations that should be considered when interpreting the findings of this study. The main limitation is the high proportion of missing data for key variables. This was mainly due to the difficulties in collecting information on patients who had ILI months before the data collection started. In addition, data on follow-up outcomes may have been be inaccurate as they were collected from clinicians who were not necessarily aware of complications that may have occurred after they saw the patients. The study may also lack representativeness. In most of the countries, patients who underwent virological testing were selected neither randomly nor systematically, and clinicians may have preferentially tested patients with specific clinical characteristics or pre-existing conditions. In addition, since reporting for the sentinel cases was based on the standard case definition used for surveillance purposes, milder cases or those presenting with unusual clinical features may have been excluded from the study population. An information bias could have occurred if data for cases with oseltamivir-resistant virus infection were collected in more accurately than for cases with susceptible virus infection. We could not demonstrate this from the data available, but some of the participating countries that considered this issue found that clinicians were unaware of the oseltamivir resistance status of their patients at the time of the interview.

Even considering these limitations, this study has relevant public health implications. Subsequent results of global antiviral surveillance found that influenza A(H1N1) viruses resistant to oseltamivir have become predominant over susceptible strains, similarly to the evolution of circulating A(H3N2) viruses, most of which have become resistant to M2 inhibitors [23-26]. In Europe, preliminary results from the 2008-9 season show that while the A(H3N2) subtype predominated, almost all the influenza A(H1N1) viruses tested were oseltamivir-resistant [25]. Therefore, it is important that results from antiviral susceptibility surveillance are used to guide therapeutic decisions at an individual level. The US Centers for Disease Control and Prevention (CDC) issued recommendations for the use of antiviral medications in 2008-9. These took into account the strain-specific prevalence of oseltamivir resistance among circulating influenza A viruses in the US, where resistant influenza A(H1N1) viruses predominated in the 2008-9 influenza season, and advised to use zanamivir or a combination of oseltamivir and rimantadine rather than oseltamivir alone when influenza A(H1N1) virus infection or exposure is suspected [27]. These guidelines do not apply to Europe, where influenza A(H3N2) fully susceptible to neuraminidase inhibitors dominated during the season 2008-9 [28]. The findings of the present study suggest that influenza viruses naturally resistant to the currently available antivirals can rapidly emerge and circulate in the community. It is therefore important that new antiviral drugs against influenza are developed. Although the main tool for the prevention of influenza remains annual vaccination, there are circumstances when the use of antiviral drugs could play a pivotal role in preventing and reducing influenza morbidity. These would include the situation of a mismatch between the circulating and vaccine influenza strains, the control of outbreaks in special settings (e.g. nursing homes), or an influenza pandemic where vaccine is unlikely to be available until some months after the start of the pandemic.

The emergence of the 2009 H1N1 influenza pandemic raised concerns over the possible emergence of oseltamivir resistance. Despite the wide use of neuraminidase inhibitors both for prophylaxis and treatment during the pandemic, oseltamivir resistance has so far only been detected sporadically and resistant viruses did not efficiently transmit in the community [29,30]. Diversification of national antiviral stockpiles to include different types of antivirals has been advised in some European countries [1,31]. The pandemic influenza A(H1N1)v virus is currently fully resistant to adamantanes but susceptible to both available neuraminidase inhibitors, zanamivir and oseltamivir [32].

In general, the unexpected emergence of high levels of oseltamivir resistance in Europe during the season 2007-8 highlights the evolving nature of the influenza virus and the requirement for a flexible approach to disease control including regular review and updating of treatment guidelines and pandemic plans [33].

What are the implications from this experience for the rapid, early assessment that is essential following the appearance of a pandemic [34]? Important lessons learnt are: 1) Reliance on referred specimens, especially from hospitalised or otherwise severe cases is likely to give a biased view of the pattern of infection in the community. 2) Multi-national approaches are more difficult once countries have started independent analytic approaches. It would be preferable for countries to develop and agree in advance on proposals (i.e. mock-up study protocols) to obtain the epidemiological information that is needed at the beginning of a pandemic to guide control measures. This is the approach being taken by the ECDC in collaboration with WHO and such plans should take into account the limitations identified in this study.

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Large measles epidemic in Switzerland from 2006 to 2009: consequences for the elimination of measles in Europe

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Switzerland adheres to the objective of eliminating measles within the European region of the World Health Organization (WHO) by 2010. After several years with a relatively low annual incidence rate (0.3 to 1 case per 100,000 inhabitants), there has been a large epidemic of measles from November 2006 to August 2009. By mid September 2009, 4,415 cases were notified by physicians and laboratories, corresponding to an incidence rate of 15 per 100,000 in 2007 and 29 per 100,000 in 2008; by far the highest rates in Europe. This exceptionally long nationwide epidemic comprised three successive waves, with peaks in August 2007 (171 cases), March 2008 (569 cases) and March 2009 (417 cases). It mainly affected children aged from five to 14 years (48% of cases). Most cases were not vaccinated (93%) or were incompletely vaccinated (5%). In total 656 patients (15%) suffered complications or were hospitalised. Insufficient, spatially heterogeneous immunisation coverage (87% for at least one dose at the age of two years at the national level) has allowed a sequence of numerous outbreaks to occur, despite the gradual strengthening of measures to control the disease. Several exportations to Europe (81 in 2007 and 2008) and to the rest of the world (10 for the whole of the epidemic) have in some instances caused large outbreaks. The epidemic was a threat to the goal of eliminating measles in Switzerland and in Europe. The Federal Office of Public Health (FOPH) and its partners are currently working on a national strategy to eliminate measles.

Introduction

Interruption of the endemic transmission of measles by 2010 is one of the objectives of the World Health Organization (WHO) for its European region [1]. The strategy proposed consists in particular of achieving and maintaining ≥95% vaccination coverage among young children (preferably before the age of two years), with two doses of MMR (measles, mumps and rubella) vaccine. Finland for example has achieved this objective, and many others are close to it [2,3]. Nevertheless, large-scale outbreaks have still been observed in Europe over the last ten years, for instance in the Netherlands, Italy, France, Germany and the United Kingdom, or in Israel [4-12].

In Switzerland, vaccination against measles has been recommended since 1976 (one dose at 12 months), with MMR vaccine being used since 1985. A catch-up vaccination has been recommended since 1985 for teenagers aged 12 to15 years. A second dose of MMR was introduced in 1996 for children aged four to seven years, and this age was lowered to 15 to 24 months in 2001 to increase immunity before entering kindergarten or school. In addition, catch-up vaccination, to reach a total of two doses is recommended since 1996 for anyone born after 1963, who has not been completely vaccinated, and has not had measles. Vaccination of young children and catch-up vaccination of children and adults are performed by pediatricians and general practitioners in private practice and reimbursed by mandatory health insurance. In some cantons, school medical services also ensure catch-up vaccination, usually during the first and the last year of compulsory school. For at least one dose at two years of age, vaccination coverage was stable at about 82% in Switzerland from the early 1990ies to the early 2000s, before increasing to 87% during the period from 2005 to 2007 [13,14]. At that stage it was 90% for children aged eight and 94% for adolescents aged 16 years. Coverage for a second dose only reached 71 to 76%, depending on age. Disparities in vaccination coverage are significant between the 26 Swiss cantons (range: 73–94% for at least one dose at two years). The coverage in the canton which recorded the highest amount of cases (Lucerne) was 78% in 2006 (86% at eight years and 94% at 16 years).

Despite over 30 years of vaccination against measles, this disease is still endemic in Switzerland with epidemic transmission occurring. From 1999 to 2006, an average of about 50 cases were notified per year (incidence rate 0.3 to 1 case/ 100,000) except in 2003, when there was an epidemic that affected the whole country (612 cases; 8.4/100,000) [15]. Whilst the circulation of the measles virus seemed very limited (three cases notified from July to October 2006), a new outbreak gradually spread across the country starting in November 2006 [16]. Since then, this epidemic has continued in three waves comprising numerous outbreaks [17,18]. The third wave began in the canton of Lucerne at the end of 2008 before spreading throughout the country. This report describes the measles epidemic that has been occurring in Switzerland over the past 34 months and the measures taken to control it. It also discusses causes and consequences of this particularly long nationwide outbreak.

Methods

Notification

The data analysed come from the mandatory notification system for measles (cases registered by the Federal Office of Public Health - FOPH, from 15 November 2006 to 17 September 2009). Since 1999, physicians have to notify the cantonal officers of health within 24 hours of any patient with a fever and a rash accompanied by at least one of the following three symptoms: cough, rhinitis or conjunctivitis. Laboratories must notify the cantonal officers of health and the FOPH within 24 hours of any confirmed measles case, whatever the test used. These initial rapid alerts allow the

cantonal physician to launch investigation and control measures. The physician later fills in a more detailed notification. The cantonal officers of health send the FOPH a copy of all notifications made by physicians.

Laboratory tests

The FOPH recommends laboratory confirmation of any suspect case of measles that has no epidemiological link to a confirmed case [19]. The analyses are carried out by numerous private laboratories or by public hospitals. Usually, Ig M and IgG are tested for in serum, using commercial tests. Two laboratories are able to test for the presence of measles virus RNA in clinical samples (throat smear or saliva) by RT-PCR. To trace the pathways of viral transmission, the WHO measles and rubella reference laboratory for Central Europe at the Robert-Koch Institute in Berlin, Germany, has genetically characterised 137 viruses and determined their genotype by sequence analysis of the variable part of the N-gene (456 nt) [20]. Since autumn 2008, genotyping of the measles virus has also been carried out at the Central Virology Laboratory of Geneva University Hospital.

Classification of cases

The definition of a clinical case corresponds to the notification criteria listed above. A case is considered confirmed if it i) is confirmed by a positive laboratory test and presents at least one of the typical signs of measles or ii) meets the clinical case definition and is epidemiologically linked to another laboratory confirmed case. A probable case is a clinical case that is not epidemiologically linked to a laboratory confirmed case. Possible cases include all reported cases without a positive laboratory result, which do not meet the clinical case criteria (clinical manifestations incomplete or unknown). In the current outbreak many possible cases had an epidemiological link with another probable or confirmed case, or belonged to space-time clusters of measles. Cases with a double negative laboratory result (two negative IgM tests or one negative IgM test with absence of RNA by RT-PCR) are discarded, as are those with a single positive IgM test without any clinical symptoms of measles, due to a high probability of false positive tests.

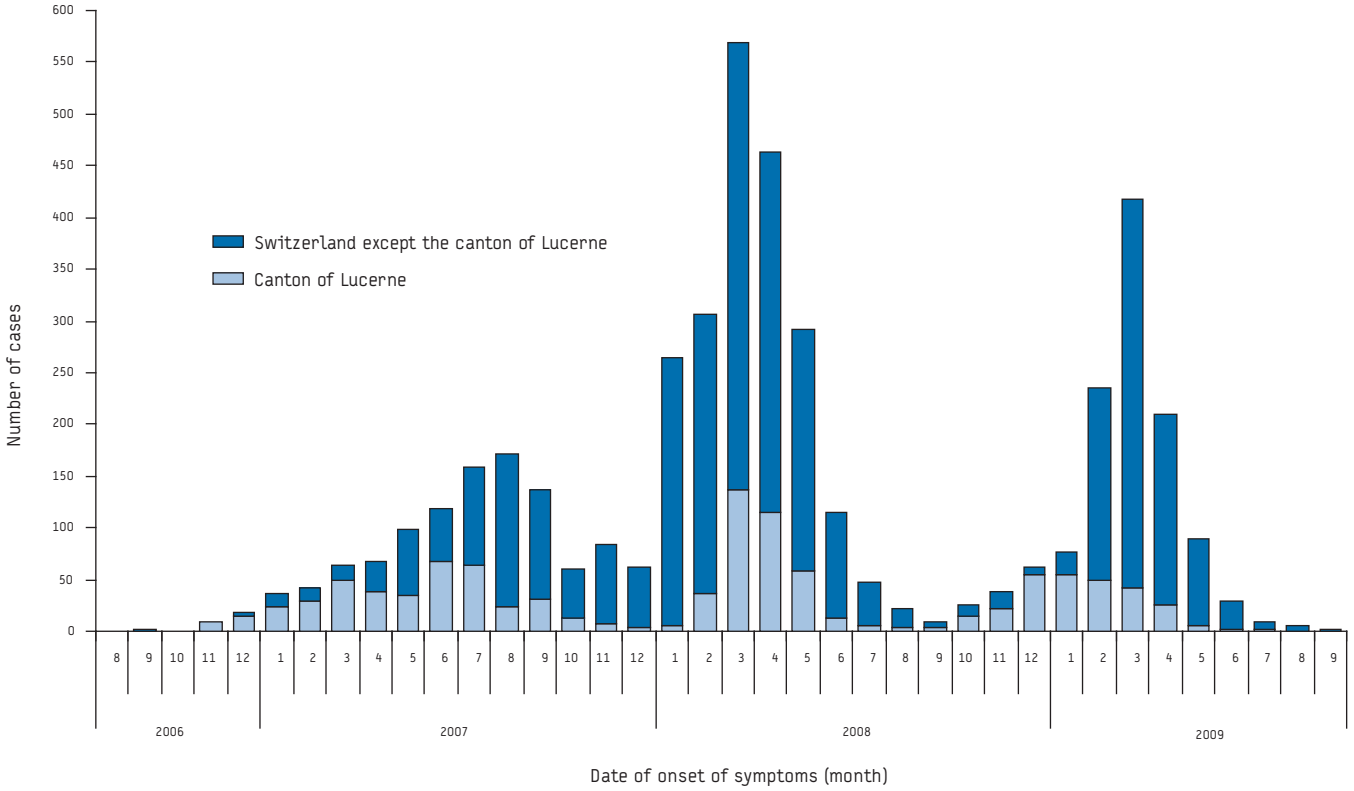
Description of the epidemic

The measles epidemic started in the canton of Lucerne in November 2006, probably following importation [16]. A first wave reached its peak in August 2007 (171 cases) (Figure 1). A second wave appeared in the Basel region around the end of 2007, with a surge from January 2008 and reinforced from February onwards by a strong return of measles in the canton of Lucerne (second peak in March 2008, with 569 cases). The number of cases then fell to a minimum of 10 in September, before constantly rising again, first in the canton of Lucerne, until March 2009 (417 cases). With only 29 cases in June, 10 in July, six in August and one case up to 17 September 2009, we consider that this epidemic has now come to an end. In total, 4,415 cases have been notified, 29 (1%) by the end of 2006, 1,098 (25%) in 2007, 2,214 (50%) in 2008 and already 1,074 (24%) by mid September 2009.

Of the total number of notified cases (4,565), 150 (3%) were discarded. Of the remaining 4,415 cases, 1,886 (43%) were confirmed, either by a positive laboratory result (35%), or by an epidemiological link with a laboratory confirmed case (7%). Of all cases, 48% were probable and 9% were possible.

Figure 1

Notified cases of measles by month, Switzerland, 1 August 2006 to 17 September 2009 (n=4,416)



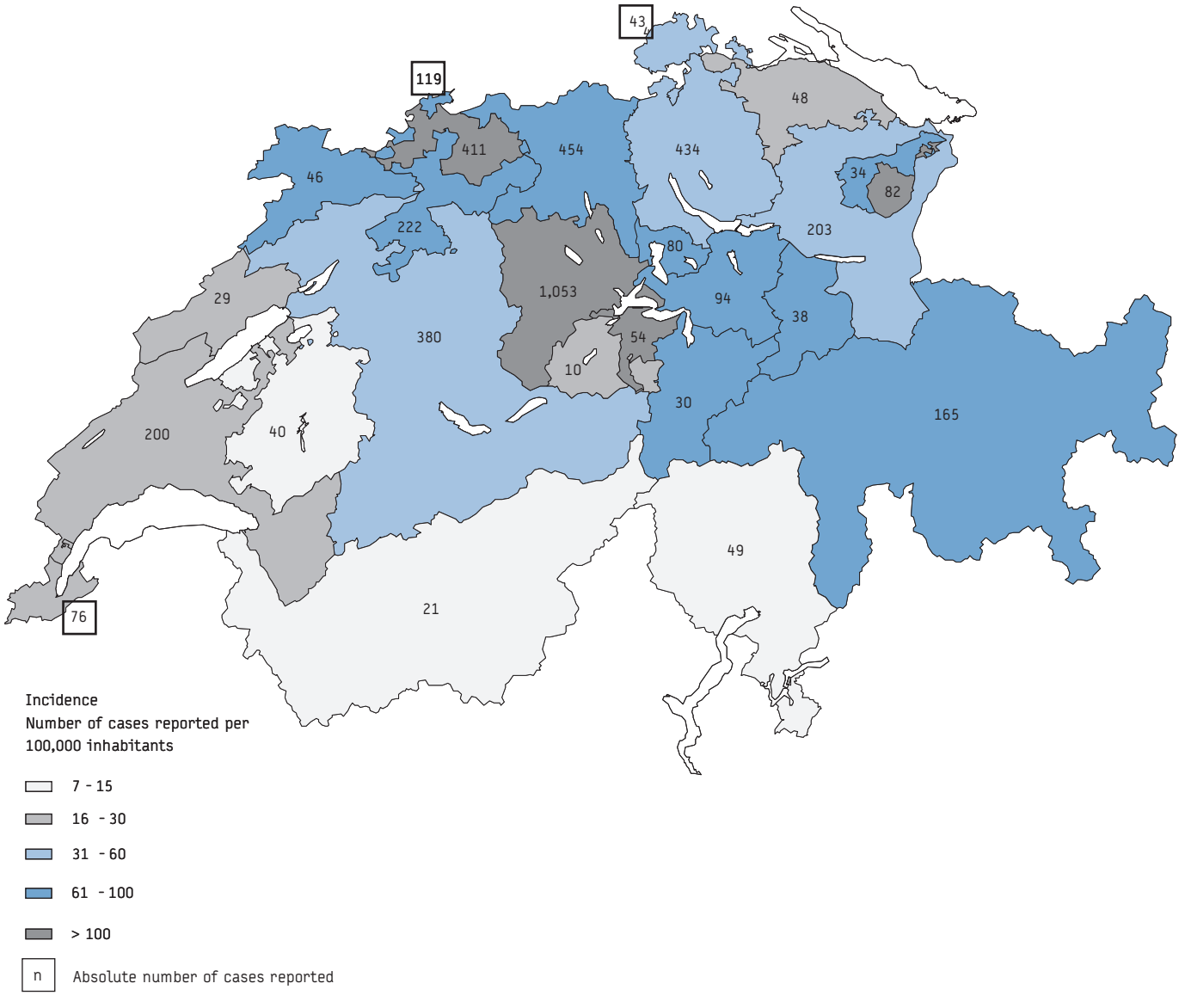
The epidemic has affected all 26 Swiss cantons. However, the total incidence rate for the whole of the epidemic has varied considerably from one canton to another, with a maximum of 530 per 100,000 in Appenzell Innerrhoden and a minimum of seven per 100,000 in the canton of Valais, giving a national average of 58 per 100,000 (Figure 2). The cumulative incidence rate per canton has tended to be lower with increasing vaccination coverage (Figure 3). It reached 74 per 100,000 in the German-speaking part of Switzerland, compared with 21 per 100,000 in the French and Italian-speaking parts, with vaccination coverage of 84.7% and 92.3% respectively for at least one dose at two years of age. The first and third wave of the epidemic started in the canton of Lucerne and Lucerne contributed significantly to the second wave (Figure 1). Overall, that canton recorded 1,053 cases, 24% of the total (cumulative incidence rate 290/100,000).

The sex of 99.8% of the patients is known. The cumulative incidence rates were virtually identical for men and for women (59 and 57/100,000 respectively). Among the 99.5% of patients whose age is known, children aged five to nine years were most affected (25% of cases, cumulative incidence rate 285/100 000) (Table). They were followed by children aged 10 to 14 years and then adolescents from 15 to 19. Adults aged 20 or over made up 19% of cases, whereas cases in infants under one year were rare (< 3%). The median age of patients was 11 years.

The genotype of the measles virus is available for 105 of the 137 samples, with positive RT-PCR sent to the regional reference laboratory in Berlin, since the beginning of 2006. The genotype of further 20 virus samples was provided by a Swiss laboratory. In Switzerland in 2006, before the beginning of the epidemic

Figure 2

Incidence and number of notified cases of measles by canton, Switzerland, 15 November 2006 to 17 September 2009 (n=4,415)



Souce: Swiss Federal Office of Public Health

in November, only the B3 genotype was identified (Figure 4). It was found in a sporadic measles case returning from London in late January and an outbreak lasting from March to May in the neighboring canton of Lucerne. Genotype D5, which was the source of the recent epidemic, was identified in a total of 91 samples from 14 cantons, between November 2006 and March 2009. Also, 13 measles cases caused by D4 virus were identified between October 2008 and March 2009, in four cantons of the German-speaking part of Switzerland. In addition, two D4 viruses were found in June in Geneva. In March 2009, there was an outbreak of genotype B3, mainly affecting the students from the Ecole polytechnique fédérale and from the University of Lausanne, following an importation of measles from Mali. B3 virus was identified in 13 patients, including the index case. In addition, two cases of B3 virus were detected in 2007 in isolated patients returning from abroad, as was a case of genotype A-related vaccine virus in a woman non-immune for rubella who developed a typical measles 12 days after a postpartum vaccination with MMR [16].

Among the 3,916 (88.7%) patients for whom the vaccination status is known through a written document or by history, 92.9% had not been vaccinated, 4.5% had been incompletely vaccinated (one dose), 2.1% had been completely vaccinated (two doses) and 0.5% had been vaccinated with an unknown number of doses. There was a high preponderance of people who had not been vaccinated in each age group, although the proportion tended to decrease from adolescence, with more people who had been vaccinated and, in particular patients whose vaccination status was unknown (Figure 5).

A detailed notification is available for 4,278 cases (96.9%), of whom 339 (7,9%) were hospitalised. No complications were reported for 207 (61%) of hospitalised cases.The frequency of hospitalisation was significantly dependent on age (chi-squared test, $p < 0.0001$). It was 13% for infants, between 4 and 5% for each of the three five-year age categories covering children from one to 14 years old, 8% for adolescents from 15 to 19 years of age, 20% for adults from 20 to 29 years and 29% for adults aged 30 years or more. Among cases with detailed information available, 452 (10.6%) suffered from complications, of which 175 were pneumonia, 219 otitis and nine encephalitis. No follow-up information is available for the latter cases, however some were probably not severe because three of them were not hospitalised and a fourth was only a suspected case of encephalitis. Among cases with a complication only 135 (29%) were hospitalised. A 12-year-old girl living in the Haute-Savoie region of France, who had previously been in good health, died of measles encephalitis in late January 2009 at Geneva University Hospital.

In 2007 and 2008, thirteen and 68 importations respectively from Switzerland were reported by European countries participating to the European surveillance network for vaccine-preventable diseases (EUVAC.NET), corresponding to 15% and 31% of the total of imported cases with a known origin [21,22]. Moreover, through the Swiss notification system and publications were are aware of at least 10 additional exportations outside of Europe during the epidemic: seven in North America; one in Asia, one in Africa and one in Australia. A number of these led to outbreaks, some of which were large, for instance in Germany, Austria, France and the United States [9,23-29]. Conversely, 54 possible or certain importations into Switzerland were reported during the epidemic, of which 33 were from Europe (in particular Italy, Germany and France), nine from Asia, seven from America (four from Latin America and three from the United States), four from Africa and one from an unknown Mediterranean country.

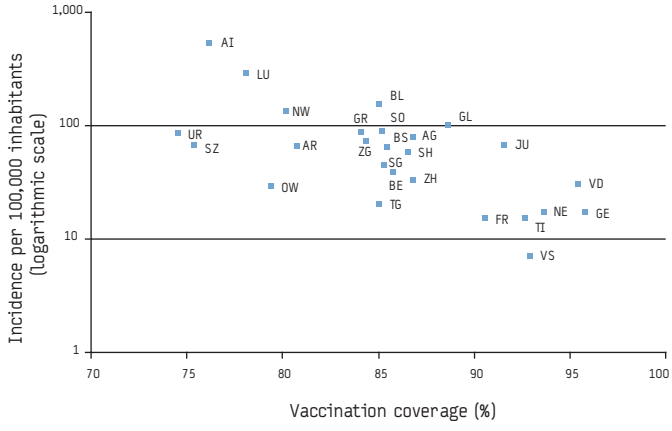
Public health measures

Control of outbreaks

In Switzerland, public health measures to control outbreaks of infectious diseases are the responsibility of the cantons. The FOPH has no detailed overview on the measures taken by the cantonal health authorities and physicians, and their results. The FOPH has developed national guidelines to standardise the cantonal measures

FIGURE 3

Cumulative incidence of measles by canton, Switzerland,15 November 2006 to 17 September 2009



AG: Aargau	GE: Geneva	OW: Obwalden	UR: Uri
AI: Appenzell Innerrhoden	GL: Glarus	SG: St. Gallen	VD: Vaud
AR: Appenzell Ausserrhoden	GR: Graubünden	SH: Schaffhausen	VS: Valais
BE: Bern	JU: Jura	SO: Solothurn	ZG: Zug
BL: Basel Land	LU: Lucerne	SZ: Schwyz	ZH: Zürich
BS: Basel-Stadt	NE: Neuchâtel	TG: Thurgau	
FR: Fribourg	NW: Nidwalden	TI: Ticino	

Note: Incidence displayed on logarithmic scale as function of vaccination coverage, ≥ 1 dose at two years of age according to the latest information available.

TABLE

Notified cases of measles and cumulative incidence per 100,000 inhabitants by age group, Switzerland, 15 November 2006 to 17 September 2009 (n=4,391)

Age (years)*	Number of cases	Proportion of all cases (%)	Incidence
< 1	114	2.6	153
1-4	531	12.1	180
5-9	1,095	24.9	285
10-14	1,033	23.5	244
15-19	775	17.6	170
20-29	399	9.1	43
≥ 30	444	10.1	9
Total	**4,391**	**100.0**	**58**

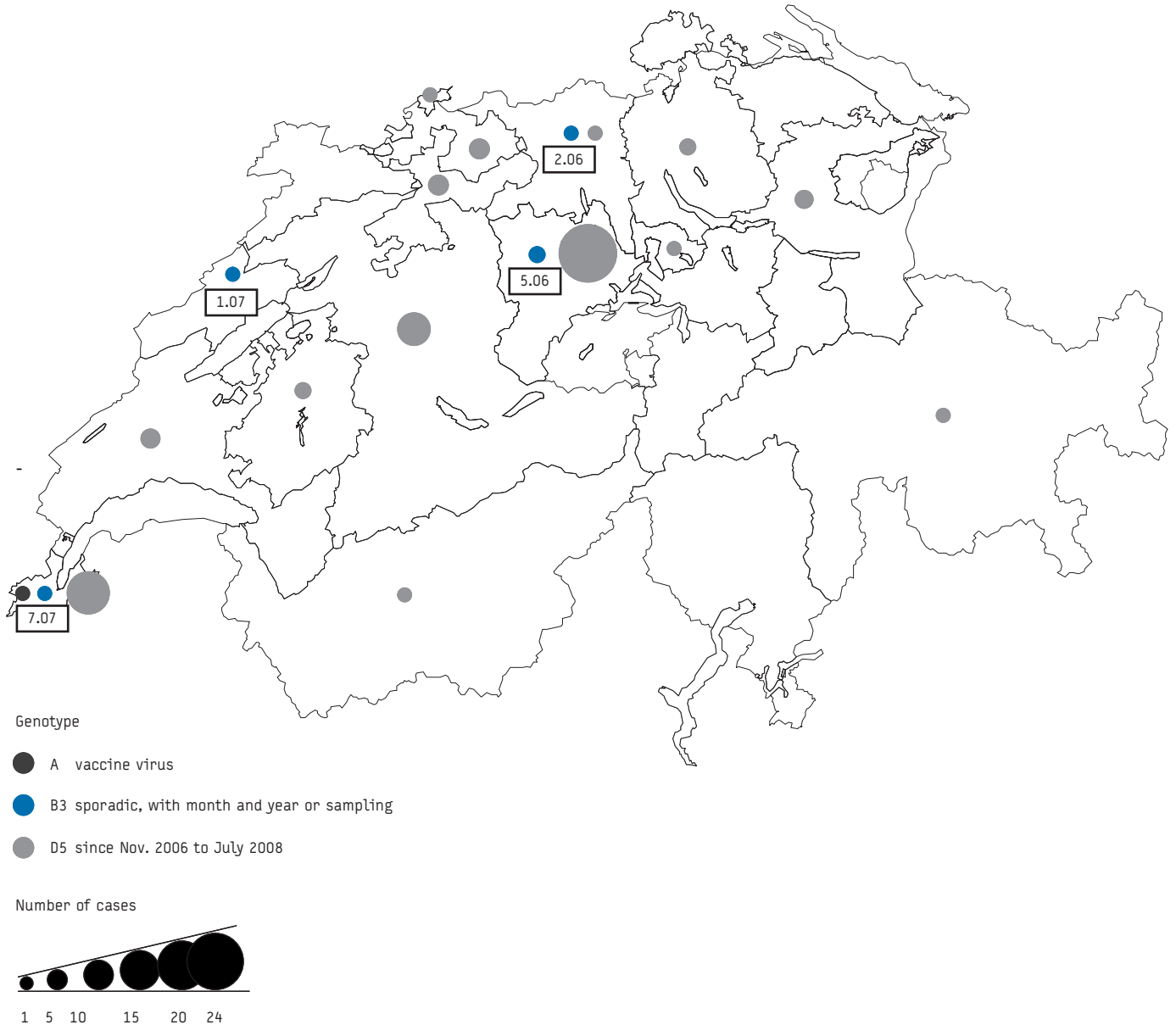
*information missing for 24 cases

intended to limit or stop transmission. Although they have not yet been finalised, they have already been widely applied in some cantons. These measures include, in particular, information for contacts of the case in settings such as schools, kindergartens, and universities, with recommendations on vaccination, active case finding and identification of susceptible contacts, post-exposure vaccination of contacts within 72 hours after exposure, exclusion of the sick from kindergartens and schools for four days after the appearance of the rash, exclusion of susceptible contacts (except if they had post-exposure vaccination) for 18 days after their last exposure and actions to vaccinate the extended circle of contacts. Post-exposure immunoglobulin is recommended for high risk groups. However, certain cantons, including some with a high incidence of measles, are not yet taking any measures or merely provide general information to the population or potential contacts.

In some instances, large-scale actions were carried out, in particular in the canton of Vaud. Following the notification of a case at the beginning of February 2009, an investigation of the contacts showed that there were already about ten non-notified cases in an anthroposophic school near Lausanne. As it was not possible to distinguish between people who had and had not been

Figure 4a

Circulating genotype of measles virus by canton, Switzerland, January 2006 to July 2008 (just before and during the first two waves of the epidemic, n=85)



NB the number of samples per canton is not proportional to the number of cases per canton

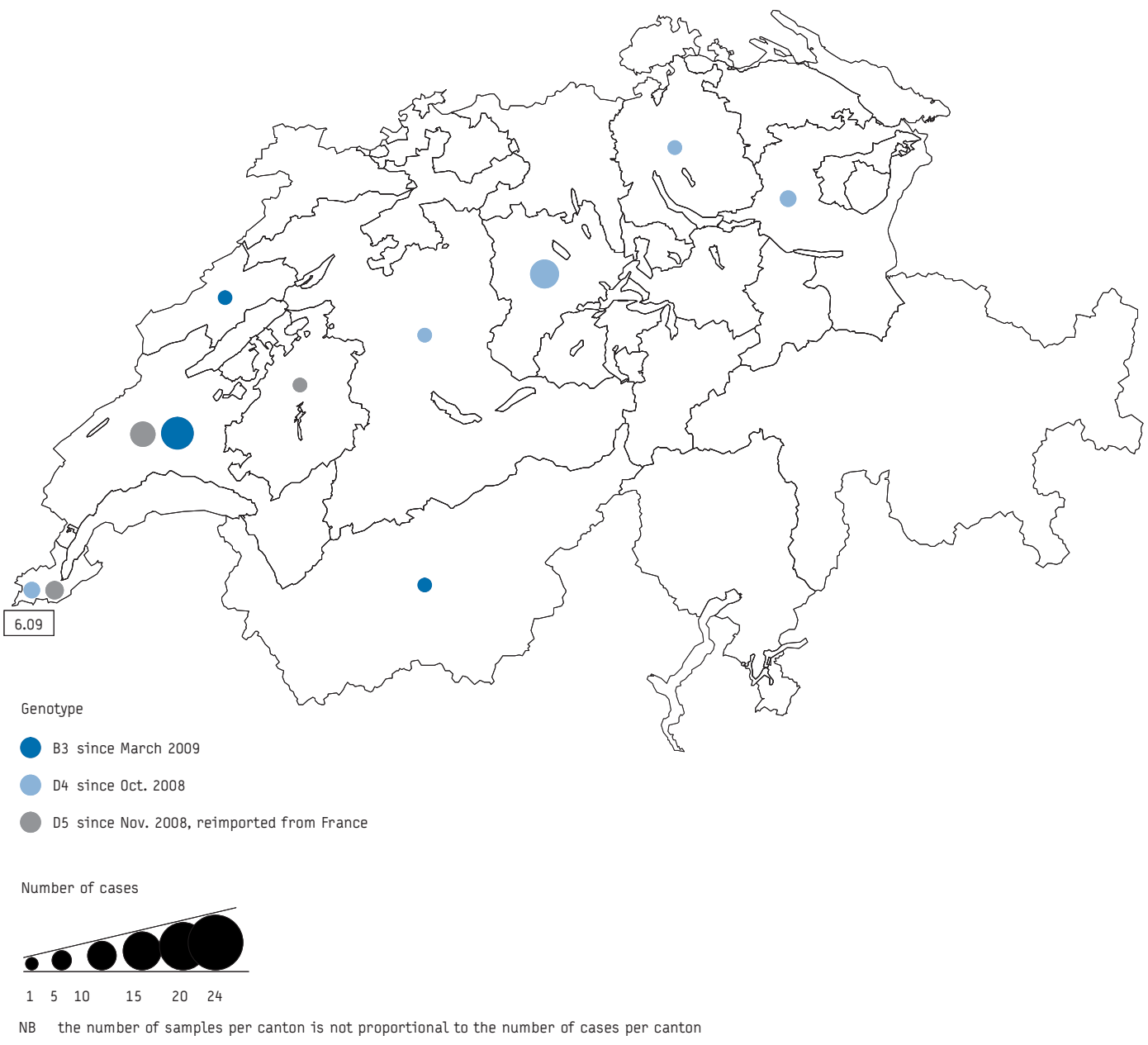
Source: Swiss Federal Office of Public Health

exposed, the cantonal officer of health immediately ordered that any pupil or teacher who had not been vaccinated at all and had not already had measles be excluded from the school and remain at home for 21 days which affected around 200 people. In March 2009, the campus of Lausanne was the centre of an outbreak of measles comprising about fifty cases. A large catch-up vaccination campaign was organised, to stop the transmission of the virus. All students and teachers were informed by email. More than 3,800 doses of MMR were administered within two and a half weeks, bringing vaccination coverage up to 97% for at least one dose of MMR vaccine from an estimated 90%.

For the first time following a risk linked to measles, in February 2009 the FOPH launched an international warning for passengers on two flights (Tel Aviv – Geneva via Zurich), with a direct search for some of the passengers. A girl, who had been infected in Switzerland before leaving for Israel, developed a rash soon after returning to Switzerland. She was thus infectious during the flights. At least one of the potentially exposed passengers sitting three rows in front and behind the girl were vaccinated.

Figure 4b

Circulating genotype of measles virus by canton, Switzerland, October 2008 to June 2009 (third wave of the epidemic, n=40)



NB the number of samples per canton is not proportional to the number of cases per canton

Source: Swiss Federal Office of Public Health

Intensification of primary prevention

Primary prevention of measles has been intensified through information and vaccination in kindergartens, schools, universities etc. In 2008, a MMR catch-up action enabled 4,500 pupils in compulsory education in the canton of Vaud to be vaccinated. Following an outbreak in an army barracks at the beginning of 2009, which led to post-exposure vaccination of about forty soldiers, the army health directorate introduced free, voluntary catch-up MMR vaccination for all conscripts. In order to improve coverage for vaccines recommended by the FOPH, in particular the MMR vaccine, Switzerland took part in the European vaccination week for the first time in 2009. On that occasion, the FOPH revised its Internet site dedicated to the promotion of vaccination [30] and distributed two new brochures to the population via physicians and pharmacists, one brochure being specifically about measles.

Media coverage of the third wave of measles reached an unprecedented level for measles. The messages of the federal and cantonal health authorities, in particular calls for vaccination, were transmitted on a large scale.

Political dimension of the elimination of measles

This epidemic has also become a political topic. The conference of cantonal health ministers has publicly committed to fight against measles in February 2009, with a view to its elimination, and to make further efforts to achieve ≥95% vaccination coverage [31]. It will consider introducing compulsory vaccination against measles before children go to kindergarten or to school, if this objective cannot be achieved by other means. Parliamentary interventions originating in both federal chambers have also successfully requested that the federal government launch a national plan to eliminate measles. This political impetus speeds up the preparation of such a plan, which was already underway at the FOPH. The main strategic focuses are to obtain the commitment of political and public health stakeholders, to reinforce the promotion of MMR vaccination through communication campaigns, to facilitate access and encourage vaccination through organisational measures, to control outbreaks of measles and to strengthen the surveillance of measles.

Discussion

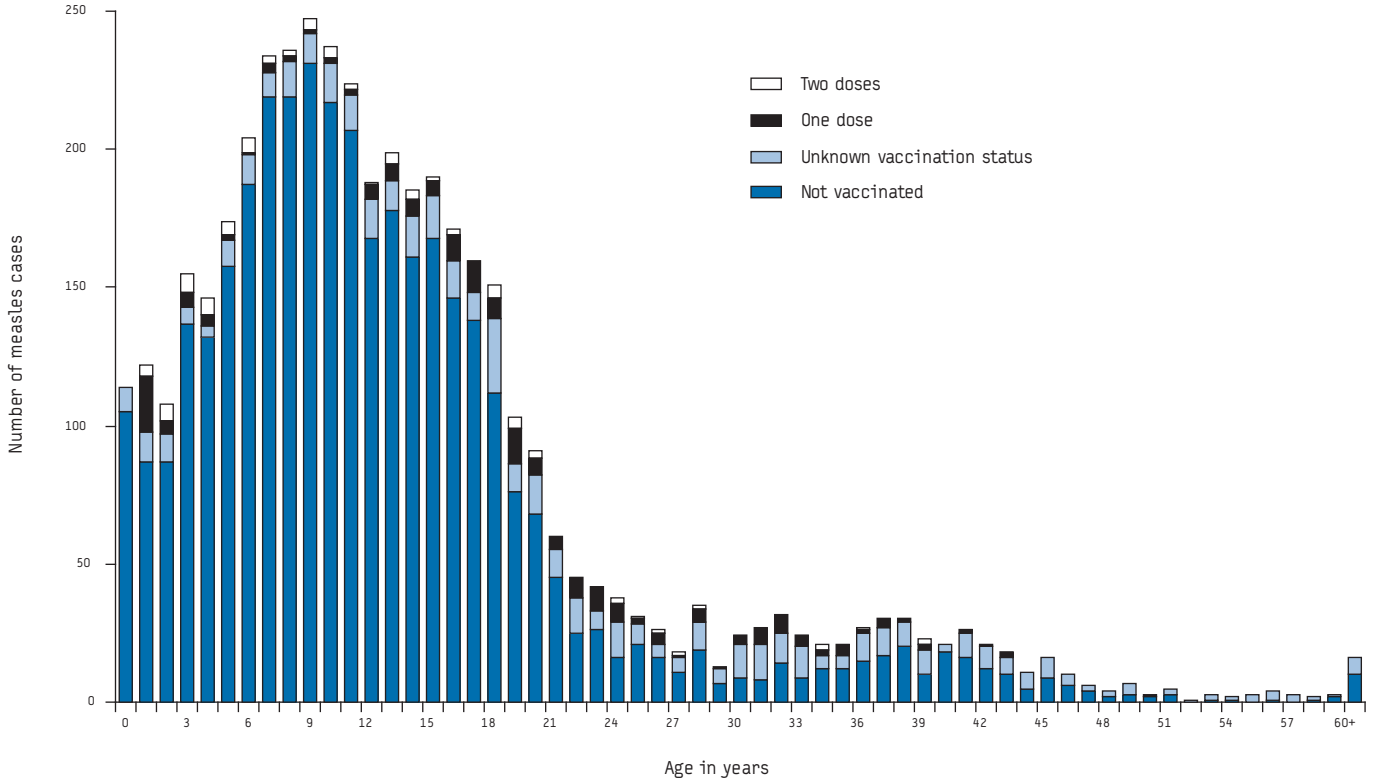
With 4,387 reported cases, since the end of 2006, Switzerland has recorded the largest and longest lasting measles epidemic since compulsory notification of this disease was introduced ten years ago (82% of all cases notified). However, the actual number of cases is certainly higher: an intensive survey of contacts suggests that only about one out of two cases were diagnosed by a physician and notified [personal communication Dr. E. Masserey]. The epidemic mainly affected younger school children and to a lesser extent adolescents and adults who had not been vaccinated. Ninety eight percent of patients had not been vaccinated or had been incompletely vaccinated.

In 2007 and 2008, Switzerland reported more cases, over a quarter of the total, with a 20-times greater incidence rate than the average, than any of the other 31 countries taking part in EUVAC.NET network [21,22].

The current epidemic is unusually long for Switzerland: 34 months with three distinct waves. In comparison, the 2003

Figure 5

Vaccination status by age for notified cases of measles, Switzerland, 15 November 2006 to 17 September 2009 (n=4,391)



epidemic only lasted six months, with six times less cases. Epidemics with several thousand or tens of thousands of cases, lasting for two to three years have been recorded recently in Europe, in particular in Romania, Georgia and Ukraine [32]. The proportion of people susceptible to measles in the Swiss population, their spatial distribution and the intensity of their contacts with parts of the world where measles are endemic are factors that allowed this prolonged though fluctuating circulation of the measles virus at the national level. During the last three years it led to numerous local and regional outbreaks, occurring successively or simultaneously, sometimes reaffecting regions that had already been affected.

Despite many importations of measles, only the D5 virus was circulating widely throughout Switzerland from the start of the epidemic until summer 2008. The beginning of the third wave, in autumn 2008, seems to coincide with the appearance of a new virus, D4, MVs/Enfield.GBR/14.07, that is endemic in the United Kingdom since April 2007 [33]. It was found in Eastern and Central Switzerland, from where the previous D5 virus was no longer reported. However, the same variant of the D5 virus reappeared in the French-speaking part of Switzerland at the beginning of 2009, following reintroduction from France, where it had been imported from the German-speaking part of Switzerland in spring 2008 [27]. Before this epidemic in Switzerland and the secondary outbreaks in neighbouring countries, the D5 virus had recently only been reported in Europe as rare, with sporadic cases or limited outbreaks, generally related to importations [34].

Inadequate vaccination coverage for many years and relatively low incidence of measles since 2004 has allowed the number of non-immune individuals to build up, feeding the current outbreaks. As expected, the incidence of measles per canton tends to increase with lower vaccination coverage. In addition, the high proportion of unvaccinated patients among cases confirms that this large epidemic was mainly due to inadequate vaccination coverage. The number of people in Switzerland who are under 20 years of age and are not immune to measles is currently estimated to be 214,000 (13% of this age group) from data on vaccination coverage and on notified cases. No seroepidemiological survey has been performed recently. The proportion varies from 9% to 18% depending on the canton, but is always above 5%, the threshold below which herd immunity establishes itself [35]. In addition, an unknown but likely small proportion of adults, in particular those under 45 years of age, is not immune.

This unsatisfactory situation can be explained by the deliberate choice not to vaccinate, made by certain parents, rather than by limited access to vaccination. Indeed, vaccination is widely available through paediatricians and family doctors. Up to 90% of the cost is covered by the compulsory health insurance scheme and several cantons offer free catch-up MMR vaccination in schools. The low amount payable by parents is probably just a minor barrier to access to vaccination. Indeed, vaccination coverage with at least three doses of a vaccine against diphtheria, tetanus, pertussis and poliomyelitis reaches approximately 95% compared with 87% for measles, while the recipient must also pay at least 10% of the invoice. In addition, vaccination coverage for measles decreases with the increasing level of education of the mother, and children of foreign nationality have a higher rate of vaccination than Swiss children [36]. As a result, vaccination coverage for measles is most probably higher in families with a lower income than in affluent families. Children of families using alternative medicine are in particular less often vaccinated than others. The canton of Lucerne where there are relatively high numbers of homoeopathic medical practitioners, has recorded about a quarter of all cases, often notified by such physicians. Some of these families who chose not to vaccinate their children also favour alternative education, in particular in private anthroposophic schools, which are often major foci as soon as measles are introduced. This was recently observed in Switzerland in the area of Basel, in Lausanne and in Berne, and elsewhere in Europe [25,26,37,38]. In addition to reluctance to vaccinate, missed opportunities certainly contribute to the accumulation of non-immune people. However, they seem to relate in particular to the second dose in children and catch-up vaccination for adults born after 1963.

Although they are still insufficient, interventions to control outbreaks of measles have continuously increased throughout this epidemic. In general they are well accepted by the population, but still have to be extended to the country as a whole. The prior aim of the measures is to stop the transmission of the virus rapidly, if not to prevent it. To this end, rapid notification of cases is crucial. This is why the delay for notification was reduced from one week to 24 hours in 2006. However, sometimes physicians are slow in notifying or do not notify cases at all. In these instances intervention is more difficult and its effectiveness reduced. Where implemented, measures such as exclusion of susceptible contacts from school have encouraged vaccination: parents have preferred to vaccinate their children rather than risking their eviction.

Consequences for the elimination of measles

Despite its magnitude, the current epidemic has only slightly (-1.4%) decreased the proportion of non-immune people in Switzerland aged less than 20 years. Although the epidemic is now over, a new one could start at any time. Therefore, it is essential to achieve very high vaccination coverage (≥95%) of each new birth cohort with two doses of MMR vaccine; but this will not be enough to eliminate measles in Switzerland: in parallel, catch-up vaccination has to be intensified for susceptible people born after 1963 ensuring that they are vaccinated with two doses of MMR.

The situation in Switzerland is a national challenge and a threat for the elimination of measles from the WHO European Region, as shown by the numerous exportations of measles. Further efforts are necessary and are planned by the national and cantonal health authorities so that with the help of partners and of the population, vaccination coverage can be increased to ≥95% and measles can be eliminated in Switzerland.

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Rubella seroprevalence in children in Dogankent, a rural area of Adana province in Turkey, January-February 2005

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A cross-sectional study was performed to determine the rubella seroprevalence in 331 children aged between 0 and 59 months in Turkey who were not vaccinated for rubella and lived in the area covered by Dogankent Health Center, a rural area with a large proportion of residents of low socioeconomic status. Rubella seropositivity was found to be low, with 17.5%, increased with age and low socioeconomic level, and was particularly high in children who live in a household with one member going to school, and in children of uneducated parents ($p<0.05$). The asymptomatic infection rate was 98.3%. There was no significant difference in seropositivity with regards to the gender, history of rubella infection, size of the household, or number of children at home ($p>0.05$). Rubella vaccine has only been included into the national vaccination programme in the form of the measles-mumps-rubella (MMR) vaccine since 2006 and is performed at the age of 12 months, in the first year of primary school and at the age of about 15 years. In order to eliminate rubella and congenital rubella syndrome, it is necessary that use of MMR vaccine is expanded to include the children born before 2006.

Introduction

Although rubella is a self-limiting disease in childhood, it can cause congenital rubella syndrome (CRS) when the mother is infected during the first trimester of pregnancy. In CRS, fetus and placenta are infected following maternal viraemia, which can result in abortion, premature birth or cataract, retinopathy, deafness, cardiac defects, hepatitis, haemolytic anaemia, thrombocytopenia, endocrinopathies, microcephaly, psychomotor retardation and progressive rubella encephalitis. The risk of clinical manifestations in the fetus or newborn decreases with the gestational age at the time of vertical transmission [1-3]. The most effective way to eliminate CRS is vaccination against rubella. A rubella elimination strategy should be based on universal childhood vaccination as well as immunisation of susceptible women at childbearing age. Unfortunately, there is no information about the CRS rate in Turkey.

In Turkey, rubella vaccine has been on the market since 1989 and has been administered in the form of the combined measles-mumps-rubella (MMR) vaccine, mainly in private practices and paid by the parents. A study conducted in Istanbul in 2002 reported that 13.3% of children were vaccinated by MMR [4]. Rubella vaccine has been incorporated into the Turkish national immunisation programme only in 2006. In the beginning of the vaccination programme, it was applied as MMR vaccine at the ages of 12 months and ca. seven years (in the first year of primary school), and as rubella vaccine at the age of about 15 years.

In studies on rubella seropositivity carried out in children in Turkey, Aksit *et al*. reported a seropositivity of 38.3% in 1-4 year-olds in Izmir in 1999 [5] and Cavusoglu *et al*. one of 12.5% in 2-5 year-olds in Istanbul in 2001[6]. Ay *et al*. reported 66.7% rubella seropositivity in primary school students in a rural district in Istanbul in 2003 [7]. In 2006, Gurgoze *et al*. reported a seropositivity of 47.3% in 1-4 year-olds and of 89.2% in 13-16 year-olds in Elazig, a city in eastern Turkey [8]. In Adana, Karakoc *et al*. found the seropositivity to be 92.5% in adolescent girls in 1999 [9] and in 2006, Oner *et al*. found it to be 93.7% in the same age group in Edirne, a city in northwest Turkey [10]. In pregnant women and women of childbearing age, reports from Turkey indicate that rubella seropositivity varies widely, ranging from 55.0% in Mersin province to 100% in Istanbul city [11,12]. Therefore, many women may be susceptible to rubella infection especially in rural areas. In the beginning of the rubella vaccination policy, children aged 1-6 years may not be vaccinated until they go to primary school, and as most of them are seronegative for rubella, they may be a risk for pregnant women. Hence, the objective of this study was to determine rubella seroprevalence in 0-59 months-old unvaccinated children in Dogankent, a district in Adana, Turkey.

Materials and Methods

Adana is an industrialised city in the southern part of Turkey with a population of approximately two million. Between 11 January and 17 February 2005, a cross-sectional study was conducted in Dogankent, a rural district, 20 km from of Adana, with a low socio-economic level and a population of 12,000. Dogankent has three elementary schools and one health centre. Main employment is in agriculture and stockbreeding. Although the mean size of a household in Turkey is four members, the mean household in Dogankent had seven members. Most of the adults were unemployed [13].

A systematic sampling method stratified by age and sex was applied, on the basis of data from the Dogankent primary health centre. This primary health centre was established in 1982 and

is under the supervision of the Department of Public Health of Cukurova University for which it serves as research and training area. The lowest seropositivity in 0-59 month-old children reported in all areas in Turkey was 12.5% [6]. At the time of study, 1,233 children between 0 and 59 months of age were living in Dogankent. The sample size of the study was calculated as 330 based on the 12.0% estimate of rubella immunity, with a 95% confidence level and worst acceptable result as 9%. The list of the subjects was obtained from the directorate of the Dogankent health centre. An additional 33 reserve subjects were also defined from the same age group, to be called if any of the 330 children could not be reached.

This sample size of 331 subjects comprised 26.8% of the 0-59 month-old children in the district. A maximum of one child from every house was included to the study. If we could not reach a child, a subject was chosen from 33 children on a reserve list. Subjects who had a telephone number were called to the primary health centre; those who did not were visited at home by one of the investigators. Twenty-nine subjects could not be included in the study for the following reasons: three did not want to participate, 26 moved away. Instead, 29 children from the reserve list were included in the study.

A questionnaire was completed about socio-demographic features, rubella vaccination and history of rubella infection of each child and family. Parents were asked if their child had ever been diagnosed for rubella by a physician or vaccinated with rubella vaccine, about the number people living in the household and the number of siblings aged 0-14 years living at home, the number of siblings going to school, and about the parents' employment and education level. Educational level of the parents was classified either as no education (not even primary school) or as having attended primary school (not necessarily graduating). Employement was defined according to the International Labour Organization [14]. There were four types of health insurance schemes in Turkey at the time of study: one for civil servants, one for self-employed people, one for workers, and a green card which covers the very poor people. People not included in any of these four insurance systems had to pay for healthcare. Since 2008 children under the age of 18 years have been entitled to free healthcare.

With permission of the parents, 3-5 ml venous blood was obtained from each child. Serum samples were stored at -20ºC and tested for rubella antibodies. Anti-rubella IgG was analysed by ELISA (DSL–05–10-RBG; Diagnostic System Laboratories). Values over 0.283 were defined as positive for the presence of antibody. The statistical analysis was done using SPSS- 10.0, and chi-square test. A p value of <0.05 was accepted as statistically significant.

The study was approved by the local ethics committee of the faculty of medicine and informed consent was obtained from all parents.

Results

The study was carried out in 331 children (162 boys, 169 girls). The mean age of the children was 30.3±16.0 months (range: 1-59 months, median age: 29 months). There was no statistically significant difference in the number of children in terms of sex and age group (p>0.05). The age distribution of the children is shown in Table 1.

Of the 331 children, 135 (40.8%) had no social health insurance, 141 (42.6%) had the green card, and 55 (16.6%) belonged to a social insurance system. Half of the fathers (48.9%) and almost all (99.1%) of the mothers were unemployed; 23.9% of fathers were workers, and 25.1% were self-employed.

None of the children participating in the study had received the rubella vaccine. Fifty-eight children (17.5%) were positive for rubella antibodies. The Figure shows the rubella seropositivity in different age groups. Rubella seropositivity increased with age (p<0.05). There was no significant difference between boys and girls in terms of rubella seropositivity (p>0.05).

Only five children were reported by the parents to have a history of rubella infection and one of those five had rubella antibodies. However, 57 (17.5%) of the 326 children without reported rubella history were positive for rubella antibody. Thus 57 of 58 children had negative rubella history, although they had had the infection in the past. Rubella seropositivity was not different between children who had a family member with (20.0%) or without (17.5%) rubella history (p>0.05). Neither was there any statistically significant relation between household size and the number of children in the house (p>0.05). The rubella seropositivity was higher in children living in a household with members who were going to school (Table 2, p<0.05). The parent's educational level was inversely associated with the prevalence of anti-rubella antibodies in the sense that as the educational level increased rubella seropositivity decreased (Table 2, p<0.05).

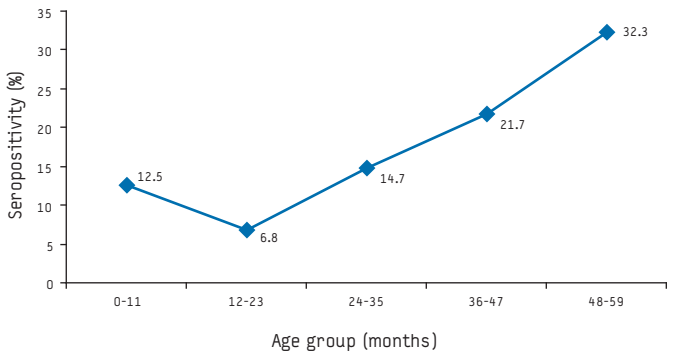
Table 1

Children, by age and sex, participating in the rubella seroprevalence study in Dogankent Turkey, January-February 2005 (n=331)

Age group (months)	Boys		Girls	
	n	%	n	%
0–11	24	50.0	24	50.0
12–23	38	51.4	36	48.6
24–35	33	44.0	42	56.0
36–47	37	53.6	32	46.4
48–59	30	46.2	35	53.8
Total	**162**	**48.9**	**169**	**51.1**

Figure

Age-specific rubella seropositivity in children of low socioeconomic status in Dogankent Turkey, January-February 2005 (n=331)



Discussion

In our study, rubella seropositivity was 17.5% among children aged 0-59 months. The major limitation of our study is that the results are from a single centre. Therefore our results may not necessarily be representative for other parts of Turkey. However, they could provide a benchmark for future assessments. In other studies carried out in Turkey, rubella seropositivity ranged from 22.5% [15] to 38.3% [5] in 0-1 year-olds, 47.3% [8] to 51.3% [16] in 1-4 year-olds, 12.5% in 2-5 year-olds [6] and 73.1% in 2-6 year-olds [15]. In adolescents, seropositivities of 92.5% [9] and 93.7% [10] have been reported. In developing countries, rubella seropositivity was found to be 97.0% in 2-7 year-olds in Iran [17], 69.2% in 1-5 year-olds in India [18] and 58.0% in 2-4 year-olds in Zaire [19]. The rubella seropositivity we found in our study was higher than the 12.5% observed by Çavusoglu *et al*. [6], but lower than those found in the other studies in similar age groups. This low level of rubella seropositivity we observed in Dogankent may be due to the geographic region or the fact that the study was performed in a rural area.

In the present study, rubella seropositivity was highest in the age group of 48-59 month-olds. Although rubella can be encountered at all ages, it is generally seen in the age group of 5-9 year-olds in countries that do not have routine rubella vaccination and rarely in those under one year of age due to maternal antibodies [1]. Several studies report that maternal antibodies are eliminated rapidly in the first 5-8 months of life [20-22]. The drop in seropositivity from 12.5% in the age group 0-11 months to 6.6% in the age group 12-23 months that we observed is probably due to the elimination of maternal antibodies.

Serologic studies conducted in Jordan [23], Nigeria [24], Yemen [25], Saudi Arabia [26], Lebanon [27], Taiwan [28], Italy [29], Ethiopia [30] in the past 20 years show that seropositivity increases with age. Seroprevalence data from the European Sero-Epidemiology Network (ESEN) study performed between 1996 and 2003 showed that women in several countries were not sufficiently protected against rubella infection. According to the European Centre for Disease Prevention and Control (ECDC), 1,498 rubella cases were reported from 22 European countries in 2005, with the highest incidences in Lithuania (3.44 per 100,000) and the Netherlands (2.23 per 100,000) [31]. Rubella susceptibility studies in our country also showed that seropositivity increases by age [6-11, 32]. Similarly, our study revealed that seropositivity increased from the 12th month of age, and this is in line with the findings in the literature.

Of 331 children in our study, only five had a history of rubella (as reported by the parents) and only in one of them, rubella seropositivity was determined. However, rubella seropositivity was found in 57 (17.5%) of the 326 children for whom no rubella history was reported. Thus, 98.3% of the children with rubella antibodies must have experienced an asymptomatic infection. However, the parents may have been unaware of infection symptoms or there may be recall problems. In a study by Kanbur *et al*. in adolescents [33], 66.0% of the seropositive cases had a positive history; however, the children in that study had been asked if they had had an eruptive disease, not specifically rubella. Two possible explanations for the lower rate of rubella history in seropositive children in our study, in comparison to Kanbur *et al*. [33] could be that the children in our study were younger and that we asked whether they had a history of rubella rather than any eruptive disease. The fact that the majority of cases were asymptomatic emphasises the importance of serological studies in determining the definite prevalence of rubella in a community.

In our study, the rubella seroprevalence in children with parents who had education of any level was statistically lower than that in children with parents who never had any education. We have no explanation why this would be the case for an air-borne infection such as rubella. A higher number of infectious diseases in children of parents (especially mothers) with low education and low socioeconomic status is to be expected, as also observed by other authors [8,34]. However, Karakoc *et al*. [9] did not find a relation between rubella seropositivity and socioeconomic status.

Table 2

Rubella seropositivity in children according to parents' education, siblings going to school, sex and number of people living at home in children of low socioeconomic status in Dogankent Turkey, January-February 2005 (n=331)

		Seropositivity		Seronegativity		All		p value
		n	%[a]	n	%[a]	n	%[b]	
Gender	Boys	26	16.0	136	84.0	162	48.9	0.49
	Girls	32	18.9	137	81.1	169	51.1	
Mother's educational status	Not educated	39	22.4	135	77.6	174	52.6	0.02
	Primary school or high school	19	12.1	138	87.9	157	47.4	
Father's educational status	Not educated	18	26.5	50	73.5	68	20.5	0.045
	Primary school or high school	40	15.2	223	84.8	263	79.5	
Siblings going to school	No	20	12.3	142	87.7	162	48.9	0.02
	Yes	38	22.5	131	77.5	169	51.1	
Number of people living at home	3-4	10	12.5	70	87.5	80	24.2	0.11
	5-6	22	17.2	106	82.8	128	38.7	
	7+	26	21.1	97	78.9	123	37.1	
Total		**58**	**17.5**	**273**	**82.5**	**331**	**100**	

[a] Percentage refers to the total in the same row.
[b] Percentage refers to the sum of totals in the column.

Whether or not the children had a family member with a history of rubella infection did not make a statistical difference in terms of rubella seropositivity. One reason for this may be the fact that rubella infection is not as contagious as measles and chickenpox. While one measles case can infect 10-14 other people, a rubella case can spread to five or six people [2], and rubella inter-household infection is 50-60% [3]. Also, as 25-60% of rubella infections are asymptomatic [2,3], it is not possible to know whether people reporting no history of rubella are actually seronegative or not, which would result in an underestimation of cases in households with a history of rubella. Another reason may be the fact that poor people from low socioeconomic background might not have a chance to see a doctor. This finding of our study can therefore not be considered to be reliable.

Although crowding is known to play a role in the dissemination of rubella, we did not observe a statistically significant difference between rubella seropositivity and the number of household members. However, the risk of rubella was 1.6 times higher in children living in a household of seven or more members than in children living in a household of three or four people (21.1% versus 12.5% seropositivity).

In our study, the number of siblings did not increase the seropositivity, but seropositivity was higher if the child had a sibling going to school (22.5% versus 12.3%). It is well known that rubella is less frequent in children before they have started school. Cengiz *et al*. [16] reported that rubella seropositivity was 12.5% before school and increased to 65.3% in primary school. Moreover, our study did not detect a statistically significant difference between rubella seropositivity and the presence in the household of children aged between 0-6 years. Rubella is seen mostly in five to nine year-old children and the rubella incidence reaches its peak in this age group [3]. The infection rate of the disease is about 100% in susceptible people in closed quarters such as schools and military barracks and 50 60% in the home environment [3]. Higher rubella seropositivity in children with brothers or sisters in school is therefore an expected finding,

Conclusion

Rubella vaccination was integrated into the national immunisation programme in Turkey in the form of MMR vaccination only in 2006. In our study, rubella seropositivity was low in children aged between 0-59 months. For this reason, it is necessary to ensure that MMR vaccination is expanded nationwide to cover the children born before 2006. Epidemiological studies should continue as the epidemiological characteristics of the disease may change depending on the uptake of MMR vaccination, while seroprevalence studies should continue in order to determine the seroconversion rate and period of preventive effectiveness of MMR vaccination. In order to eradicate rubella and CRS, it is necessary to vaccinate women at child-bearing age who are found to be susceptible as a result of serological tests and children born before 2006 with rubella vaccination.

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Results of a vaccination campaign against human papillomavirus in the province of La Spezia, Liguria, Italy, March-December 2008

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Sexually transmitted diseases caused by human papillomavirus (HPV) are being diagnosed more frequently than others. It is accepted that HPV infection is a necessary cause for all cases of cervical carcinoma and a large number of other anogenital and oral cancers. Two vaccines have been developed and were licensed in 2007, which can prevent infections and pre-cancerous lesions due to HPV. In Italy pre-adolescent age (12 years-old) was identified as the ideal age for vaccination against HPV. In Liguria, the first free HPV vaccination campaign was started on 8 March 2008 in 12 year-old girls. We assessed the adherence to the vaccination during the 2008 campaign as 80.6%, 79.0% and 64.1%, respectively, for the first, second and third dose of vaccine in the target population.

Introduction

Sexually transmitted diseases caused by human papillomavirus (HPV) are being diagnosed more frequently than others. Today it is universally accepted that HPV infection is the necessary, although not sufficient, cause of all cases of cervical carcinoma and of a large number of other anogenital cancers and oral squamous cell carcinoma [1].

Certain viral genotypes, defined as high-risk (HR) carcinogenic genotypes (e.g. HPV types 16, 18, 31, 33, 35, 45, 52, 58) are associated more strongly with the development of tumours than others [4], and among these, genotypes 16 and 18 are most relevant in the context of cervical carcinogenesis [5-8]. The low-risk viral genotypes, including HPV6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, cause benign laesions such as anogenital condyloma and laryngeal papilloma [9].

Cervical cancer affects approximately 1.4 million women in the world, with an annual incidence of 500,000 cases [2] and causes an estimated 274,000 deaths each year. In Europe, cervical cancer affects approximately 60,000 women and 30,000 die because of this disease every year [3]. In Italy, recent numbers indicate that an estimated 3,500 women are diagnosed with cervical cancer every year and approximately 1,200 die. Cervical cancer occupies the tenth position for cancers affecting women in Italy and is the third most common cancer in women between the ages of 15 and 44 years. It is estimated that about 10.3% of women in Italy have an HPV infection and that 71.7% of invasive cervical cancers are attributable to the high-risk HPV genotypes 16 and 18 [3].

Vaccines

Two vaccines have been developed, Cervarix and Gardasil, that can prevent infections and pre-cancerous laesions caused by HPV infection. They consist of recombinant viral capsid protein L1 (or a combination of L1 and L2) of HPV genotypes 6, 11, 16 and 18, assembled into virus-like particles (VLPs), and induce the production of neutralising antibodies against these genotypes. Since these vaccines do not contain HPV DNA they cannot cause infection or have an oncogenic effect by integrating into the DNA of the host cell [10-12]. Cervarix is a bivalent vaccine developed by GlaxoSmithKline, containing VLPs of the L1 proteins of HPV16 and 18, 20 µg of each, with an adjuvant of aluminium salts and a lipid agent (ASO4). The vaccination protocol foresees three intramuscular doses of 0.5 ml (at 0, 1 and 6 months) for girls from 10 to 25 years of age. The quadrivalent vaccine Gardasil was developed by Sanofi Pasteur MSD. The vaccine contains 20 µg L1 VLPs of HPV6 and 18 and 40 µg L1 VLPs of HPV11 and 16. The purified particles were adsorbed with aluminium salts that act as adjuvant. The protocol for the vaccine foresees three intramuscular doses of 0.5 ml (at 0, 2 and 6 months) for girls from nine to 26 years of age [13,14].

Both vaccines are considered to be safe and several studies document seroconversion to all types of HPV contained in the vaccine in more than 98% of cases. The antibody peak occurs a month after the third dose, then it decreases slowly until 18 months. In general, the antibody titres decrease 10-fold in the first one or two years post vaccination and stabilise after three to five years at levels higher than those induced by the natural infection. The quadrivalent vaccine showed 100% and 99% efficacy, respectively, against cervical intraepithelial neoplasia grade 2/3 and condyloma. The bivalent vaccine proved 100% effective in the prevention of cervical dysplasia [15-18]. At this point in time, it is not known how long the protection by the HPV vaccine lasts and whether a later booster vaccination will be necessary. However, preliminary results have shown that a booster with monovalent HPV16 vaccine induced a quick, very high and prolonged immune response [19].

The bivalent vaccine shows cross-reactivity to other HPV types, in particular to HPV45 and 31, which are phylogenetically similar to HPV18 and 16, respectively [17].

Target population

Genital HPV infection is usually transmitted sexually, and immunisation should therefore precede the start of sexual activity. It implies that the target population for vaccination is prepubertal girls or young adolescents. In addition, the antibody response induced by vaccines is generally higher in prepubertal children [20].

The United States Advisory Committee on Immunization Practices (ACIP) recommends the routine use of the vaccine for 11-12 year-old girls (minimum age nine years) and a catch-up vaccination for women between 13 and 26 years of age, regardless of whether they are sexually active or not [20]. The Canadian National Advisory Committee on Immunization (NACI) advises that girls aged between nine and 13 years should be vaccinated before their sexual debut and that women between 14 and 26 years of age should be vaccinated, regardless of whether they are sexually active or not [21].

The target populations in some European countries are shown in Table 1. In Germany and in the United Kingdom, the HPV vaccine is offered to the target population free of charge. In France, 65% of the cost is borne by the welfare system and the remaining 35% are paid by the individual or by a voluntary private insurance [22].

A recent survey on the sexual habits of young Italians indicates that 4% of girls report to have had their first sexual intercourse at the age of 14 years and 10% at the age of 15 years. Moreover, the data stratified by age showed that the age of the first sexual intercourse is decreasing within the cohort of 18-29 year-olds (both in men and in women) [23].

Another study compared 16 vaccine strategies in different age cohorts and the corresponding number of infections prevented by HPV. It found that vaccinating 12 year-old girls can be effective in the prevention of HPV infections. Indeed, the majority of 12 year-old girls are not yet sexually active and therefore represent the best target for vaccination [24]. The Superior Council of Health in Italy identified in its opinion on 11 January 2007 pre-adolescence (12 years) as the ideal age for vaccination because of the following considerations:

- Almost none of the children have previously had any sexually transmitted infections;
- The immune response at that age tends to be stronger;
- Children of that age attend the first two classes of secondary school where parents are still much involved and therefore both children and parents can be reached with adequate and relevant information about infection and vaccination;
- There is the possibility to catch up on missed doses of the vaccine in the third class of secondary school;
- Children of that age are under the responsibility of their parents who may insure adherence to the vaccination course;
- The vaccination can be included in the national vaccination schedule [25].

On 22 February 2008, the Italian Minister of Health announced the start of the first public vaccination campaign against HPV for 12 year-old girls [26].

The HPV vaccination campaign in Liguria

As foreseen in the Regional Decree (DRG) No. 54 on 25 January 2008, the vaccination campaign started on 8 March 2008 and targeted 12 year-old girls (born in 1997) who were offered free vaccination. Moreover, a free not active offer is in place for girls at the age of 13 years (born in 1996), and girls and women between the ages of 14 and 26 years can get the vaccine at a partial price of EUR 105, the cost of the vaccine and its administration incurred for the local public health authority (Azienda Sanitaria Locale, ASL). The objective was to achieve a coverage of >95% of the 12 year-old-girls with three doses of vaccine within five years after the start of the vaccination programme [27]. The bivalent vaccine was chosen for the campaign.

The aim of this work was to assess the adherence to HPV vaccination in 12 and 13 year-old girls during the vaccination campaign in the ASL 5 "Spezzino", from March to December 2008. The study analysed all girls (12-13 years old girls) vaccinated as part of the active free offer as well as the not active free offer, and also noted the adherence to vaccination in people who paid the partial costs for their vaccination.

Materials and methods

This study shows the results of the HPV vaccination in the province of La Spezia in the region of Liguria. This province is served by the ASL 5 "Spezzino". The resident population of La Spezia on 31 December 2007 was 218,032 people [28]. Healthcare is provided by four hospitals and three social health districts.

The recruitment of birth cohorts 1996 and 1997 for vaccination was made using the municipal registers. The other birth cohorts were not recruited, but signed up for the vaccination themselves. An invitation letter was sent to the girls' parents to explain the campaign. It included a regional information brochure, the informed consent form, and the date on which to present to the outpatient clinics for vaccination. A second invitation letter was sent if parents did not respond. Moreover, if girls stopped the vaccination cycle after the first or second dose of vaccine, a reminder letter was sent, containing a consent or dissent form to be completed and returned.

Table 1

Details of HPV vaccination programmes introduced in some European countries as of 31 October 2007*

Characteristics	Austria	France	Germany	United Kingdom
Target population	Girls and boys before sexual debut	14 year-old girls	12-17 year old girls	12-13 year-old girls
Catch-up	No	15-23 year-old women, sexually active or who started sexual activity in the 12 previous months	No	16-18 year-old women from autumn 2009 and 15-17 year-old women from autumn 2010

HPV: human papillomavirus
*According to reference [22]

A similar invitation letter was sent to the 1996 birth cohort together with the regional information brochure and a phone number to call for an appointment.

Information campaigns on prevention interventions and health promotion targeting adolescents are often fragmented and without continuity. They often do not integrate the work of health and education services and voluntary associations. Therefore, the ASL 5 coordinated the activities for the HPV vaccination campaign, involving different areas of expertise such as general practitioners, paediatricians, local nursery and infant health services, school authorities, local press and families.

The general practitioners, paediatricians, and local nursery and infant health services received posters to be displayed in their waiting rooms, containing the email addresses and internet links of where to obtain information about the vaccination. They also actively informed parents and girls about transmission, consequences of HPV infection and the protection the new vaccine could offer. ASL staff also prepared and distributed brochures in all schools in the province of La Spezia. In a simple and understandable way, the girls were informed about the benefits of vaccination and invited to ask their paediatricians for further information.

Gynaecologists and local public health experts held a press conference explaining in detail how the immunisation campaign was organised and the benefits it offered. Local newspapers reported on the beginning of the immunisation campaign, with an invitation to call the vaccination clinics for any information about it. Finally, the ASL 5 website posted a link to the HPV vaccination campaign site containing frequently asked questions about vaccination (prepared by the National Screening Observatory) and the procedures for access to the public health clinics throughout the territory.

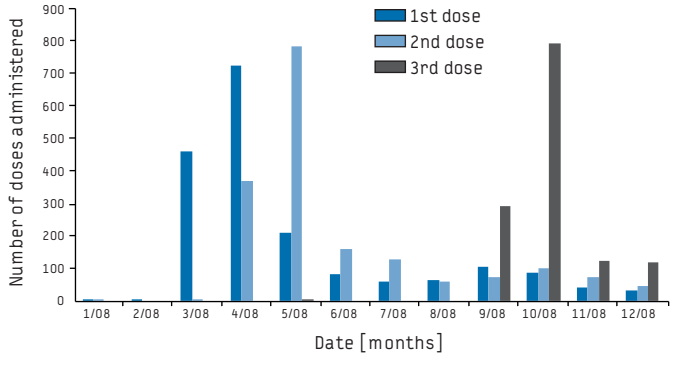
Vaccination teams were established to reduce outpatient waiting times. These vaccination units consisted of a physician, a nurse and an administrative technician. In addition, compensation was provided for staff working outside normal office hours to enable vaccination sessions in the afternoon. Data were collected from March to December 2008 in a computerised vaccination registry. The percentage of adherence to the vaccination was calculated as the number of doses administered per target resident population x 100. Adverse reactions to the vaccine were reported to the regional Department of Health, following the established routine for adverse reactions to other vaccines.

Results

The figure shows the number of doses administered between March and December 2008. The majority of first doses were

Figure

HPV vaccine doses administered from March to December 2008 to girls born in 1997 and 1996, by month, in La Spezia province



HPV: human papillomavirus.

Table 2

Number of HPV-vaccinated girls per birth cohort (1997-1982) and relative vaccination adherence, Italy, March to December 2008

Birth cohorts	Resident girls (no.)	Vaccinated girls first dose (%)	Vaccinated girls second dose (%)	Vaccinated girls third dose (%)
1997	825	80.6	79.0	64.1
1996	854	74.5	73.5	58.1
1995	782	12.3	11.9	7.2
1994	761	10.1	8.9	5.4
1993	753	10.1	9.6	5.8
1992	841	12.5	11.7	6.3
1991	782	8.2	7.5	3.6
1990	836	6.7	6.2	5.0
1989	844	2.3	2.1	1.3
1988	842	2.5	2.0	1.2
1987	797	1.9	1.6	0.9
1986	846	1.7	1.4	0.7
1985	852	1.8	1.2	0.8
1984	902	1.4	1.2	0.6
1983	862	1.3	1.3	0.8
1982	978	0.9	0.6	0.4
total 1982-1995	**11,678**	**5.0**	**4.6**	**2.7**

HPV: human papillomavirus

administered by the vaccination clinics during the months of March and April, following the administration of the first dose on 19 March, with a slight increase in September, which, according to statements from girls and parents, may be related to the Nobel Prize in Medicine awarded to Harald zur Hausen who is dedicated to the study of HPV.

The percentage of adherence to the vaccination was 80.6%, 79.0% and 64.1%, respectively, for the first, second and third dose in the 1997 birth cohort, and 74.5%, 73.5% and 58.1% in the 1996 birth cohort. As expected, the adherence in older age groups was lower: 5.0%, 4.6% and 2.7%, respectively, in those born between 1995 and 1982. Table 2 shows the number of vaccinated girls born between 1982 and 1997 and the relative vaccination adherence.

As of 31 December 2008, only three girls born in 1997 had stopped taking the vaccine after the first dose. Twenty girls had stopped after taking the second dose. In the 1996 birth cohort were two girls hat stopped after the first dose, one of them due to an adverse reaction, and 22 had interrupted the vaccination after the second dose (one due to an adverse reaction).

In the assessment of side effects due to the vaccine (data not shown) that occurred within seven days after administration, local effects were the most frequent, especially pain and redness in the inoculation site. The most frequently observed systemic side effects were fatigue, general malaise and gastrointestinal symptoms, which is in agreement with the literature [15-17].

Two adverse reactions involved girls born in 1996. One was characterised by redness and induration at the breast ipsilateral to the inoculated arm. It appeared about 12 hours after the first vaccination and resolved spontaneously within a few days. This reaction led to the decision to suspend the vaccination cycle. The other one, following administration of the second dose of vaccine, was characterised by a severe form of atopy which resolved spontaneously in a girl with a history of atopic dermatitis. This girl had already presented erythema and itching with lower intensity after the first dose of vaccine. Again, as a precaution, it was decided to suspend the vaccination course.

Discussion and conclusion

HPV vaccination is a new important instrument to prevent the occurrence of a specific cancer. The success of a vaccination campaign depends on several factors including support from policy makers, the presence of qualified and expert health professionals and the cost-effectiveness of the vaccine. It is necessary to provide the population with clear, concise and simple information about HPV infections, cervical cancer, prevention and vaccination. It is important that healthcare workers are well trained in communicating with patients to insure professional credibility and aid the promotion and implementation of coordinated vaccination campaigns.

There are as yet no published national or international data on how many vaccine doses were administered in countries that have already run HPV vaccination campaigns, and how many people were vaccinated or completed the vaccine course. It is estimated that the target population, 12 year-old girls, was about 280,000 in Italy [26] and 6,000 in the region of Liguria [27]. Considering the expected objective for Liguria to achieve a coverage of >95% of the 12 year-old girls with three doses of vaccine within five years after the start of the vaccination programme [27] and the Ligurian pooled data which show an adherence to HPV vaccination of about 62% among 12 year-old girls [29], the results obtained during the HPV vaccination campaign in 2008 in the ASL 5 "Spezzino" (80% adherence) are to be considered very good.

Adverse reactions to all vaccines have to be reported to the regional Department of Health to ensure post-licensure monitoring of the safety of the vaccine. In the 2008 HPV vaccination campaign the ASL 5 "Spezzino" observed only two moderate adverse reactions that did not require hospitalisation or medication and resolved spontaneously. With regard to the tolerability of the vaccine, these side effects were comparable to those observed in the literature [15-17] and the number of girls who interrupted their vaccination course was limited (two girls).

The involvement of girls and their parents in the vaccination campaign was very high and they showed a considerable interest in HPV and the consequences that the infection may have. General practitioners and paediatricians received many requests for information from parents of girls involved in the campaign and their number of patients increased. In the first month of the campaign the website was accessed more than 500 times and 300 phone calls were made to the dedicated numbers. The main points of our programme were the implementation of educational campaigns targeted according to age and sex, the involvement of educational institutions, information about the transmission of the infection and an increase in staff at vaccination clinics during the campaign.

In conclusion, we can say that the information campaign carried out throughout the province was conducted successfully and appropriately. However, this is only a starting point. To further raise the awareness of girls and parents regarding HPV vaccination, the quality of the information and especially the quality of healthcare and vaccination services needs to be improved.

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Estimating diagnostic accuracy of tests for latent tuberculosis infection without a gold standard among healthcare workers

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The evaluation of diagnostic accuracy of new *in vitro* diagnostic assays for tuberculosis infection has been hampered by the lack of a standard reference test. The aim of this study was to compare sensitivity and specificity of interferon gamma assays for latent tuberculosis infection by assessing the association of test results with tuberculosis occupational exposure and by using latent class analysis. We analysed data from 115 healthcare workers on whom tuberculin skin test (TST) and the following *in vitro* tests were performed: in-house ELISPOT for RD1 proteins, T.SPOT-TB and Quantiferon-TB Gold. Results of all tests were associated with increased occupational risk of exposure to *Mycobacterium tuberculosis*, but only TST was associated with *Bacillus* Calmette-Guérin (BCG) vaccination. Sensitivity/specificity (95% confidence intervals) estimated by a latent class model were: 99.9%/64.2% (53.0-74.1) for TST, 95.3% (61.8-99.6)/87.5% (78.0-93.2) for in-house ELISPOT, 96.7% (69.3-99.7)/85.6% (75.3-92.0) for T.SPOT-TB, and 76.3% (55.9-89.1)/93.6% (85.4-97.3) for Quantiferon. The estimated specificity of *in vitro* assays was higher than that of TST also among individuals who were not BCG-vaccinated. In conclusion, when used in healthcare workers, *in vitro* assays may provide a significant increase of specificity for tuberculosis infection compared to TST, even among non vaccinated individuals, at the cost of some sensitivity.

Introduction

Identification and treatment of individuals with latent tuberculosis infection is an important component of tuberculosis elimination strategies in low incidence countries, and may contribute to the global tuberculosis control efforts [1-4]. In this context, healthcare workers represent an important target population for latent tuberculosis infection screening programmes [5]. The effectiveness of these programmes, however, has been limited by the fact that the standard tool used to diagnose latent tuberculosis infection, the tuberculin skin test (TST), has a limited diagnostic accuracy, mainly because it relies on the use of protein purified derivative (PPD), which is a mixture of antigens shared by many pathogenic and non-pathogenic mycobacteria, including *Bacillus* Calmette-Guérin (BCG) strains used for vaccination [6].

Recently, new immunologic tests have been introduced for diagnosing tuberculosis infection [7,8]. These tests, often referred to as interferon gamma release assays (IGRAs) are based on the detection of *in vitro* response to proteins encoded by genes located within the region of difference 1 (RD1) of *M. tuberculosis* genome, the early secreted antigenic target 6 protein (ESAT-6) and the culture filtrate protein 10 (CFP-10), that are not shared with BCG strains or most environmental mycobacteria [9,10]. Two of these tests have been made commercially available. Both measure interferon gamma released *in vitro* in response to RD1-encoded antigens, although they use different antigen preparations (overlapping peptides spanning the entire length of these proteins) and different assay formats (ELISA and ELISPOT) [11,12]. Recent guidelines recommend that these tests be used instead of [1,2] or in addition to [13] TST.

A number of studies have evaluated IGRA, in comparison to TST, as a tool for screening latent tuberculosis infection among healthcare workers [14-19]. To our knowledge, however, no study has compared different IGRAs in this population group.

The lack of a gold standard for the diagnosis of latent tuberculosis infection has hampered the assessment of the diagnostic accuracy of IGRAs. Different strategies have been used so far to address this issue, including the evaluation of the proportion of positive tests among individuals with active tuberculosis (as a proxy for sensitivity), and of the proportion of negative tests among individuals at low risk for tuberculosis infection (as a proxy for specificity) [1,2,7]. Another approach that has been proposed for the validation of IGRAs is based on the assessment of the association of test results with risk factors for tuberculosis infection

[11,20]. Finally, latent class analysis, a statistical method which has been proposed for the assessment of diagnostic tests in the absence of a gold standard, could be used in this context [21]. In the frequentist statistical approach used in the present study, this analysis requires availability of results from at least three different diagnostic tests on the same individual, and it is based on the concept that different tests for the same disease are influenced by a common latent variable, the disease status, which cannot be measured directly [21-23].

Healthcare workers remain at risk for tuberculosis infection also in countries with low tuberculosis incidence [24]. However, especially in countries such as Italy where until recently BCG vaccination has been widely used in healthcare workers, surveillance of tuberculosis infection has been hampered by the low specificity of TST. In the present paper, we analysed data on healthcare workers in Italy who were tested by TST and by three *in vitro* interferon gamma tests, an in-house ELISPOT assay based on RD1 proteins [25], a commercial ELISPOT assay and a commercial whole blood ELISA using RD1 peptides. To validate the use of these tests in this population group, we assessed their association with occupational tuberculosis risk and estimated their sensitivity and specificity by using a latent class analysis.

Methods

Study design and participants

We conducted a cross-sectional study in 2004-2005 at two tertiary care hospitals in Rome, Italy, which include wards that routinely treat pulmonary tuberculosis patients. Healthcare workers at these institutions who had had a routine periodic health check in 2004 or 2005 were considered for inclusion, if they had a positive TST result in the 12 months, or a negative TST result in the three months before we did the *in vitro* tests. There was no formal calculation of the sample size prior to the study. No incentive was offered for participation. The study was approved by the ethics committees at participating institutions and study participants gave written informed consent.

For each individual enrolled in the study, the following data were abstracted from personal charts: age, sex, place of birth, job category, ward or service of present and past employment, BCG vaccination, household tuberculosis contacts. Ward or service of employment were classified either as high risk if more than one patient with tuberculosis was cared for per year, or as low risk if that was not the case.

Diagnostic assays

The TST was administered by trained nurses at participating institutions by the Mantoux procedure using 5 IU of PPD (Chiron). Results were read after 48 to 72 hours. For the purpose of the present analysis an induration of at least 10 mm was scored as a positive response [1,2].

The in-house ELISPOT assay based on ESAT-6 and CFP-10 proteins (Lionex) was performed as previously described [25], and results were scored positive if the average number of spot-forming cells (SFCs) in cultures stimulated with these antigens was at least three-fold higher than the average number of SFCs in the control. Interferon gamma values are presented as number of SFCs per million PBMC, after subtraction of the appropriate control according to the described criteria.

The commercial ELISPOT assay used was the T-SPOT.TB (Oxford Immunotec) and it was performed as previously described [11]. Responses were scored positive if the test wells contained a mean of at least six spot-forming cells more than the mean of the negative control wells, and if this number was at least twice the mean of the negative control wells.

The commercial ELISA assay was the enhanced 'in-tube' version of QuantiFERON-TB Gold (QFT-G, Cellestis Limited).This assay is based on peptides spanning the entire sequences of ESAT-6 and CFP-10 as well as another peptide representing a portion of the TB7.7 antigen [12]. It involves two stages: incubation of whole blood with the antigens, and measurement of interferon gamma production in harvested plasma by ELISA. As recommended by the manufacturer, the cut-off value for a positive test was 0.35 interferon gamma IU/ml.

All blood test were performed on the same blood sample. For 47 individuals (45.3%), the blood sample was taken on the day the TST was performed, while for the remaining individuals, it was taken eight to 365 days after the TST. ELISA and ELISpot were performed at the study site, and all assays met quality control standards.

Statistical methods

Standard univariable methods were used to describe the association between participant characteristics and results of diagnostic assays.

The association of test results with risk factors for tuberculosis infection was studied by fitting four multivariable logistic regression models, one for each diagnostic test, with the same covariates, and results were shown as odds ratios (OR) with the associated 95% confidence intervals (CI). Risk factors introduced in the models were age (as a continuous variable), sex and all variables that were significant in the univariable anlaysis for at least one diagnostic test. Whether the association with each risk factor varied by type of diagnostic assay was assessed by testing the hypothesis of homogeneity of the relative odds ratios. The test was performed using seemingly unrelated regression that takes into account the correlation between diagnostic test results of the same participant.

To estimate sensitivity and specificity of different diagnostic tests we performed a latent class analysis [21-23,26] a family of statistical models based on the concept of 'latent variable', that can simply be thought as an unobservable random variables. LCA is appropriate to study situations in which categorical responses are observed on n subjects and these responses are dependent by a categorical unobservable characteristic of the subject. Briefly, parameters of interest were estimated by modelling the relations between an unobservable (latent) and observable variables. In this respect, the observed results of the diagnostic tests are considered as a measure, prone to error, of an unobservable dichotomous latent variable, the true disease status. From these imperfect measures we can estimate a 'consensus' gold standard used, in turn, to evaluate sensitivity and specificity of the tests as well as the prevalence of the disease [22].

Let us assume that D represents the unknown disease status for each subject (1 for diseased and 0 for not diseased) and θ_d (d=0,1) its probability. Moreover let t_j be the observed result of our jth test (j=0,...,p) that can take on the values 0, negative,

or 1, positive. If we denote with π_{jd} the conditional probability of a positive response at the jth test given D=d, the parameters of interest for our study, i.e. the sensitivity and specificity of each test, are π_{j1} and $1-\pi_{j0}$, respectively. Each subject i (i=1,.....,n) will have a vector of observed responses, $T_i=(t_1,\ldots,t_p)$, and the marginal probability of T_i that follows a multivariate Bernoulli distribution is given by

$$Pr(\mathbf{T}_i) = \sum_{d=0}^{1} \theta_d Pr(T_i \mid D = d) \quad (1).$$

Assuming for each subject the independence between responses to the p tests, given the true disease status, equation (1) can be written as:

$$Pr(\mathbf{T}_i) = \sum_{d=0}^{1} \theta_d \prod_{j=1}^{p} \pi_{jd}^{t_j}(1-\pi_{jd})^{1-t_j} \quad (2).$$

Both θ_d and π_{jd} were modelled on a log odds, or logit, scale and we could also account for the effect of covariates using the usual approach of logistic model. The equations describing prevalence and conditional probabilities of positive response were as follows:

$$Logit(\theta_d \mid \mathbf{v}) = \alpha \quad (3) \text{ and}$$

$$Logit(\pi_{jd} \mid \mathbf{x}) = \gamma_j + \boldsymbol{\lambda}_j \eta_d + \mathbf{x}'\boldsymbol{\beta} \quad (4),$$

where:

1. x was a vector of covariates for the ith subject, with their relatives vectors of parameters β;
2. η_d was the (random) effect, common for all tests, exerted by the unknown true disease status;
3. λ_j were the factor loadings that allow the effect of ηd to differ between tests and
4. γ_j represented the (fixed) effect of each test on conditional probability [22,26].

In order to make a latent class model estimable, the number p of diagnostic tests used on the same study sample must provide at least as many degrees of freedom as the number of parameters to be estimated, in other words the condition $(2^p-1)\geq(2p+1)$ has to be satisfied and this imply that at least three tests are requested for our study. Prevalence as well as sensitivity and specificity were modeled as logit (log odds). We included BCG as a covariate in the model for sensitivity and specificity. The fit of the model without covariates was assessed by using the Pearson's chi-squared statistic (the sum of squared difference between observed and expected frequencies over the expected). Nested models were compared using the log-likelihood ratio (LR) test [27-29].

The significance of the difference in accuracy between pairs of diagnostic assays was evaluated by using Wald test for fixed coefficients of the latent class model.

In traditional latent class analysis, it is assumed that the results of each individual for a given disease status are independent (the so-called conditional independence) or, in other words, that the observed associations between tests are explained only by the latent variable. In our study this condition could not be satisfied, regarding the similarities in technological characteristics of assays. To verify whether a lack of conditional independence between tests could have influenced our estimates, we introduced in the equation (4) an additional subject-specific random variable z with Gaussian distribution to take into account the correlation between the assays that was not due to the disease status [27,29]. The results from the traditional latent class analysis were then compared with those from the model with random effect using the Akaike Information Criterion (AIC) and Pearson's statistic.

Statistical analyses were performed with Stata, Release 9 (Stata Corp). The programme "gllamm" in Stata [30] and "randomLCA" package for R [31] were used to fit latent class analysis models.

Results

Study population

Included in the present analysis were 115 healthcare workers. Of these, 39 (33.9%) were currently employed in wards in which the risk of being exposed to tuberculosis was high (such as wards for infectious diseases and respiratory diseases), and 76 (66.1%) were employed in hospital services in which the risk of exposure to tuberculosis was low (such as paediatrics, internal medicine and hospital epidemiology). Of those currently employed in low-risk services, seven had worked in services with high exposure risk in the past. The median age of the participants was 41 years and the majority were female. BCG vaccination was documented for 43 participants (37.4%).

Association of results in the four diagnostic assays with participants characteristics

Overall 61 individuals (53.0%) were TST-positive, 40 (38.4%) were positive by in-house ELISPOT, 42 (36,5%) by T-SPOT.TB and 29 (25,2%) by QFT-G . The results of the different diagnostic assays by participant characteristics are shown in Table 1. A higher proportion of positive tests was observed among those who had at one point been employed in high-risk services, compared to those employed only in other hospital services. This difference was statistically significant for all tests except for the QFT-G test. In addition, older study participants were more likely to be positive in all tests. A positive result in the TST only was associated with a previous BCG vaccination. Physicians had the lowest prevalence of positive results in all tests, but this difference was significant for QFT-G only. Surprisingly, the prevalence of positive results in the three *in vitro* assays was not elevated among those reporting household tuberculosis contact, and differences were not statistically significant.

As shown in Table 2, 40 individuals (34.8%) were negative in all the four tests, while 75 (65.2%) individuals were positive in at least one test. Of those 75, 22 (19.1%) were positive in all the four tests. Nineteen individuals (16.5%) were positive only in the TST.

In a multivariable analysis (Table 3), having worked in high-risk tuberculosis services increased the probability of a positive result for all diagnostic tests (homogeneity test: p=0.52), although the effect was significant only for the T-SPOT.TB and the in-house ELISPOT. Sex was not significantly associated with the probability of a positive result and the odds ratios were not significantly different among diagnostic tests (p=0.41). Older individuals, however, had a significantly higher probability of a positive result for all tests. The effect of BCG vaccination was not homogeneous among diagnostic tests (p=0.001) and significant only for the TST, with a higher odds ratio for a positive result for BCG-vaccinated compared to not vaccinated subjects. Physicians were at a lower risk of a positive result compared to nurse assistants; this result was significant for TST and QFT-G.

Table 1

Results of diagnostic tests for tuberculosis infection by characteristics of healthcare workers in Rome, Italy (n=115)

Characteristic (no.)	Tuberculin skin test no. of positives (%)	In-house RD1 ELISPOT no. of positives (%)	T-SPOT.TB no. of positives (%)	QuantiFERON TB Gold no. of positives (%)
Ward/service				
Low TB risk (69)	30 (44)	17 (25)	18 (26)	16 (23)
High TB risk* (46)	31 (67) †	23 (50) †	24 (52) †	13 (28)
Sex				
Male (48)	22 (46)	17 (35)	19 (40)	11 (29)
Female(67)	35 (52)	23 (34)	23 (34)	18 (27)
Place of birth				
EU (110)	57 (53)	38 (35)	40 (37)	26 (24)
Non-EU (5)	3 (60)	1 (20)	1 (20)	2 (40)
BCG vaccination				
No (72)	30 (42)	26 (36)	24 (33)	22 (31)
Yes (43)	31 (72) †	14 (32)	18 (42)	7 (16)
Household TB contact				
No (102)	53 (52)	37 (36)	40 (39)	27 (27)
Yes (13)	8 (62)	3 (23)	2 (15)	2 (15)
Job category				
Physician (18)	6 (33)	4 (22)	6(33)	1 (5.6)
Nurses (67)	40 (60)	24 (36)	23 (34)	16 (24)
Nurse assistant (30)	15 (50)	12 (40)	13 (43)	12 (40) †
Age (years)				
≤41 (59)	41 (36)	11 (19)	12 (20)	8 (14)
>41 (56)	40 (71) †	29 (52) †	30 (54) †	21 (38) †

BCG: Bacillus Calmette-Guérin; EU: European Union; TB: tuberculosis.
* currently or in the past
† p<0,05

Table 2

Response patterns to four different diagnostic tests for tuberculosis infection observed among healthcare workers in Rome, Italy, and predicted by a latent class analysis model with and without a random effect (n=115)

Response pattern				Observed		Predicted LCA	Predicted LCA with random effect
Tuberculin Skin test	In-house RD1 ELISPOT	T-SPOT.TB	QuantiFERON TB Gold	No.	%	No.	No.
-	-	-	-	40	34.8	37.8	39.9
+	+	+	+	22	19.1	21.8	21.9
+	-	-	-	19	16.5	21.1	19.4
+	+	+	-	7	6.1	7.3	7.1
+	-	+	-	7	6.1	3.9	4.7
-	+	-	-	5	4.3	5.4	4.6
-	-	-	+	4	3.5	2.6	2.1
+	+	-	-	3	2.6	3.2	3.9
-	-	+	-	3	2.6	6.4	5.3
-	+	+	-	2	1.7	0.9	1.0
+	+	-	+	1	0.9	1.0	0.9
+	-	+	+	1	0.9	1.3	1.3
+	-	-	+	1	0.9	1.5	1.8
-	+	+	+	0	0.0	0.1	0.2
-	+	-	+	0	0.0	0.4	0.3
-	-	+	+	0	0.0	0.4	0.5

LCA: latent class analysis.

Estimation of the accuracy of the assays by latent class analysis

The tuberculosis infection prevalence in the population estimated in the latent class analysis model was 26.9% (95% CI: 18.1% to 35.7%). The predicted frequencies for the patterns of response to the four tests (Table 2) showed a good fit with the observed data (Pearson's statistic p-value=0.25).

In the latent class analysis (Table 4), TST had the highest estimated sensitivity but a very low specificity. The two ELISPOT-based tests, the in-house ELISPOT and the T-SPOT.TB, both had a sensitivity close to that of the TST, while their estimated specificity was still high. QFT-G had a very high estimated specificity, although its sensitivity was lower than that of the other three tests. When

Table 3

Multivariable odds ratios (95% confidence intervals) of a positive result for selected risk factors by diagnostic test among healthcare workers in Rome, Italy (n=115)

	Diagnostic test assumed as outcome variable				
	Tuberculin Skin test	In-house RD1 ELISPOT	T-SPOT.TB	QuantiFERON TB Gold	
	MOR# (95% CI)	MOR# (95% CI)	MOR# (95% CI)	MOR# (95% CI)	p*
Ward/service					
Low TB risk	1.00	1.00	1.00	1.00	
High TB risk	2.48 (0.97-6.35)	3.88 (1.52-9.91)	3.10 (1.28-7.48)	1.68 (0.63-4.49)	0.519
p**		0.472	0.681	0.491	
BCG Vaccination					
No	1.00	1.00	1.00	1.00	
Yes	4.32 (1.56-11.95)	0.62 (0.23-1.67)	1.49 (0.58-3.81)	0.41 (0.14-1.23)	0.001
p**		0.001	0.060	<0.001	
Gender					
Male	1.00	1.00	1.00	1.00	
Female	2.13 (0.73-6.21)	1.23 (0.46-3.26)	1.28 (0.50-3.26)	0.82 (0.29-2.31)	0.413
p**		0.449	0.401	0.107	
Age (per five years increase)	1.86 (1.39-2.48)	1.69 (1.29-2.22)	1.56 (1.21-2.02)	1.50 (1.16-1.95)	0.485
p**		0.599	0.231	0.215	
Job category					
Physician	0.20 (0.04-0.92)	0.25 (0.05-1.23)	0.39 (0.09-1.63)	0.07 (0.01-0.70)	0.480
p**		0.758	0.393	0.377	
Nurses	1.64 (0.49-5.51)	1.21 (0.38-3.87)	0.67 (0.22-2.04)	0.63 (0.21-1.91)	0.211
p**		0.721	0.159	0.156	
Nurse assistant	1.00	1.00	1.00	1.00	

BCG: Bacillus Calmette-Guérin; CI: Confidence Interval; MOR: multivariable odds ratio. TB: tuberculosis.
Adjusted for all the variables in the table by fitting a logistic regression model.
* p-value for the hypothesis of no difference among OR, obtained by fitting a seemingly unrelated regression model.
**p-value for the hypothesis of no difference to the OR for tuberculin skin test, obtained by fitting a seemingly unrelated regression model.

Table 4

Specificity and sensitivity of four diagnostic assays for tuberculosis infection estimated among 115 healthcare workers in Rome, Italy by a latent class analysis model

	Specificity [%]			Sensitivity [%]		
	Estimate	95% confidence interval		Estimate	95% confidence interval	
Tuberculin skin test	64.2	53.0	74.1	99.9	NC	NC
In-house RD1 ELISPOT	87.5	78.0	93.2	95.3	61.8	99.6
T-SPOT.TB	85.6	75.3	92.0	96.7	69.3	99.7
QuantiFERON TB Gold	93.6	85.4	97.3	76.3	55.9	89.1

NC: not computable.

the tests were compared in pairs to evaluate differences in their diagnostic accuracy, statistically significant differences were recorded for the comparison between TST and the other three tests (p=0.003, p=0.005 and p<0.001, respectively, for the comparison with in-house ELISPOT, T-SPOT.TB and QFT-G), while the difference between the T-SPOT.TB and QFT-G was of borderline statistical significance (p=0.057).

To explore the impact of BCG vaccination on the diagnostic accuracy of the TST, we also fitted a latent class analysis models solely for those subjects who had not been vaccinated against BCG. In this analysis, the estimated prevalence of tuberculosis infection was 26.3%. As shown in Table 5, the sensitivity of the TST was similar to that estimated for the entire population. In contrast, an increased specificity was estimated for TST among not BCG-vaccinated subjects (79.1%), although it remained lower than that estimated for the *in vitro* assays. The estimated accuracy of IGRAs did not vary markedly in this analysis, except for QFT-G sensitivity which increased from 76.3 to 94.8.

Finally, we compared the traditional latent class analysis model to a model with a subject-specific random effect in order to assess whether the removal of conditional independence assumption among tests had an impact on the results. The estimate of tuberculosis infection prevalence in the latter model was 25.0%, and the predicted frequencies for the patterns of response to the four tests were similar to the former model with a slight worsening of the AIC (476.97 and 477.77 in the latent class analysis and the model with subject-specific random effect, respectively), and an equally slight improvement in Pearson's statistic (p=0.267). The estimates of diagnostic accuracy were remarkably similar in the two models (Table 6).

Discussion

We compared the results obtained in the TST and three *in vitro* assays for tuberculosis infection in healthcare workers. We found that positive results in all four assays were associated with increased occupational risk of exposure to *M. tuberculosis*, but only the TST was correlated with BCG vaccination. Taking advantage of the fact that the results of four different assays for tuberculosis infection were available for the same groups of individuals, we provided an estimate of the diagnostic accuracy of these assays by using a latent class analysis model. In this analysis, the *in vitro* tests were found to be more specific for tuberculosis infection than the TST, even among non-vaccinated individuals, at the cost of some sensitivity. Moreover, our data suggest that ELISPOT-based tests may differ in accuracy from the ELISA-based test.

Previous studies conducted among healthcare workers in countries with low and high tuberculosis incidence [14-17] have shown an association between QFT-G results and occupational exposure to patients with active tuberculosis. Our results are consistent with these findings and show an even stronger association with occupational exposure for ELISPOT-based assays, although no statistically significant differences were recorded when association coefficients for the four different tests were compared. Moreover, as in previous studies [32,33], we found that TST results were associated with previous vaccination, while this was not the case for *in vitro* assays.

We also used latent class analysis to estimate and compare the sensitivity and specificity of different tests for tuberculosis infection. Latent class analysis allows addressing a major issue in the evaluation of diagnostic tests, i.e. the estimation of diagnostic accuracy when a gold standard test is not available, and for this reason it has been used in different infectious conditions in which a

Table 5

Comparison of specificity and sensitivity of four diagnostic assays for tuberculosis infection estimated among 72 not BCG-vaccinated healthcare workers by a latent class analysis model

	Specificity %			Sensitivity %		
	Estimate	95% confidence interval		Estimate	95% confidence interval	
Tuberculin skin test	79.1	65.9	88.1	100.0	N.C.	N.C.
In-house RD1 ELISPOT	84.6	72.2	92.1	94.4	65.8	99.3
T-SPOT.TB	90.4	78.4	96.1	100.0	N.C.	N.C.
QuantiFERON TB Gold	92.3	81.3	97.1	94.8	63.1	99.5

NC: not computable.

Table 6

Comparison of specificity and sensitivity of four diagnostic assays for tuberculosis infection estimated among 115 healthcare workers by a latent class analysis model with and without a subject-specific random effect

	Specificity %		Sensitivity %	
	LCA	LCA with random effect	LCA	LCA with random effect
Tuberculin skin test	64.2	64.4	99.9	100.0
In-house RD1 ELISPOT	87.5	88.5	95.3	97.5
T-SPOT.TB	85.6	86.9	96.7	98.8
QuantiFERON TB Gold	93.6	94.3	76.3	81.4

LCA: latent class analysis.

definitive demonstration of the infecting organism was not feasible [22].

As reported in a recently published systematic review, the sensitivity of IGRAs for tuberculosis infection has previously been estimated in a number of studies by calculating the proportion of positive patients among those diagnosed with culture-proven tuberculosis [32]. The sensitivity in these studies ranged from 55% to 93% for QFT-G with a pooled estimate of 78% for the first version of the QFT-G or 70% for the in tube version of this assay, and from 83 to 100% for T-Spot.TB with a pooled estimate of 90%. In the studies in which both IGRAs were performed on the same group of patients, the positivity rate tended to be higher for the ELISPOT assay. Our estimates of the sensitivity of interferon gamma tests for latent infection, obtained by latent class analysis, were above 95% for ELISPOT-based assays and 76.3% for the ELISA assay, thus consistent with those obtained from patients with active tuberculosis. Nevertheless, the TST had the highest estimated sensitivity (99.9%) in our study, which is in contrast to the results of studies on patients with active tuberculosis, most of which reported a higher sensitivity for interferon gamma assays compared to the TST [34]. However, there is evidence that estimates of sensitivity of TST for active infection may differ from that for latent infection: On average 10 to 25% of patients with active TB do not respond to the TST, and reactivity may be restored after initiation of treatment in most of the patients who were initially negative [35]. In contrast, sensitivity estimates derived from studies on healthy individuals may exceed 95% [36]. Moreover, some studies conducted to assess the accuracy of diagnosis of latent tuberculosis infection suggest that the sensitivity of interferon gamma tests may indeed be somewhat lower than or equal to that of the TST [33,37,38]. On the other hand, in a recent study carried out among healthcare workers in India, in which a Bayesian latent class analysis was used to compare accuracy of QFT-G and TST, Pai *et al.* estimated that the QFT-G had an higher sensitivity than the TST (89.9% and 79.5 %, respectively) [39]. The results reported by Pai *et al.* are not directly comparable to those of the present study since a different statistical approach was used to construct the latent class model and results from only two different tests were available for each subject. Moreover, the subjects in the two studies were enrolled in countries with very different tuberculosis incidence.

In this study, specificity was estimated to be consistently higher for IGRAs compared to the TST. This finding was not unexpected since these *in vitro* assays are based on antigens that, differently from the PPD antigens used in the TST, are present almost exclusively in bacteria of the *M. tuberculosis* complex. Previous studies included in the aforementioned systematic review [34] have shown that, among individuals at low risk for tuberculosis infection, QFT-G is negative in 92-98% of cases (estimated pooled specificity 99% and 96% in BCG-vaccinated and non-vaccinated individuals, respectively), and T-SPOT.TB in 85-100% of cases (estimated pooled specificity 93%). These figures are consistent with specificity values estimated for IGRAs in our study. Moreover, there is indirect evidence that these tests have higher specificity for latent tuberculosis infection than the TST. It has in fact been shown that, when used in contact tracing studies, these tests yield a better correlation to the degree of exposure to tuberculosis cases than the TST, and that their results are not influenced by the BCG vaccination status [32,33,37]. The specificity of the TST estimated in our study was quite low. It has been shown that large variations in the specificity of the TST can be observed when the test is applied to different populations [38], and in our study, the high prevalence of previous BCG vaccination among healthcare workers may be one cause of low specificity. However, TST specificity was estimated to be low also among non-vaccinated healthcare workers. A similar finding has been reported for healthcare workers in the United States, and it has been attributed to infection with non-tuberculous mycobacteria [40]. In contrast, a higher value for the specificity of the TST (87.4%) resulted from the application of a Bayesian latent class model in spite of the fact that 71% of subjects were BCG-vaccinated [39].

The statistical model we used also allowed an overall comparison of diagnostic accuracy of the tests analysed. We found that the diagnostic accuracy of the TST was significantly different from that of blood tests. This finding is not surprising if it is considered, in addition to the higher specificity of the antigens used, that the *in vitro* tests avoid a series of operational problems that may affect the accuracy of the TST, including variability in the intradermal injection of the antigen and in the reading of the response [8].

When the three *in vitro* tests were compared, we found a difference of borderline significance between QFT-G and T-SPOT.TB. The reasons for this difference are unclear. One may speculate that the ELISPOT technique, thanks to the ability to detect single cells that secrete interferon gamma in response to specific stimuli, may provide a higher sensitivity at the cost of some specificity. The cut-off value used to define positivity could also account for differences in sensitivity and specificity, at least in part. In fact, a study in which the commercial T-SPOT.TB and ELISA were used, has shown that the differences in diagnostic accuracy between the two tests become negligible when new cut-off points are used that have been optimised on the same population [41].

Before drawing firm conclusions, it is important to appreciate the limitations of the statistical method we used [21,22]. Latent class analysis assumes the existence of a 'true disease status' which influences the results of diagnostic tests, and this mathematically defined entity does not necessarily have a clear clinical or biological sense. There is consistent evidence that the TST predicts the development of active tuberculosis [6]. Thus the presence of latent tuberculosis infection, as identified by a positive TST, is associated with an increased risk of active disease. It remains to be determined if the same meaning could be attributed to the random variable identified as 'latent tuberculosis infection' in the present analysis.

Another drawback of the traditional version of latent class analysis is the assumption of conditional independence, i.e. the absence of correlation among test results given the disease status. This is often unrealistic in practice due to similarities among tests. However, following the approach proposed by Qu *et al.* [27] to relax this assumption, we used an additional random effect, with which it is possible to model all the non-observable factors at the subject level that could introduce correlation between test results. The estimates of diagnostic accuracy for the model with subject-specific random effect were very similar to those obtained in the traditional latent class analysis, and the measures of goodness of fit were comparable in the two models as well.

Other limitations of the present study need to be mentioned. First, all the individuals included were healthy adults, and thus our results should not be generalised for different populations, in particular for children or immunocompromised individuals in whom a significant proportion of indeterminate results may be observed,

in particular when using ELISA-based assays [40]. Similarly, the diagnostic accuracy estimated for latent tuberculosis infection is not necessarily similar to that obtained when using these tests to diagnose active tuberculosis infection. Second, tuberculin skin tests have been administered and read by different trained nurses, and thus inter-reader variability in interpreting the results should be expected. Third, the confidence intervals around our estimates of association coefficients and of sensitivity and specificity were rather wide because of the limited size of the population studied. Nevertheless, we were able to demonstrate statistically significant differences in the diagnostic accuracy of the different tests used.

Longitudinal studies comparing the ability of the TST to predict the risk of active tuberculosis with that of interferon gamma assays would be needed to establish the usefulness of the new tests for tuberculosis infection. Preliminary data suggest that positive IGRAs results may indeed be associated with the risk of active tuberculosis [42]. However, these studies will be difficult to perform in populations such as healthcare workers. In this context, the present study provides further evidence on the advantages in terms of specificity, and on the potential loss of sensitivity for latent tuberculosis infection of blood tests in comparison to the TST. Moreover, it provides comparative estimates of diagnostic accuracy of different blood tests and thus may contribute to choosing the strategies for diagnosing tuberculosis infection among heath careworkers. In particular, our results may suggest the use of IGRAs, either alone or as confirmatory tests in TST-positive individuals, in a population with a high prevalence of previous BCG vaccination. These choices, however, will also need to take other considerations into account, including the economical and operational aspect, and the stability of test results over time [43].

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"I-MOVE" towards monitoring seasonal and pandemic influenza vaccine effectiveness: lessons learnt from a pilot multi-centric case-control study in Europe, 2008-9

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Within I-MOVE (European programme to monitor seasonal and pandemic influenza vaccine effectiveness (IVE)) five countries conducted IVE pilot case-control studies in 2008-9. One hundred and sixty sentinel general practitioners (GP) swabbed all elderly consulting for influenza-like illness (ILI). Influenza confirmed cases were compared to influenza negative controls. We conducted a pooled analysis to obtain a summary IVE in the age group of ≥65 years. We measured IVE in each study and assessed heterogeneity between studies qualitatively and using the I2 index. We used a one-stage pooled model with study as a fixed effect. We adjusted estimates for age-group, sex, chronic diseases, smoking, functional status, previous influenza vaccinations and previous hospitalisations. The pooled analysis included 138 cases and 189 test-negative controls. There was no statistical heterogeneity (I2=0) between studies but ILI case definition, previous hospitalisations and functional status were slightly different. The adjusted IVE was 59.1% (95% CI: 15.3-80.3%). IVE was 65.4% (95% CI: 15.6-85.8%) in the 65-74, 59.6% (95% CI: -72.6 -90.6%) in the age group of ≥75 and 56.4% (95% CI: -0.2-81.3%) for A(H3). Pooled analysis is feasible among European studies. The variables definitions need further standardisation. Larger sample sizes are needed to achieve greater precision for subgroup analysis. For 2009-10, I-MOVE will extend the study to obtain early IVE estimates in groups targeted for pandemic H1N1 influenza vaccination.

Introduction

The influenza virus has a high genetic mutation rate that frequently determines antigenic drifts and occasionally antigenic shifts. To achieve a good match between circulating and vaccine viruses, the composition of the vaccine has to be reformulated each season based on the recommendations of the World Health Organization (WHO) Global Influenza Surveillance Network [1]. Therefore, influenza vaccine effectiveness (IVE) can vary from year to year according to the degree of match between the selected vaccine strains and those actually circulating. Hence, IVE should be measured and monitored every year. In a pandemic situation, strain specific vaccines become available only four to six months after beginning the development of the vaccine. Consequently, when the vaccines start to be administered, the virus is already circulating and IVE results are needed rapidly. In addition, vaccine availability is likely to increase over time according to the speed of vaccine production and the licensing of additional vaccines, meaning that IVE measurements need to be repeated over time during the pandemic.

Many factors affect IVE in observational studies. IVE estimates vary according to the specificity of the outcome, the influenza incidence, the population targeted for vaccination and the confounding factors taken into account. Many of the case-control studies reported in the literature measured IVE against clinical outcomes (i.e. hospitalisations for pneumonia or influenza, acute respiratory infections, influenza-like illness (ILI)). Clinical outcomes for influenza are non-specific and likely to underestimate the IVE [2]. To minimise bias, laboratory-confirmed influenza is now being used as outcome in case-control studies in Canada, Australia and the USA [3-5].

Confounding affects IVE observational studies. IVE is underestimated when individuals at higher risk of acquiring influenza are more likely to be vaccinated than individuals at lower risk (negative confounding by indication) [6,7]. IVE is overestimated if individuals more cautious about their health and at lower risk of acquiring influenza are more likely to be vaccinated (positive confounding due to healthy vaccinee effect) [7,8].

In general practitioners (GP) based case-control studies, individuals who use health services more often are more likely to be vaccinated and more likely to consult their GP with influenza symptoms. Vaccinated individuals with influenza symptoms will have a higher probability of being included in the study than vaccinated individuals with no influenza symptoms. This would underestimate the IVE. To control for health seeking behaviour, recent studies suggested comparing individuals who consult for ILI and are influenza positive to individuals consulting for ILI who test negative for influenza (test-negative controls) [3-5;9]. The assumption is that test-negative controls have the same vaccination coverage as the source population giving rise to the influenza cases detected at the GP practice.

I-MOVE started in 2007 with the aim to measure IVE against seasonal and pandemic influenza in the European Union (EU) and the European Economic Area (EEA). Two cohort and five case-control studies to measure IVE were piloted in the 2008-9 season. In order to develop a sustainable system, the studies were conducted in the framework of existing GP-based influenza sentinel surveillance systems. All the country teams conducting I-MOVE pilot studies are members of the European Influenza Surveillance Network (EISN) (the successor of the Commission-funded network, EISS). EISN collects and exchanges timely information on influenza activity in Europe [11]. National Reference Laboratories participating in EISN are evaluated periodically through external inter-laboratory quality control assessments. All the EU Member States recommend seasonal vaccine for the elderly either defined as 65 years old and older or as 60 years old and older [12].

In the pilot case-control studies, we measured IVE against laboratory-confirmed influenza and collected variables to control for positive and negative confounding in the analysis. We restricted the study population to community-dwelling elderly. To increase the precision of the estimates and to provide a summary IVE for the five studies, we explored the feasibility of conducting a pooled analysis. We present here the pooled results of the pilot case-control studies conducted in Denmark, Hungary, Portugal, Romania, and Spain. We assumed that if the pooled case-control design was feasible for seasonal vaccine, the study population could later be expanded to include the age groups targeted for the pandemic vaccine.

Methods

The study population consisted of community-dwelling elderly living in selected sentinel GP practice catchment areas in the five participating European countries. Age groups included were 60 year-olds and older in Hungary and 65 year–olds and older in the other four countries. Participating sentinel GPs swabbed all community-dwelling elderly individuals consulting for ILI during 2008-9 influenza season.

For the first time, in Denmark, Hungary, and Romania sentinel GPs used the EU ILI case definition [13]. In Spain, the ILI EU case definition was used with an additional stated criterion "without any other suspected diagnosis". In Portugal, ILI was defined as in the routine sentinel surveillance, according to GPs´ criteria. Clinical symptoms were collected for all ILI cases.

ILI patients were not eligible for the study if they were institutionalised, had evidence of dementia, did not speak the local language or refused participation.

A case of influenza was defined as an ILI patient who was swabbed and tested positive for influenza using real-time polymerase chain reaction (RT-PCR) or culture. Test-negative controls included in the five studies were ILI patients who were swabbed and tested negative for influenza.

To check if vaccination coverage observed among ILI patients testing negative for influenza was different from that observed in other potential control groups, we measured vaccination coverage among systematic samples of patients from participating GPs who had not had ILI since the beginning of the influenza season (non-ILI controls; up to two controls selected around the time of occurrence of a case) (Hungary, Portugal, Spain), in the community (Denmark, Portugal) and in the participating GPs' catchment area (Hungary, Romania, Spain).

A person was considered vaccinated if s/he had received the 2008-9 influenza vaccine more than 14 days before date of onset of ILI symptoms or of selection as a control.

The minimum set of common confounding variables for the five countries included age, sex, presence of chronic conditions and their respective severity measured in number of hospitalisations for the chronic diseases in the previous 12 months or any hospitalisation in the previous 12 months (Hungary and Portugal), smoking history (none, past, current smoker), functional status (help for bathing and/or help for walking), and influenza vaccination in the previous two seasons.

All ILI patients had a nasal or throat swab taken, which was tested for influenza at the respective countries' National Influenza Reference Laboratory (in Spain, all laboratories integrated in the Spanish Influenza Sentinel Surveillance System) using RT-PCR techniques and/or culture. In each country, all or a subset of influenza isolates were antigenically characterised. Laboratory viral detection, typing, subtyping and variant analysis performed in each of the National Reference Laboratories are described elsewhere [14].

The sentinel GPs carried out face-to-face interviews with ILI patients and non-ILI control patients using country-specific standardised questionnaires. Trained interviewers conducted telephone interviews with community controls using a standardised questionnaire in Denmark and Portugal. Each country study team entered and validated data.

A previously agreed minimum dataset for pooling, including information on case or control status and exposure status and several covariates, was sent to EpiConcept, the I-MOVE coordination focal point. EpiConcept checked the data again for inconsistencies, outliers and logical errors and conducted the pooled analysis.

We created a common restricted dataset of ILI patients meeting the EU case definition, older than 64 years and with a delay between onset of symptoms and swabbing of less than eight days. For each of the country specific datasets, we excluded the controls identified before the week of the first case and after the week of the last case, in order to include only ILI cases within the influenza season.

IVE estimates were obtained using the formula: 1- odds ratio, with 95% exact confidence intervals (CI) [10,15].

We computed study specific crude IVE and adjusted for the pre-defined set of confounders (including age, sex, chronic disease, smoking, previous influenza vaccination and functional status) where possible, using logistic regression. We evaluated heterogeneity between studies qualitatively by assessing the standardisation of the case and covariate definitions. We evaluated statistical heterogeneity using the Q-test and the I2 index [16,17]. To estimate a pooled IVE, we used a one-stage method with study as fixed effect in the model. Results were stratified according to influenza strain and two age groups: 65-74 and >74 years.

According to country specific requirements for ethical approval, all participants provided oral or written consent.

Figure 1

Influenza-like illness (ILI) incidence (cases per 100,000 population) reported by the national influenza sentinel surveillance systems in Denmark, Hungary, Portugal, Romania, and Spain, influenza season 2008-9*

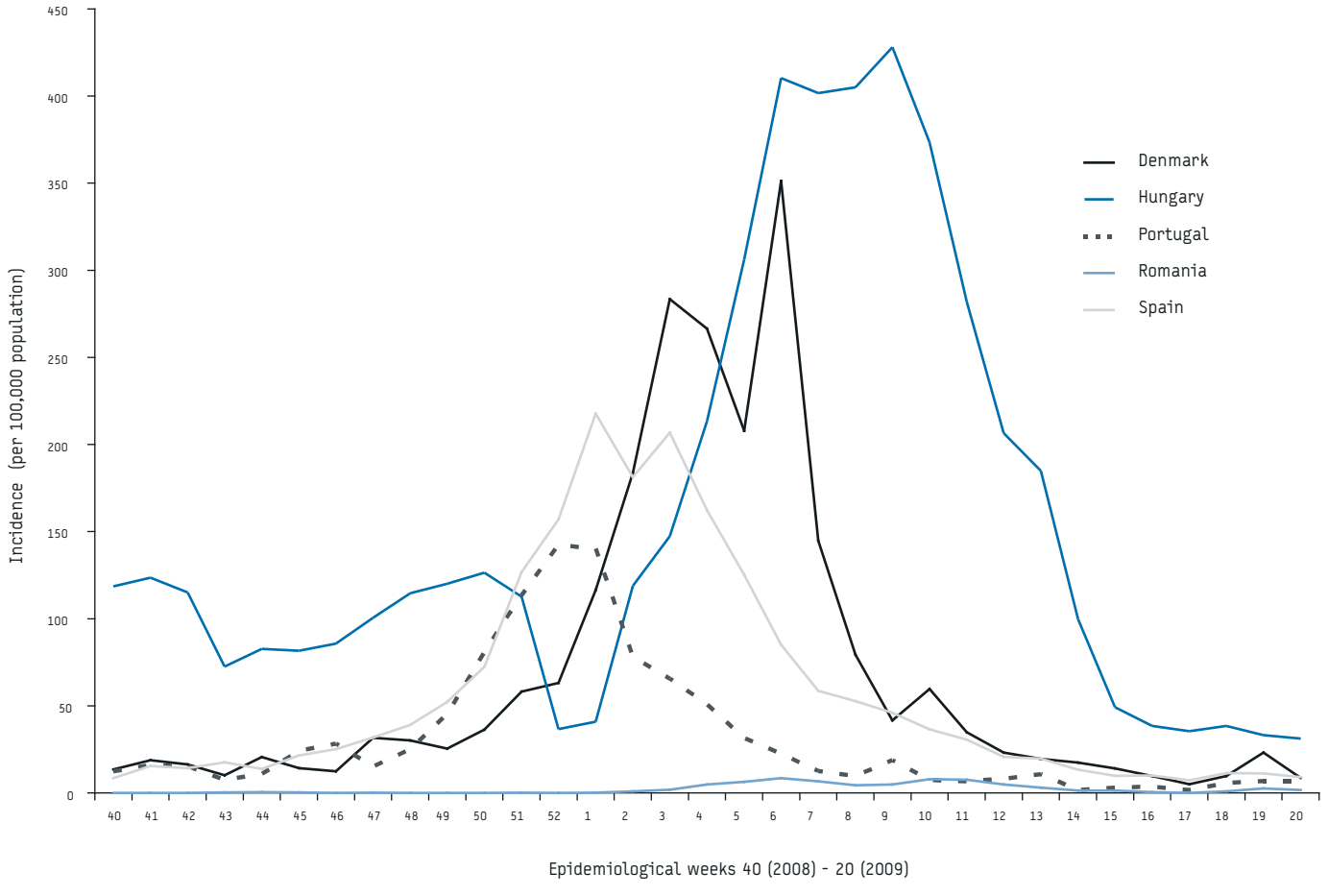

Table 1

General practitioner (GP) participation and influenza-like illness (ILI) cases recruitment by study, Denmark, Hungary, Portugal, Romania, and Spain, influenza season 2008-9

Study	Number of GPs accepting to participate	Number of GPs recruiting at least one ILI patient (%)	Number of ILI patients recruited by GPs	Number of ILI patients positive to influenza (%)	Number of non-ILI GP patients	Number of community controls
Denmark	40	29 (73)	63	25 (40)	N/A	80
Hungary	50	27 (54)	144	45 (32)	89	N/A
Portugal	42	9 (21)	42	15 (36)	40	136
Romania	47	28 (60)	103	30 (29)	N/A	N/A
Spain	164	67 (40)	103	44 (43)	88	N/A
Total	**343**	**160 (47)**	**455**	**159 (35)**	**217**	**216**

Figure 2

Diagramme with study exclusion criteria, Denmark, Hungary, Portugal, Romania, and Spain, influenza season 2008-9

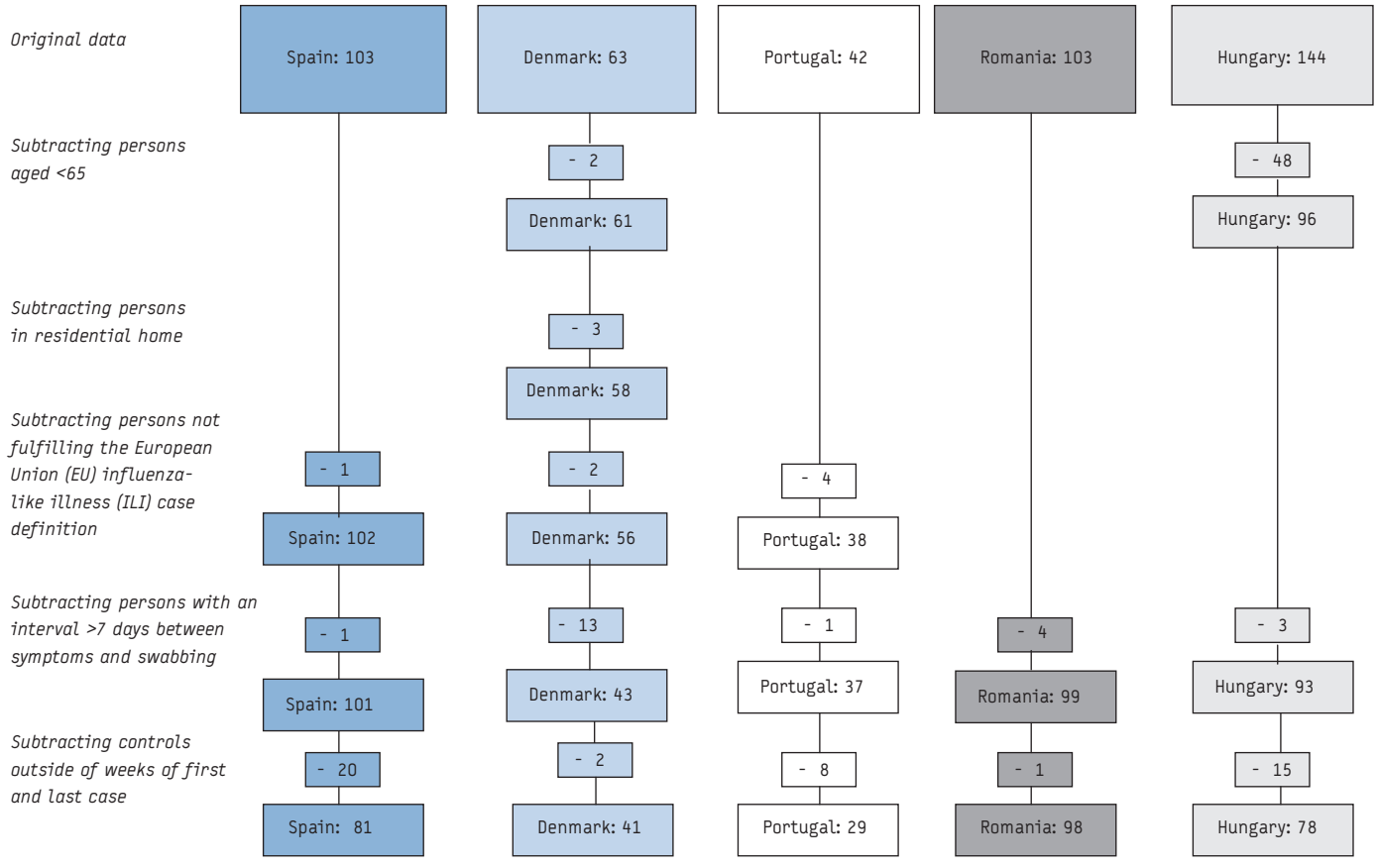

Table 2

Influenza cases and test-negative controls by study and characteristic, Denmark, Hungary, Portugal, Romania, and Spain, influenza season 2008-9

Characteristics	Country	ILI patients		p
		Influenza cases	Test-negative controls	
Interval from symptom onset to swab sample collection (mean in days)	Denmark	3.05	4.25	0.001*
	Hungary	2.1	3.08	0.036*
	Portugal	1.5	2.33	0.030*
Mean age	Portugal	70	75	0.040*
		Number with characteristic / total	Number with characteristic / total	
Any influenza vaccination in past 2 seasons	Portugal	4/14	10/14	0.023**
	Spain	28/43	33/36	0.005**
Fever	Denmark	19/20	14/21	0.022**
	Romania	30/30	57/68	0.019**
Cough	Denmark	20/20	16/21	0.020**
Chronic pulmonary disease	Romania	1/30	13/68	0.040**

*Mann-Whitney U test, **Chi square

Results

In the participating pilot countries, the 2008-9 seasonal influenza epidemic started in Portugal at the end of 2008 (epidemiological week 49) and spread to the east of Europe (Hungary) in spring 2009 (week 4) (Figure 1).

The duration of the epidemic period ranged from seven weeks in Denmark to 13 weeks in Romania. The influenza peaks were reached between week 52 in 2008 (Portugal) and week 10 in 2009 (Romania).

In the five participating countries, the population was vaccinated with a trivalent inactivated influenza vaccine. In the 2008-9 influenza season, different vaccine brands were used in each of the countries. The number of GPs enrolled in each of the studies ranged from 40 in Denmark to 164 in Spain. Overall, 160 GPs recruited at least one patient ranging from 21% in Portugal to 73% in Denmark (Table 1). GPs swabbed and interviewed a total of 455 ILI patients. Among them, 159 (35%) were positive for influenza (from 29% in Romania to 43% in Spain). The completeness of the variables in the returned questionnaires varied from 85% to 100%.

Among 147 isolates typed before the restriction criteria were applied, 131 (89%) were influenza A and 16 (11%) B. Ninety-five of the A isolates were H3N2. All H3N2 strains genetically characterised were A/Brisbane/10/07 similar to the H3N2 vaccine component of the 2008-9 northern hemisphere vaccine. The B strain included in the 2008-9 vaccine did not match the circulating strain. Eight out of the 16 type B isolates were from cases enrolled in Hungary.

After applying the study restriction criteria we included 138 cases and 189 test-negative controls in the analysis (Figure 2).

Table 3

Vaccination coverage for the seasonal 2008-9 influenza vaccine by control group and country study, Denmark, Hungary, Portugal, Romania, and Spain, 2008-9

Study	Vaccine coverage (%) in ILI positive cases	Vaccine coverage (%) in test-negative controls	Vaccine coverage (%) in non-ILI GP patients	Vaccine coverage in community controls	Vaccine coverage in participating GPs catchment area
Denmark	55	71.4	N/A	53.6**	N/A
Hungary***	41.9	48.7	42.7	N/A	38.5
Portugal	42.9	53.3	70	54.4*	N/A
Romania	46.7	67.6	N/A	N/A	86.9
Spain	61.4	89.2	80.7	N/A	65.3

N/A : not applicable
*Community controls sample selected for national telephone survey (Lisboa: Instituto Nacional de Saúde Dr. Ricardo Jorge. Observatório Nacional de Saúde)
** Community controls randomly selected from the Danish population register
*** Results apply to ages 65 years and above, apart from Hungary where the study was carried out for 60 year-olds and older

Table 4

Country specific and pooled crude and adjusted vaccine effectiveness (VE), Denmark, Hungary, Portugal, Romania, and Spain, influenza season 2008-9

		Crude analysis				Adjusted analysis		Variables used for adjustment
		N	VE	95% CI	N	VE	95% CI	
Country specific estimates	Spain	81	80.8	36.0 - 94.2	76	82.9	30.6 - 95.8	age, sex, chronic disease, smoking, functional status
	Portugal	29	34.4	-184.3 - 84.9	28	82.3	-70.5 - 98.2	age, sex, chronic disease, smoking
	Denmark	41	51.1	-78.2 - 86.6	34	90.9	-43 - 99.4	age, sex, chronic disease, smoking, previous influenza vaccination
	Romania	98	58.2	-0.8 - 82.6	92	86.8	38.0 - 97.2	age, sex, chronic disease, smoking, previous influenza vaccination
	Hungary	78	28.6	-78.6 - 71.5	72	43.6	-119.8 - 85.6	age, sex, chronic disease, smoking, previous influenza vaccination
Pooled estimates	65+	327	55.1	27.8 - 72.1	292	59.1	15.3 – 80.3	study, age, sex, chronic disease, smoking, previous influenza vaccination, functional status, previous hospitalisation
	65-74 years				196	65.4	15.6 - 85.8	study, sex, chronic disease, smoking, previous influenza flu vaccination, functional status, previous hospitalisation
	75+ years				96	59.6	-72.6 - 90.6	study, sex, chronic disease, smoking, previous influenza vaccination, functional status, previous hospitalisation
	A(H3) strain				259	56.4	-0.2 - 81.0	study, age, sex, chronic disease, smoking, previous influenza vaccination, functional status, previous hospitalisation

In Romania and Denmark, the proportion of ILI patients presenting with fever was higher among cases than among test-negative controls (Table 2). In Denmark, all of the cases and three quarters of the controls had a cough (p=0.02). In Romania, the proportion of ILI patients with pulmonary chronic disease was lower among cases than among controls (3% vs. 19%).

The mean delay between onset of symptoms and swab collection was shorter for cases than for test-negative controls in Portugal, Denmark and Romania (Table 2). In Spain and Portugal, the proportion of people having received influenza vaccines in at least one of the two previous seasons was lower among cases than among test-negative controls.

Vaccination coverage among controls varied according to country and control group; no specific pattern was identified (Table 3).

The country specific adjusted VE estimates ranged from 43.6% (95% CI: -119.8 - 85.6) in Hungary to 90.9% (95% CI: -42.6 - 99.4) in Denmark (Table 4).

In terms of heterogeneity between studies, two out of the five studies used a different ILI definition. Three variables (number of hospitalisations, presence of chronic diseases and functional status) were collected differently in the five studies. The Q test for heterogeneity was 2.87 (p = 0.579) and the I2 index was 0%.

In the pooled analysis the crude IVE was 55.1% (95% CI: 27.8-72.1%). The IVE adjusted for study, age, sex, presence of chronic conditions, previous hospitalisations, smoking history, functional status, and previous influenza vaccination was 59.1% (95% CI: 15.3-80.3%) (Table 4).

The adjusted IVE was 65.4% (95% CI: 15.6-85.8%) in the 65-74 year-olds and 59.6% (95% CI: -72.6 -90.6%) in the age-group of ≥75 years. The adjusted IVE against the A(H3) strain was 56.4% (95% CI: -0.2-81.0%).

Discussion

We estimated influenza VE against laboratory-confirmed medically attended influenza using test-negative controls, within existing sentinel GP networks in five EU countries. The country specific and the pooled IVE estimates suggest a protective effect of the 2008-9 seasonal vaccine in the elderly population in a year with a good match between the seasonal vaccine and the A(H3) strain predominantly circulating in Europe [18]. However, the estimates have wide confidence intervals.

The case-control design using test-negative controls was performed easily in the framework of the established GP sentinel surveillance networks. Participating GPs had previous experience in collecting swabs and in completing a form for each patient swabbed. Among the GPs who accepted to participate in the study, less than half interviewed and swabbed ILI patients. This may be explained by the overall low incidence of ILI in the elderly in 2008-9 [18] rather than a low acceptability of GPs, as swabbing and interviewing ILI patients is a simple way of recruiting cases and test- negative controls. The questionnaires used for data collection were short leading to a high completeness of all variables. At the end of the season, the study coordinators in Denmark, Romania, and Spain interviewed GPs who participated in the 2008-9 study. Most of them (95% in Spain, 78% in Romania, 74% in Denmark) would be willing to participate in the study in 2009-10 (data not shown). In 2006 in Denmark (one of the current study sites), Mazick *et al.* showed similar acceptability results following an influenza VE case-control study based on the sentinel GP network [19].

The recruitment procedure minimised selection bias as all ILI cases were swabbed. Furthermore, GPs did not know the case or control status when recruiting ILI patients. This was the first season in which the EU ILI case definition was introduced into the sentinel GP networks. For most ILI patients recruited, the case definition was correctly used: of 455 ILI patients reported, only 17 were excluded because they did not match the EU ILI case definition. However, we cannot rule out that some GPs did not include all patients corresponding to the EU case definition. If the sensitivity of GPs´ ILI case definition were dependent on the vaccination status, IVE might have been over- or underestimated.

Various studies suggest that ILI test-negative controls represent the source population of influenza cases seen at GPs offices and that the study design adjusts for propensity to seek care. This would mean that the propensity to seek care is equal between ILI patients who test positive and those testing negative for influenza. Our results indicate that in three out of the five studies, the delay between onset of symptoms and swabbing was shorter for cases than for test-negative controls. Similar results were found in the Wisconsin study [3]. This may indicate a different health-seeking behaviour or a different severity of ILI in cases and in controls. Health-seeking behaviour of ILI cases and ILI test-negative controls should be further studied and compared.

To further assess the representativity of test-negative controls, we measured the vaccine coverage in other potential control groups. The vaccine coverage differed by control group (test-negative controls, non-ILI GP controls, community controls) and between countries with no specific pattern. This could suggest that the source population of influenza cases consulting a GP may be country specific. In general, the vaccine coverage in the community or in the GPs catchment area was lower than the vaccine coverage of GP clients indicating that community controls do not represent a good control group for medically-attended ILI influenza cases. In a recent study in Wisconsin, VE for laboratory confirmed medically attended-ILI was estimated for three seasons using two control groups: test-negative controls and controls randomly selected from individuals in the source population who did not have a clinical encounter for acute respiratory illness prior to the week of recruitment [3]. In the three seasons, the vaccination coverage of the test-negative controls was higher than among the other controls.

We took into account the main confounding factors identified in the literature. Most of them were based on patients´ report for which validity is unknown. The pooled crude and adjusted IVE were similar suggesting a low distortion of effect due to confounding. In our study, a small proportion of ILI patients had indicators of frailty (4.3% had poor functional status and 6.4% were hospitalised in the previous year). Elderly ILI patients consulting GPs at their office may have a better health status than those not consulting. Therefore, functional status and severity may not be relevant confounding factors within this study population and study design. Our results may also reflect that using specific outcomes decrease the amount of confounding observed [5,7]. In Canada, using the same study design, IVE did not change when adjusting for chronic diseases [20].

The excellent collaboration between the study teams made the pooling of data from the five studies possible. Pooling increased the precision of the estimates. Given the small samples sizes of the individual studies, we used a one-stage pooling model that assumes that the effect of the exposure (the seasonal vaccine) and the effect of the covariates are the same in all the studies. We do not know if the difference in virus circulation in the various countries and a potential different health-seeking behaviour may violate this assumption. The pooled estimates of the pilot phase have to be interpreted with caution as heterogeneity between studies may exist. Futhermore, different vaccine brands were used. However, the aim of I-MOVE is not to guide the Member States in deciding which seasonal vaccine to purchase. In order to assess VE for the various vaccine brands, the sample size would have to be increased significantly. The definitions of some covariates were not exactly the same in the different studies. Tests for interaction between study and covariates did not suggest the presence of heterogeneity. However, the small sample size may have led to an insufficient power to detect heterogeneity.

Conclusions

In 2008-9 the match between the seasonal influenza vaccine and the predominant circulating strains was good and the IVE in the elderly relatively high. Our results suggest that GP based case-control studies using test-negative controls to estimate seasonal IVE against laboratory-confirmed medically- attended influenza, are feasible in Europe. The use of a laboratory confirmed outcome may reduce the magnitude of confounding. If other studies confirm this, the number of confounders documented may be reduced, thus simplifying the data collection. The representativity of test-negative controls should be further evaluated.

Pooling of country specific data is needed to have early seasonal or pandemic VE estimates and to increase the precision of the estimates for subgroup analysis. In 2009-10 we will increase the sample size, by increasing the number of countries participating in the study and including more GPs per country. The larger sample size will allow the use of a two-stage model that better takes into account the potential heterogeneity between studies [18,21]. The studies will use common definitions for all variables to minimise heterogeneity between studies. During H1N1 influenza pandemic, interim analyses will be conducted in different periods according to the available sample size. The timing for conducting each of the interim analyses will depend on the time necessary to reach the appropriate sample size. This will depend mainly on the ILI incidence, the influenza incidence and the vaccination coverage.

The suitability of the case-control studies based on sentinel GPs to measure pandemic IVE will depend on the vaccination and control strategy. If pandemic cases are seen by the sentinel GPs and GPs have the possibility to ascertain patient vaccination status, then the case-control design piloted in 2008-9 would be adequate to estimate pandemic VE. All age and risk groups targeted by the vaccine should be included in the study. The design will be adapted to reduce the GPs' workload by simplifying the questionnaire and revising the procedure to select patients to swab.

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*Erratum: The x-axis in Figure 1 indicated the wrong weeks and this was corrected on 6 November 2009.

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Introduction of human papillomavirus (HPV) vaccination in Belgium, 2007-2008

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This paper documents the progress of human papillomavirus (HPV) vaccine introduction in Belgium. Information on vaccine use is based on sales statistics and reimbursement claims. From November 2007 to November 2008, the National Institute for Health and Disability Insurance reimbursed the HPV vaccine for girls aged between 12-15 years. In December 2008, the age limit was extended to include girls up to the age of 18. In November 2008, the total number of HPV vaccines sold exceeded 530,000 doses. The number of vaccines reimbursed in Belgium, for the period November 2007-November 2008, corresponds to the amount required to fully vaccinate 44% of all girls aged between 12-15 years. However, the trend was decreasing over the last 10 months. By the current reimbursement policy, we can expect that maximum half of the target population can be reached. In Flanders (one of the three Communities in Belgium), the intention is to start, from September 2010, with a free school-based HPV immunisation for girls in the first year of secondary school (12 years of age), complemented with vaccination by a physician of choice. This strategy ensures a higher HPV vaccine coverage which is expected to be as high as the current coverage in the hepatitis B vaccination programme (approximately 80%) offered to boys and girls in the same age group and under the same circumstances.

Introduction

In 2004, 651 cases of cervical cancer (European-age standardised rate (E-ASR) 8.5/100,000 women-years) were reported in Belgium, and approximately 264 women (E-ASR 3.8/100,000 women-years) died from the disease [1,2]. Currently, screening for cervical cancer is mainly opportunistic in Belgium [3,4]. The screening coverage for cervical cancer, in the target age group (25-64 years), with a three-year interval, was 59% in 2000. However, the modal screening interval is 12 months, whereas the recommended interval is 36 months. Moreover, screening is often offered to women younger than 25 years of age. Therefore, the number of smears taken annually could theoretically cover the whole target population [5]. Nevertheless, organised screening according to European guidelines and in collaboration with the three Communities (Flemish, French, and Germanophonic), is planned within the new Cancer Plan [6,7]. It is estimated that 72% of all cervical cancers in Europe and North America are caused by the oncogenic human papillomavirus (HPV) types 16 and 18 [8]. The current paper updates a previous report on HPV vaccine introduction in Belgium, Luxembourg and the Netherlands [9], and provides more detailed information on the Belgian situation.

Recommendations and decision making in Belgium

On 2 May 2007, the Belgian Superior Health Council (SHC) made its first recommendations regarding vaccination against infections caused by HPV. The only vaccine available at that moment was the quadrivalent HPV-vaccine, containing virus-like particles of HPV types 6, 11, 16 and 18 (Gardasil, licensed in Belgium on 20 September 2006). Summarising the recommendations of the SHC to the health authorities:

- Organised HPV vaccination should be offered to a one-year birth cohort of girls between 10 and 13 years of age [10].
- Girls should preferably be vaccinated through the school health system within a scholar calendar year, free of charge, as currently done for hepatitis B vaccination [11]. In Belgium, 70-80% of the vaccines for school-age children are given through the school health system. Practicing physicians (general practitioners (GPs), paediatricians and gynaecologists) have a complementary role in this. The SHC therefore recommended that for HPV too, parents should have the option of having their child vaccinated by such practicing physicians.
- The additional protective effect of organised catch-up vaccination up to the age of 15 years was recognised but only recommended if health-economic evaluation would confirm that it is cost-effective.
- Vaccination at older ages (14-26 years) can be considered when delivering personal healthcare, for instance during a consultation related to contraception, taking into account prior sexual experience and stressing the importance of safe sex. Systematic preliminary HPV testing before vaccination was not recommended.
- It is considered necessary to set up an organised screening programme according to European guidelines [7,9], to register administration of the HPV vaccine and to monitor their effects.

The recommendation was updated on 5 December 2007 to include the bivalent vaccine (HPV types 16 and 18) (Cervarix, licensed in Belgium on 24 September 2007).

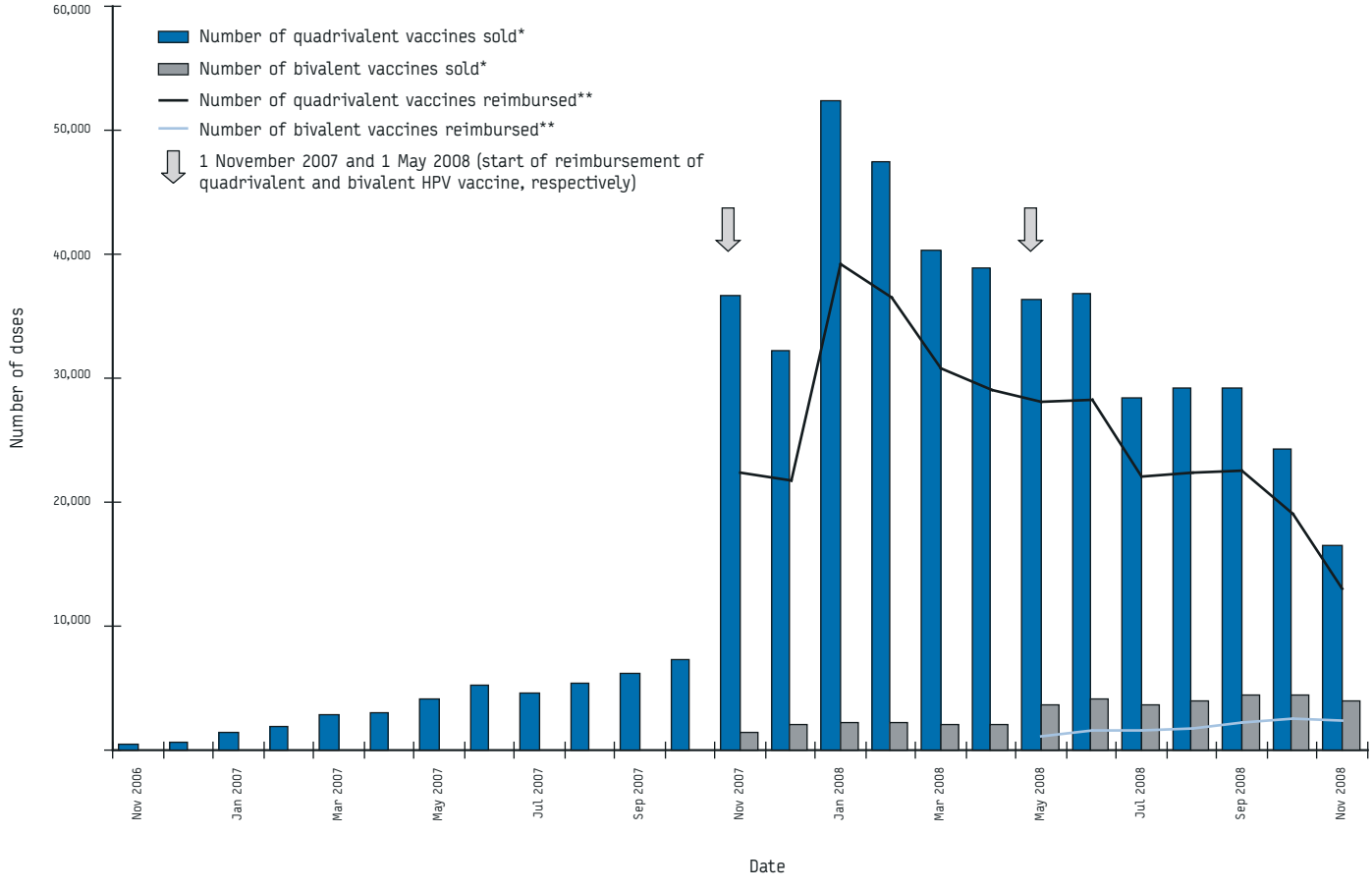
The SHC is the link between government policy and the scientific world in the field of public health. The council provides independent advice and recommendations to the Minister, on his/her specific request for information or on its own initiative. The Communities are free to implement these recommendations, even independent of each other.

The National Institute for Health and Disability Insurance (NIHDI) is a federal institution that organises, manages and supervises the correct application of the 'compulsory insurance' in Belgium [12]. It covers the whole population officially residing in Belgium. The NIHDI has decided, independently of the recommendation of the SHC, to reimburse opportunistic HPV vaccination for girls between the age of 12 and 15 years (for the quadrivalent vaccine since 1 November 2007 [13] and for the bivalent vaccine since 1 May 2008 [14]). In the framework of the National Cancer Plan, the age range for reimbursement was extended to include the age of 18 years as of 1 December 2008 [6]. This reimbursement of the HPV vaccines was communicated widely both in the scientific and the popular press.

The organisation of preventive healthcare in Belgium, including the management of the routine vaccination programme, is a responsibility of the three Communities. However, since 2004, in recognition of the high prices of some new vaccines, the NIHDI has been co-funding two thirds of the costs for vaccine purchase (only for vaccines purchased via tender, such as for the hepatitis B adolescent vaccination programme, the infant hexavalent vaccination programme, etc.). This mechanism of shared funding requires consensus on vaccination policies between all three Communities and federal authorities (the federal Ministry of Health together with the NIHDI). In 2008, the Ministry of the Flemish Community responsible for public health endorsed the recommendations of the SHC and the Flemish Vaccination Platform regarding HPV vaccination: i.e. offering HPV vaccination to a one-year birth cohort of girls between 10-13 years of age [15]. However, the Ministry of Health of the French Community did not follow the SHC advice [15]. Girls aged 12-18 years from the French

Figure

Number of vaccines sold and number of vaccines reimbursed per month for girls between 12 and 15 years of age, Belgium, November 2006-November 2008



* Source: Intercontinental Marketing Services (IMS) Health
** Source: The Belgian National Institute for Health and Disability Insurance
HPV: human papillomavirus

Community will be offered HPV vaccination by their GP or another physician, with the cost of the HPV vaccine partially reimbursed by the NIHDI and the remaining cost carried by the patient. Until now, the Germanophonic Community has not made a decision regarding a generalised immunisation programme for school girls against HPV.

Recently, legislation has changed and the consensus on vaccination policies between communities is no longer required, allowing for asymmetric immunisation policies over the different Communities [16]. The intention is to start free school-based HPV vaccination, at least in Flanders, in the school year 2010-2011, in a one-year cohort of girls in the first year of secondary school (12 years of age).

Vaccine sales and reimbursement data

Information on the total number of HPV vaccines sold in Belgium (complete wholesale data, not accounting for administration of the vaccine), was obtained from Intercontinental Marketing Services (IMS) Health (Figure: bars). IMS statistics show a cumulative amount of approximately 43,000 doses of the quadrivalent vaccine sold up to October 2007 (after the start in November 2006, sales figures gradually increased from ca. 400 to ca. 7,200 monthly doses). After the start of reimbursement in November 2007, a rapid increase in the monthly number of HPV vaccine doses sold was seen, up to 52,000 in January 2008. From then on, sales decreased progressively to 20,000 doses in November 2008. In total, about 532,000 HPV vaccine doses were sold in Belgium, up to November 2008.

The NIHDI HPV vaccine reimbursement data are also shown in the Figure (line curve), for the period November 2007-November 2008 (source NIHDI). At the start of reimbursement (in November and December 2007), the monthly number of reimbursed doses of the quadrivalent vaccine was around 22,000. In January 2008, the number increased to ca. 39,000 doses, but decreased afterwards to ca. 15,000 doses in November 2008. Over 1,000 doses of the bivalent vaccine were reimbursed in May 2008, which was the first month of reimbursement for this type of vaccine. This number increased up to 2,350 per month in November 2008. In total, over the 13-month period, 348,000 HPV vaccine doses were reimbursed. These reimbursed vaccines were administered by the GPs, paediatricians or gynaecologists of the 12-15 year-old girls.

The proportion of total vaccines sold that were reimbursed over the period where both IMS and reimbursement data were available, increased from 59% in November 2007 to about 75% in November 2008. The proportion of sold vaccines that were bivalent increased progressively from less than 4% before reimbursement to 19% in November 2008. The difference between sales and reimbursement figures (see Figure) presumably corresponds to vaccination beyond the target population, probably women older than 15 years buying it privately.

In Belgium, ca. 348,000 doses of HPV vaccine (both quadrivalent and bivalent) were reimbursed over a period of 13 months, which corresponds to an annual average of about 320,000 (ca. 27,000 per month); with this amount of vaccines one could theoretically reach a full three-dose coverage of 44% of all girls aged 12-15 years residing in Belgium. Around 61,000 monthly doses would be needed to reach complete coverage. Over the last six documented months ca. 31,500 doses were reimbursed per month and this quantity was following a negative trend. If this trend continues, we can expect that maximum half of the target population could be reached by the current reimbursement policy in Belgium.

Discussion and conclusion

The current policy of administration of the HPV vaccine in Belgium is estimated to cover maximum half of the targeted population. School-based free vaccination, complemented with vaccination by a physician of choice, is expected to guarantee a higher level of HPV vaccine coverage, effectiveness, cost-effectiveness and equity in healthcare access. Data from the recent immunisation coverage study in Flanders (2008) show that hepatitis B vaccine coverage offered at the age of 12 years achieved a coverage of approximately 90% [17]. In Flanders (one of the three Communities in Belgium), the intention is to start, from September 2010, a free school-based HPV immunisation, which is the preferred strategy option for HPV vaccine delivery in European countries proposed by the European Centre for Disease Prevention and Control [18]. In Flanders, this will be complemented by vaccination by a physician of choice (as is the situation for the national adolescent hepatitis B vaccination programme).

Current HPV vaccines are expensive, the duration of elicited immunity is still unknown and not all oncogenic HPV types are included. Therefore, careful surveillance is needed. In Belgium, the National Cancer Plan foresees registration of all organised vaccination efforts. Moreover, linkage of HPV vaccination status with the Belgian Cancer Registry is foreseen. However, international consultation is desirable, in order to orient the design of local surveillance plans allowing for international comparison.

Data on HPV vaccine sales and reimbursement will be collected continuously from the IMS and the NIHDI, both sources described in this paper. In the near future, the Scientific Institute of Public Health in collaboration with the Intermutualistic Agency, will analyse individual patient data from all reimbursed HPV vaccinations which will allow to estimate HPV vaccination coverage by number of doses, age and geographic unit.

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Competing interest

C Simoens and M Arbyn received travel funding from GSK and SPMSD, respectively (before 2008). P Van Damme has been principal investigator of bivalent and quadrivalent HPV vaccine trials, for which the University of Antwerp obtains contractual funding. All other authors declare no conflict of interest.

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Single-nucleotide polymorphism in the SCCmec-orfX junction distinguishes between livestock-associated MRSA CC398 and human epidemic MRSA strains

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A number of real-time PCR assays for direct detection of methicillin-resistant (MRSA) in clinical specimens are targeting staphylococcal cassette chromosome mec (SCCmec) right extremity sequences and the *S. aureus* chromosomal *orfX* gene sequences located to the right of the SCC*mec* integration site. When testing 184 MRSA strains of human and animal origin from geographically distinct locations, we identified several characteristic single-nucleotide polymorphisms (SNPs) within the SCC*mec-orfX* junction of livestock-associated (LA) MRSA CC398 which serve as suitable strain markers for screening purposes. Within an assay time of 60 minutes and an additional 10 minutes for the melting curve analysis, all MRSA CC398 isolates were correctly identified by their characteristic T_m value in the commercial LightCycler MRSA Advanced test. Studies to confirm the diagnostic accuracy of the SNP-based strain identification assay with a larger collection of clinical and LA-MRSA strains are ongoing.

Introduction

Rates of methicillin-resistant *Staphylococcus aureus* (MRSA) infections have steadily increased during the past two decades and the occurrence and spread of MRSA strains in healthcare facilities as well as in the community is a growing problem worldwide [1,2,3]. Although classically considered as a nosocomial pathogen, reports of MRSA carriage or its acquisition in the community have become increasingly common during the past decade [2,4]. More recently, studies have demonstrated that especially swine and swine farmers in Austria [5], Denmark [6], Germany [7,8], the Netherlands [9-11], Portugal [12], the United States [13], and many other countries are colonised with MRSA. Since it was realised that livestock animals may form a new, separate reservoir, these strains are now called livestock-associated MRSA (LA-MRSA). Molecular characterisation revealed that a distinct clone of MRSA is predominant within this collective: LA-MRSA strains are grouping within the new clonal complex 398 (CC398) with sequence type 398 (ST398) as the most prevalent type. Animals carrying MRSA represent a reservoir for transmission to humans [13,14,15]. The MRSA strains from animal origin have been shown to be pathogenic for humans and can cause severe infections such as endocarditis and ventilator-associated pneumonia [16]. A number of diagnostic strategies have been published on the molecular characterisation of the MRSA CC398 clonal lineage, using pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) or other laborious techniques based on genome sequencing [1,19,20].

Active surveillance is needed

Since livestock animals may act as a reservoir for this bacterium, the development of rapid molecular methods for screening and identification of MRSA CC398 will have important implications for surveillance, epidemiology and future infection control initiatives. As with many other bacterial pathogens, the rapid and reliable detection of certain MRSA clones is of the utmost importance to prevent the spread of infection. A number of real-time PCR assays targeting staphylococcal cassette chromosome *mec* (SCC*mec*) right extremity sequences and the *S. aureus* chromosomal *orfX* gene sequences located to the right of the SCC*mec* integration site have recently been established for direct detection of MRSA in clinical specimens. In the course of the present study, such assays were evaluated for their performance to detect and distinguish LA-MRSA strains of the new clonal complex 398 (CC398).

Materials and Methods

Study population, survey methods and diversity of investigated strains

During an on-going study conducted by the Bavarian Health and Food Safety Authority to explore the epidemiology of MRSA CC398 in Bavaria, the MRSA colonisation status among swine bred in Bavaria and the involved farmers was investigated by sampling the nares of 634 swine and 116 farmers on 60 geographically distinct farms. Epidemiological results will be available when this particular study is completed. Additional representatives of the MRSA CC398 lineage from other geographic locations and other sources including horses, dogs, guinea pigs, chicken, poultry and humans with contact to colonised animals, as well as MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates of presumed to be related spa-types were kindly provided by a number of supporting laboratories listed in the Table and in the Acknowledgements section.

Diagnostic culture and template DNA preparation

The *S. aureus* strains collected from pigs and farmers in Bavaria were maintained on Columbia blood agar and identified by colony morphology, Gram-stain characteristics, catalase reaction, coagulase production, and the results of the API Staph system (bioMérieux). Oxacillin susceptibility was determined by the agar screening method with Mueller-Hinton agar containing 2% NaCl and 6 mg/l of oxacillin for *S. aureus* [3,19]. An identical protocol was applied for characterisation and maintaining the various *S. aureus* strains provided by supporting laboratories. Template DNA for PCR was prepared from individual bacterial colonies by a simple and rapid 'boiling' procedure [20]. Briefly, colonies were suspended in 200 µl of lysis buffer containing 1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl pH 8.0 and 1 mM EDTA, and incubated for 10 min at boiling temperature. After centrifugation for 2 min at 10.000 x g to sediment the debris, a 2 µl aliquot of the clear supernatant was directly transferred to PCR. Alternatively, the reagents and the protocol of the LightCycler Advanced Lysis kit (Roche Diagnostics) were used for template DNA preparation. For cultured bacterial organisms, the efficiencies of the commercial lysis kit and the 'boiling' procedure were found to be comparable for the extraction of amplifiable *S. aureus* DNA (data not shown).

PCR amplification, DNA sequencing and molecular strain typing

Amplification and sequencing of the SCC*mec-orfX* junction was performed according to Hagen *et al.* [21]. PCR reactions and subsequent hybridisation probe melting curve analyses were carried out on a Roche LightCylcer 2.0 device. Amplicons of the expected size were purified (HighPure PCR Cleanup Micro kit, Roche Diagnostics) and sequenced on an automated ABI 310 sequencer using BigDye v. 1.1 chemistry.

Real-time PCR amplification and detection reactions were carried out according to the protocol of the LightCycler MRSA Advanced test (Roche Diagnostics). In the case of a negative result, an in-house duplex PCR assay was performed targeting a segment of the *mec*A gene and the *S. aureus*-specific genomic fragment Sa 442 [22]. For selected *S. aureus* strains, accessory testing was performed with well-established commercial PCR tests designed for direct detection of MRSA from clinical specimens, namely the GenoType MRSA Direct (Hain Lifescience), the BD GeneOhm MRSA (Becton Dickinson) and the Xpert MRSA (Cepheid) assays.

The presence of Panton-Valentine leukocidin (PVL) [23] was investigated by PCR testing for the *luk*S-PVand *luk*F-PV genes [2,4]. Typing of the *S. aureus* protein A gene (spa) was performed for all isolates obtained in this study using a standard protocol [24]. Clustering of *spa* types into *spa* clonal complexes (*spa*-CC) was performed using the Based Upon Repeat Pattern (BURP) algorithm of the Ridom StaphType software (Ridom GmbH) with the following preset parameters as recommended previously [25]: *Spa* types were clustered into the same group if the cost was four or less; spa types which were shorter than five repeats were excluded. When an isolate was indicated to be closely related to a *spa* type presumed to be associated with CC398, but the T_m values observed in the LightCycler MRSA Advanced test did not correspond to the T_m values expected for CC398 isolates, MLST- and SCC*mec*-types of the isolates were determined [1] or provided by the supporting

Table

MRSA isolates investigated in the present study (n=184)

Number of tested strains	MLST	*spa* type	T_m value (°C)	*mecA*	Sa 422	Xpert MRSA	BD GeneOhm MRSA	Source	Geographic origin	Culture result	Comment
8	ST 398	t011	55	n.d.	n.d.	pos.	pos.	Piglets	Germany (center 1)	MRSA	Bavarian LA-MRSA survey
11	ST 398	t034	55	n.d.	n.d.	pos.	pos.	Piglets	Germany (center 1)	MRSA	Bavarian LA-MRSA survey
11	ST 398	t011	55	n.d.	n.d.	pos.	pos.	Fattening pigs	Germany (center 1)	MRSA	Bavarian LA-MRSA survey
5	ST 398	t034	55	n.d.	n.d.	pos.	pos.	Fattening pigs	Germany (center 1)	MRSA	Bavarian LA-MRSA survey
5	ST 398	t011	55	n.d.	n.d.	pos.	pos.	Humans (pig farmers)	Germany (center 1)	MRSA	Bavarian LA-MRSA survey
4	ST 398	t034	55	n.d.	n.d.	pos.	pos.	Humans (pig farmers)	Germany (center 1)	MRSA	Bavarian LA-MRSA survey
1	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Pig	Germany (center 2)	MRSA	
1	ST 398	t2510	55	n.d.	n.d.	n.d.	n.d.	Pig	Germany (center 2)	MRSA	
1	ST 398	t1451	55	n.d.	n.d.	n.d.	n.d.	Pig	Germany (center 2)	MRSA	
1	ST 398	t108	55	n.d.	n.d.	n.d.	n.d.	Pig	Germany (center 2)	MRSA	
13	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Human (pig farmers conference)	Austria (center 3)	MRSA	SCC*mec* type V
1	ST 398	t2576	55	n.d.	n.d.	n.d.	n.d.	Human (veterinarian conference)	Austria, Germany, Switzerland (center 3)	MRSA	SCC*mec* type V
7	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Human (veterinarian conference)	Austria, Germany, Switzerland (center 3)	MRSA	SCC*mec* type V
3	ST 398	t034	55	n.d.	n.d.	n.d.	n.d.	Human (veterinarian conference)	Austria, Germany, Switzerland (center 3)	MRSA	SCC*mec* type V
13	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Human (pig farmers and veterinarians)	Austria (center 3)	MRSA	SCC*mec* type V
24	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Dust (from pig breeding facilities)	Austria (center 3)	MRSA	

1	ST 398	t034	55	n.d.	n.d.	n.d.	n.d.	Dust (from pig breeding facility)	Austria (center 3)	MRSA	
10	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Pigs	Germany (center 8)	MRSA	
1	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Human (wound swab)	Germany (center 1)	MRSA	
1	ST 398	t1456	55	n.d.	n.d.	n.d.	n.d.	Human (wound swab)	Germany (center 1)	MRSA	
1	ST 398	t1456	55	n.d.	n.d.	n.d.	n.d.	Human (wound swab)	Germany (center 1)	MRSA	
1	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 5)	MRSA	
7	ST 398	t108	55	n.d.	n.d.	n.d.	n.d.	Humans	Netherlands (center 7)	MRSA	
2	ST 398	t034	55	n.d.	n.d.	n.d.	n.d.	Humans	Netherlands (center 7)	MRSA	
9	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Humans	Netherlands (center 7)	MRSA	
2	ST 398	t034	55	n.d.	n.d.	n.d.	n.d.	Humans	Denmark (center 6)	MRSA	SCC*mec* type V
1	ST 398	t108	55	n.d.	n.d.	n.d.	n.d.	Human	Denmark (center 6)	MRSA	SCC*mec* type V
2	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Humans	Denmark (center 6)	MRSA	
1	ST 398	t5706	55	n.d.	n.d.	n.d.	n.d.	Human	Denmark (center 6)	MRSA	
1	ST 398	t108	55	n.d.	n.d.	n.d.	n.d.	Human	Denmark (center 6)	MRSA	PVL positive, SCCmec type V
1	n.d.	t1793	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	PVL positive
1	n.d.	t1250	55	n.d.	n.d.	n.d.	n.d.	Pig	Germany (center 2)	MRSA	
2	n.d.	t011	55	n.d.	n.d.	n.d.	n.d.	Poultry	Germany (center 2)	MRSA	
1	n.d.	t011	55	n.d.	n.d.	n.d.	n.d.	Guinea pig	Germany (center 2)	MRSA	
1	n.d.	t011	55	n.d.	n.d.	n.d.	n.d.	Dog	Germany (center 2)	MRSA	
3	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Horses	Germany (center 2)	MRSA	
1	n.d.	t1457	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t1580	55	n.d.	n.d.	n.d.	n.d.	Human (pharynx)	Germany (center 2)	MRSA	
1	n.d.	t2011	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t1451	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t2346	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t2370	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t2576	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t2741	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t3423	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t1255	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t1197	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t571	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t108	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t2582	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	n.d.	t034	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	ST 398	t011	55	n.d.	n.d.	n.d.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	ST 30	t138	59	pos.	pos.	pos.	n.d.	Human (nose)	Germany (center 2)	MRSA	
1	ST 9	t1430	59	pos.	pos.	pos.	n.d.	Chicken (wing)	Germany (center 4)	MRSA	SCC*mec* type IVa
1	ST 1	t127	59	pos.	pos.	pos.	pos.	Piglet (nose)	Germany (center 1)	MRSA	
1	ST 398	t034	neg.	pos.	pos.	neg.	neg.	Human	Denmark (center 6)	MRSA	SCC*mec* type IVa
1	ST 398	t034	neg.	pos.	pos.	neg.	neg.	Human	Denmark (center 6)	MRSA	SCC*mec* type VII
1	ST 398	t571	neg.	pos.	pos.	neg.	neg.	Human	Denmark (center 6)	MRSA	PVL positive, SCCmec non-typeable
1	ST 398	t1606	neg.	pos.	pos.	neg.	neg.	Human (nose)	Germany (center 2)	MRSA	SCC*mec* non-typeable
1	ST 753	t898	neg.	pos.	pos.	neg.	neg.	Human (nose)	Germany (center 2)	MRSA	SCC*mec* non-typeable
1	ST 398	t567	neg.	pos.	pos.	neg.	neg.	Human (nose)	Germany (center 2)	MRSA	SCC*mec* non-typeable
1	ST 30	t021	neg.	pos.	pos.	neg.	neg.	Human (nose)	Germany (center 2)	MRSA	SCC*mec* non-typeable

BD GeneOhm MRSA: test result of a commercial MRSA-specific PCR assay (Becton Dickinson); MLST: multilocus sequence typing; MRSA: methicillin-resistant *Staphylococcus aureus*; *mecA*: test result of an in-house realtime PCR assay targeting the *mecA* gene [20]; neg: no specific amplification products observed or negative test result for MRSA; n.d. not done; pos: specific amplification products observed or positive test result with the applied realtime PCR assays designed for direct detection of MRSA by targeting the SCC*mec-orfX* integration site; pSA422: test result of an in-house realtime PCR assay targeting a *S. aureus*-specific species marker gene Sa422 [20];T_m: T_m-value observed with the LightCycler MRSA Advanced Test (Roche Diagnostics); Xpert MRSA: test result of a commercial MRSA-specific PCR assay (Cepheid).

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Figure 1

Multiple sequence alignment of a selected *S. aureus orfX* segment

```
FJ830606  CCGCATCATTTGGTGTGGAAATGTCATTTTGCTGAATGATA porcine MLST 398
AB033763  ............A.....G..................... SCCmec Type I
D86934    ............A.....G..................... SCCmec Type II
AB047089  ............A.....G..................... SCCmec Type III
AB063172  ............A.....G.............A....... SCCmec Type IVa
AB063173  ............A.....G..................... SCCmec Type IVb
AB096217  ............A.....G..................... SCCmec Type IVc
DQ106887  ............A.....G..................... SCCmec Type IVg
AB121219  ............A.....G.............A....... SCCmec Type V
AF411935  ............A.....G..................... SCCmec Type VI
AB373032  ............A.....G..................... SCCmec Type VII
FJ390057  ............A.....G..................... SCCmec Type VIII
AM292304  ............A.....G..................... SCCmec Type unknown
AB425823  ............A........................... SCCmec Type IVa
U10927    ..T.......................................... SCCmec Type unknown
          |           |     |           |         |
Pos.      300         312   318         330     340  in FJ830606

FJ830606  GTGCGTAGTTACTGCGTTGTAAGACATCCTTGTGCAGGCCG porcine MLST 398
AB033763  ........................G.............. SCCmec Type I
D86934    ........................G.............. SCCmec Type II
AB047089  ........................G.............. SCCmec Type III
AB063172  ....A...................G.............. SCCmec Type IVa
AB063173  ........................G.............. SCCmec Type IVb
AB096217  ........................G.............. SCCmec Type IVc
DQ106887  ........................G.............. SCCmec Type IVg
AB121219  ....A...................G.............. SCCmec Type V
AF411935  ........................G.............. SCCmec Type VI
AB373032  ........................G.............. SCCmec Type VII
FJ390057  ........................G.............. SCCmec Type VIII
AM292304  ........................G.............. SCCmec Type unknown
AB425823  ............A...........G.............. SCCmec Type IVa
U10927    ........................G.............. SCCmec Type unknown
          |        |         |    |             |
Pos.      341      350       360  366           381 in FJ830606

FJ830606  TTTGATCCGCCAATGACGAATACAAAGTCGCTTTGCCCTTG porcine MLST 398
AB033763  ..................A..................... SCCmec Type I
D86934    ........................................ SCCmec Type II
AB047089  ........................................ SCCmec Type III
AB063172  ........................................ SCCmec Type IVa
AB063173  ..................A..................... SCCmec Type IVb
AB096217  ........................................ SCCmec Type IVc
DQ106887  ........................................ SCCmec Type IVg
AB121219  ........................................ SCCmec Type V
AF411935  ........................................ SCCmec Type VI
AB373032  ........................................ SCCmec Type VII
FJ390057  ........................................ SCCmec Type VIII
AM292304  ........................................ SCCmec Type unknown
AB425823  ........................................ SCCmec Type IVa
U10927    ........................................ SCCmec Type unknown
          |       |         |         |           |
Pos.      382     390       400       410         422 in FJ830606
```

Multiple sequence alignment of a selected *S. aureus orfX* segment located close to the SCC*mec-orfX* junction (position 253 in GenBank FJ830606). The most similar sequences found in BLAST search show either a sequence identical to GenBank sequence entries of *S. aureus* isolates carrying one of the eight *SCCmec* types or differ from the MRSA ST 398 isolates of the study by at least two nucleotides at positions 312 and 366 (GenBank FJ830606).

laboratories. Typing of SCC*mec* elements of types I to VII was carried out according to previously published PCR procedures [26].

Results

Molecular characteristics of MRSA isolates derived from the Bavarian LA-MRSA survey

By sampling the nares of 634 swine and 116 farmers on 60 geographically distinct farms in Bavaria during the course of an ongoing study, a total number of 245 MRSA strains from pigs and 34 MRSA strains from farmers were grown from the collected swabs. From this collection, 44 MRSA isolates from geographically distinct farms were chosen for further analyses (Table, rows 1 to 6). The distribution of *spa* types among these isolates was as follows: t011 (n=24) and t034 (n=20). MLST-typing of all selected MRSA isolates revealed that they belonged to MLST ST398. All 44 MRSA strains tested negative for PVL-encoding genes.

Novel single nucleotide polymorphisms in the SCC*mec-orfX* integration site of LA-MRSA isolates

By systematic sequencing of the SCC*mec-orfX* integration sites of MRSA isolates of animal origin, all of the 44 sequences obtained from Bavarian porcine isolates (Table, rows 1 to 6) were found to be identical in a multiple alignment (using pileup from the HUSAR sequence analysis package from the German Cancer Research Center (DKFZ), http://genius.embnet.dkfz-heidelberg.de, data not shown). As the sequence differed from previously published SCC*mec-orfX* integration site sequence motifs, it was deposited in GenBank with accession number FJ830606 (to be released after publication).

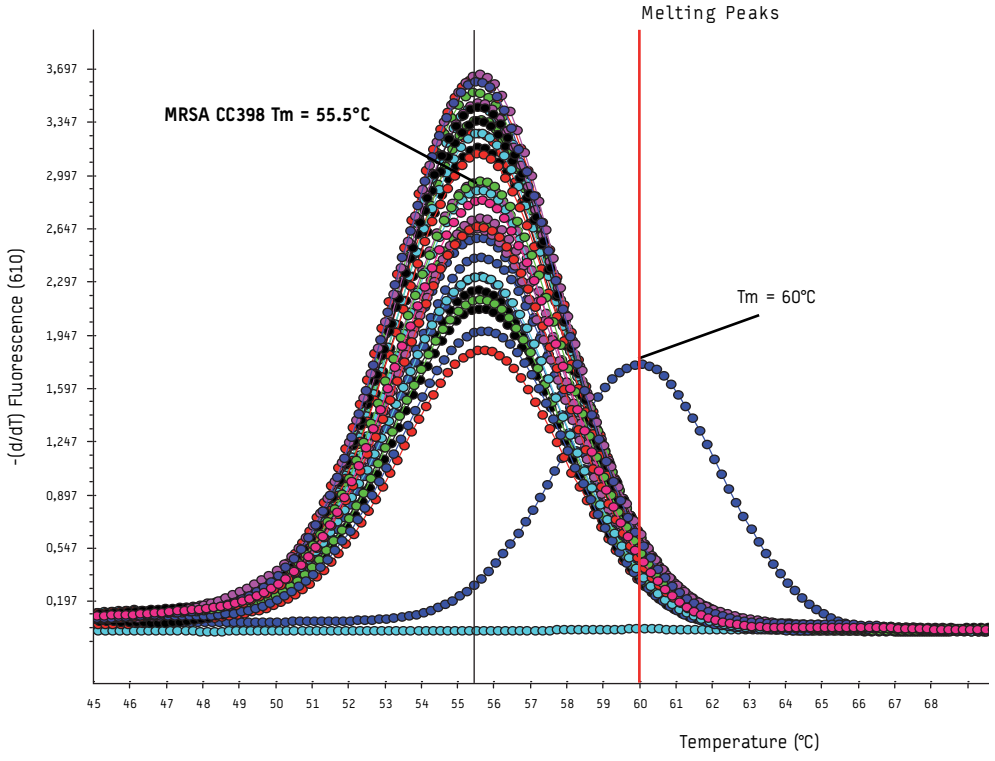
A detailed investigation of the novel sequence revealed some nucleotide positions uncommon in *S. aureus* GenBank sequences and at least three characteristic single nucleotide polymorphisms (SNPs) in the *S. aureus* chromosomal *orfX* gene sequence located to the right of the SCC*mec* integration site: guanine at position 312, adenine at position 366, and cytosine at position 441 (GenBank FJ830606). At least two of these SNPs were found exclusively in the investigated MRSA strains of animal origin and may serve as a diagnostic marker for the presence of MRSA CC398. A BLAST search (National Center for Biotechnology Information (NCBI), http://blast.ncbi.nlm.nih.gov/Blast.cgi) with the complete amplicon sequence revealed GenBank accession number AM292304 (*S. aureus* SCC*mec*ZH47 mobile element) as the most similar hit with five mismatches. GenBank AB425823 and U10927, the next similar sequences found in the BLAST search, were either identical to GenBank entries of one of the eight acknowledged MRSA SCC*mec* types deposited in GenBank, or had at least one nucleotide difference at position 366 compared with the sequence FJ830606 obtained from the investigated MRSA ST398 strains of porcine origin (Figure 1).

Practical application of the identified single nucleotide polymorphisms

In addition to the broad spectrum of unpublished in-house PCR protocols, also the proprietary sensor hybridisation probe of

Figure 2

Specificity of the Roche LightCycler MRSA Advanced test for differentiating MRSA CC398 and non-CC398 strains in hybridisation probe melting curve analysis



The curves represent the MRSA strain ATCC 33592 (T_m ≈ 60 °C) and 30 MRSA ST 398 strains of porcine origin with characteristic Tm values around 55.5 °C. A methicillin-sensitive strain of *S. aureus* (clinical isolate) was used as negative control.

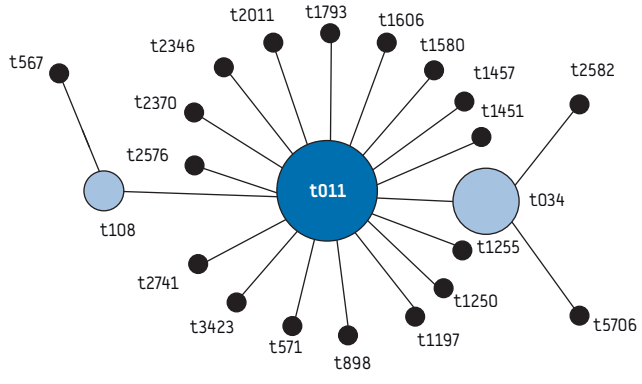
the recently developed LightCycler MRSA Advanced Test (Roche Diagnostics) covers one of these two particular nucleotide positions. This real-time PCR assay detects MRSA strains with different molecular sequences surrounding the right extremity junction of the SCC*mec* cassette with the *S. aureus orfX* gene. As a practical application of the SNPs identified in our study, we present the use of this commercial real-time PCR kit for the direct detection of MRSA and simultaneous identification of LA-MRSA CC398. For all 44 investigated Bavarian MRSA ST398 strains, specific amplification products were generated with the LightCycler MRSA Advanced test, and they all harboured at least one of the identified SNPs in the SCC*mec-orfX* junction represented by a characteristic T_m of 55.5 °C in the subsequent LightCycler hybridisation probe melting curve analysis (Figure 2). Since we have not yet observed such a Tm-shift with any non-ST398 MRSA strains of human or animal origin, this point mutation may serve as a molecular marker for the presence of MRSA CC398.

As an approved in vitro diagnostics (IVD) product, the Roche LightCycler MRSA Advanced test has already been validated with a comprehensive collection of MRSA strains of human origin for the limit of detection, inclusivity and exclusivity. The results of systematic studies on the assay's diagnostic performance will be published soon (personal communication, Roche Diagnostics). According to the product information of the test kit, the range of T_m values observed in these multicenter validation studies with various epidemic MRSA clones of human origin was from 57.0 to 62.0 °C. Therefore a T_m value of 55.5 °C observed with MRSA CC398 should be discriminative with respect to most of the clinical MRSA strains, and melting curve analysis represents a reliable surrogate marker for screening purposes.

From a technical point of view, it should be noted that melting points outside the expected range of 57.0 to 62.0 °C have to be examined manually in the LightCycler software. When testing MRSA CC398 strains of the present study, the calculation algorithms embedded in the automated assay interpretation software of the LightCycler MRSA Advanced test (Micro Analysis Software; MAS) reported "MRSA result: not detected" with a specific comment "Peak(s) outside Target TM range".

Figure 3

Population snapshot of the tested isolates based on BURP analysis (n=127)



Each dot represents a single *spa* type and the diameter of the dot reflects the number of isolates associated with the respective *spa* type. Group founders are coloured in blue, Subgroup founders are coloured in light blue.

In the course of the study, we also applied a number of other commercial PCR tests targeting the SCC*mec-orfX* junction. These included the GenoType MRSA Direct (results not shown), the BD GeneOhm MRSA, and the Xpert MRSA test. The 44 investigated Bavarian MLST CC398 strains, which had all tested positive in the LightCycler MRSA Advanced test, also tested positive for MRSA in these other assays (see Table) - but these PCR test platforms either did not have an option to perform a hybridization probe melting curve analysis or did not allowviewing such melting curve data. Since clinical sensitivity of real-time PCR assays may also depend on the annealing temperatures of the respective probes, it is currently unclear whether the point mutations in the target region will have an impact on the sensitivity when testing samples from patients or animals.

Testing of non-Bavarian MRSA strains within or related to MLST CC398

In addition to the 44 strains of the Bavarian porcine LA-MRSA survey, 140 MRSA strains recovered from animals and humans in other geographical regions or from other animal sources as well as *S. aureus* isolates of spa-types sharing similar *spa* repeat patterns, were included in the present study to further address the diversity among isolates within the MLST CC398 clonal complex. Overall, 133 of the 140 isolates were successfully detected by the LightCycler MRSA Advanced test. The collection of investigated strains is shown in detail in the Table, together with the characteristic T_m values observed in the LightCycler MRSA Advanced test and the corresponding results of supplementary S. aureus- and *mec*A-specific PCR assays, as well as the results obtained in other commercial PCR tests targeting the SCC*mec-orfX* junction. While seven isolates were not detectable, 130 isolates were associated with T_m values of 55.5 °C in the LightCycler MRSA Advanced test, indicative of the presence of the novel SNPs, and three isolates were associated with T_m values of 59 °C, known to be within the range observed for the epidemic MRSA clones of human origin.

All of the applied real-time PCR assays, which are designed for direct detection of MRSA by targeting the SCC*mec-orfX* integration site, failed to generate specific amplification products with seven (3.8%) of the investigated MRSA strains (Table). The MRSA phenotype of these strains was confirmed by diagnostic culture including oxacillin susceptibility testing. In addition, the MRSA genotype was confirmed by an in-house duplex PCR assay targeting the *mec*A gene and a *S. aureus*-specific species marker. SCC*mec* typing of these seven isolates revealed that one was associated with SCC*mec* IVa, one with SCC*mec* VII and five isolates were not typeable using a common SCC*mec* typing approach.

A population snapshot based on the BURP algorithm was performed for all MRSA isolates included in the study (Figure 3). For arithmetical reasons, three isolates characterised by a T_m of 55.5 °C (two t1456 isolates and one t2510 isolate, all typed as MLST ST398) were excluded from spa cluster formation by BURP because they were shorter than five repeats. The snapshot showed that all remaining 127 isolates associated with T_m values of 55.5 °C clustered into one *spa*-CC. This *spa*-CC comprised the major *spa* types t011 and t034 shown to be associated with MLST ST398. This spa-CC contained a further 20 spa types sharing closely related spa repeat patterns: t108, t567, t571, t898, t1197, t1250, t1255, t1451, t1457, t1580, t1606, t1793, t2011, t2346, t2370, t2576, t2582, t2741, t3423 and t5706.

Moreover, six of the seven isolates not detected by the LightCycler MRSA Advanced test clustered in this *spa*-CC. MLST typing revealed that five isolates (two of *spa* type t034 and one each of types t567, t571 and t1606) were associated with ST398, and one isolate associated with *spa* type t898 was MLST ST753 (90-35-19-2-20-26-39), which is closely related to ST398 (3-35-19-2-20-26-39). Thus, all these six isolates were part of the CC398 complex. The remaining isolate not detected by the LightCycler MRSA Advanced test was associated with *spa* type t021 (ST30).

Those three strains that were characterised by a T_m of 59.0 oC in the LightCycler MRSA Advanced test showed spa types t127 (ST1), t138 (ST30) and t1430 (ST9).

Discussion and conclusions

Although a number of comprehensive studies have been published on the molecular characterisation and detection of the CC398 clonal MRSA lineage using PFGE, MLST or other techniques based on genome sequencing [1,16], this is the first report on a truly rapid detection and/or screening method for this livestock-associated clonal lineage based on characteristic SNPs within a popular target sequence of MRSA-specific PCR assay.

Here, 184 different LA-MRSA isolates obtained from various geographic regions in several European countries and from different sources including pigs, horses, dogs, guinea pigs, chicken, poultry as well as associated in-contact humans were systematically investigated for a characteristic SNP-induced Tm-shift in the LightCycler MRSA Advanced test.

The novel SNPs within the *S. aureus* chromosomal *orfX* gene detected in the investigated LA-MRSA isolates seemed to represent a conserved sequence motif for these MRSA strains. Even if seven of 184 MRSA strains (six of which were LA-MRSA CC398) were not picked up by the assays due to the presence of uncommon SCC*mec* elements, it can be stated that the investigated commercial PCR tests targeting the SCC*mec-orfX* junction showed acceptable inclusivity rates for members of the MRSA CC398 complex. A *spa* type population snapshot applying the BURP algorithm showed that all MRSA isolates characterised by the SNP-induced Tm-shift in the LightCycler MRSA Advanced test clustered into a distinct *spa* clonal complex indicative for CC398. Therefore, the novel SNPs within the *S. aureus* chromosomal *orfX* gene sequences could serve as a discriminative marker for MRSA belonging to the CC398 complex.

It is a well known fact that primer and probe sequences of the current PCR assay concepts are designed to cover the most common SCC*mec* types encountered in clinical MRSA isolates. With our increasing knowledge about the enormous sequence diversity of SCC*mec* sequences, rational primer selection and assay design can only be a best compromise between the coverage of as many SCC*mec* variants as possible and loss of analytical sensitivity due to primer multiplexing problems in the PCR reaction mixture.

In the course of the present study, we identified a powerful additional feature of the commercial Roche LightCycler MRSA Advanced test. This observation is another example for the fact that the natural diversity of MRSA is also reflected on genomic level. The more isolates are tested for a given target sequence, the more nucleotide mutations or deletions may be encountered. This fact has also implications on the design of specificity panels when developing assays. The assay panel covering epidemiologically relevant clones frequently encountered in patients at risk for MRSA infection is not necessarily congruent with the spectrum of variant isolates that may be found in a specific geographical or epidemiological setting (e.g. introduction of LA-MRSA lineages into a hospital setting). A recent study by Bartels *et al.* [25] highlighted this problem reporting on a variant SCC*mec* type IVa clone (*spa* t024 ST 8) circulating in Copenhagen, which was not detected by a commercial real-time PCR assay targeting the SCC*mec-orfX* junction.

Now that characteristic SNPs have been identified, colleagues may verify our findings with their collections of animal-associated MRSA strains and may check the primer and probe sequences of their individual in-house PCR protocols targeting the SCC*mec-orfX* junction for the ability to cover and/or to discriminate MRSA CC398 from human MRSA clones.

If the LightCycler MRSA Advanced test was implemented in a diagnostic laboratory for the intended purpose of direct detection of MRSA in clinical specimens, the occurrence of presumptive MRSA CC398 strains could be monitored without extra work or extra cost just by looking at the melting curve screen. In combination with the simple 'boiling'-protocol for template DNA preparation, it can be easily integrated into the workflow of any clinical or veterinary laboratory routinely using molecular techniques for diagnostic purposes. Once growth of staphylococci is observed on agar plates, a portion of the colony can be transferred to PCR and discriminative MRSA results can be available within 80 minutes. Moreover, knowing about our study results, users of this assay will no longer be confused by the comment "Peak(s) outside Target TM range" generated by the automated assay interpretation software.

In conclusion, the characteristic SNP-induced Tm-shift found in the LightCycler MRSA Advanced test was shown to be suitable to rapidly identify LA-MRSA CC398 clones. By simultaneous screening for general MRSA carriage as well as for MRSA CC398 carriage, this commercial real-time PCR test or comparable assay designs may help to monitor the spread of MRSA CC398 in the human population and, in particular, its importation into healthcare settings. Moreover, this approach may be helpful in screening for MRSA CC398 carriage among animals, farmers or other risk groups.

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Viral hepatitis, HIV, human herpes virus and Treponema pallidum infection in haemodialysis patients from Kosovo, 2005

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The serological status of hepatitis viruses and other infectious diseases in the 66 dialysed patients of one haemodialysis unit in Kosovo were studied, comparing the data with a large group of blood donors and out-patients. All dialysed patients were hepatitis A virus (HAV) positive. Prevalence of hepatitis B surface antigen (HBsAg), hepatitis B surface antibodies (anti-HBs), and hepatitis B core antibodies (anti-HBc) was 14 of 66, 21% (95% confidence interval (CI): 12-33%), 5 of 66, 8% (95%CI: 5-22%), and 50 of 66, 76% (95%CI: 64-85%), respectively. Antibodies to hepatitis C virus (anti-HCV) prevalence was 57 of 66, 86% (95%CI: 76-94%). No human immunodeficiency virus (HIV) positive case was found. Prevalence of past herpes simplex virus type 2 (HSV-2) infection was 29% (95%CI: 18-41%). Two patients (3%, 95%CI: 0-10%) were positive for *Treponema pallidum* and 18% (95%CI: 10-30%) were human herpesvirus 8 (HHV-8) antibody positive. Four hundred and fifty-two subjects were recruited for comparison. Markers of past HAV infection was associated with haemodialysis (Fisher's exact test p-value=0.037). Dialysed patients were at a higher risk of being HBsAg positive than others: the sex- and age-adjusted odds ratio (OR) was 5.18 (95%CI: 1.87-14.32). Anti-HBc positivity was strongly associated with haemodialysis: the sex- and age-adjusted OR was 6.43 (95%CI: 3.22-12-85). Anti-HCV positivity was 86% and 1% in presence and absence of haemodialysis, respectively. The Fisher's exact test for association proved a strong association between haemodialysis and HCV (p-value<0.0001). The OR for association between haemodialysis and HSV-2 positivity was 3.20 (95%CI: 1.46-7.00). Significant associations were also observed between haemodialysis status and antibodies to *Treponema pallidum* (Fisher's exact test p-value=0.044). In Kosovo, the prevalence of viral hepatitis infection and other viral infections and *Treponema pallidum* among dialysed patients is high, indicating major ongoing nosocomial transmission.

Introduction

The population of Kosovo has suffered substantially after the break-up of the former Yugoslavia in the early 1990s and the consequent armed conflict in 1999. Recently, the region has acquired a national autonomy, with some limitations of sovereignty and with the support of the European Union [1]. In 2006, the population was estimated at 1.9 million and was one of the youngest in Europe. About 37% lived in poverty; unemployment was estimated at around 40%, with a gross domestic product per capita of 834 EUR in 2006 (468 EUR in 2000) [2]. Health indicators remained among the most unfavourable in the Balkan region. The annual per capita government expenditure on health care was 35 EUR, the lowest in Europe. Kosovo had one of the highest perinatal mortality rates (23 per 1,000 live births) in Europe and the number of physicians per 1,000 inhabitants was 0.94 [3]. The transition to more modern concepts of health care management presented a challenge to health personnel and the population after the war. Currently, the healthcare system consists of primary centers located in each municipality, secondary health care facilities at the regional level (five hospitals), and tertiary health care centers (University of Pristine and a few other specialised institutions).

After the conflict, the number of end-stage renal disease (ESRD) patients progressively increased in Kosovo: from 190 in 1999 to approximately 600 in 2007. The rate of patients in DC treatment in Kosovo is 286 per million, lower than in other Central and Eastern European countries [4]. At the time of our study, patients were treated in six different dialysis centres (DC), with standard twice or three times a week five hour dialysis sessions (10% and 90%, respectively). We examined patients at the DC in Peja hospital which had no special areas dedicated to patients with positive history of hepatitis.

A number of reports have shown that viral hepatitis B (HBV) and viral hepatitis C (HCV) are common among ESRD patients [5-7]. In the dialysis centres of Kosovo and of other Eastern European countries, the prevalence of such infections has been poorly investigated. The few existing studies suggest that the prevalences are higher in patients dialysed in this part of Europe compared

with other European countries [8-11]. The aim of this study was to analyse the prevalence of viral hepatitis and other infections such as HIV, HVS-2, HHV-8 and syphilis in the ESRD patients of the hospital in the Peja region. Furthermore, we wanted to investigate whether the haemodialysis was associated with an elevated risk of infections. Our study was part of a survey carried out in the period 2004-2007 during a training project for healthcare workers at

Table 1

General characteristics of the 66 haemodialysis patients, compared to 452 non-haemodialysed patients (n=518)

Characteristics of patients		Haemodialysis				Chi-square homogeneity test
		Yes		No		
		N	%	N	%	p-value
Sex	Females	27	41	296	65	<0.01
	Males	39	59	156	35	
Age	18-30	3	4	185	41	<0.01
	30-50	21	32	220	49	
	50+	42	64	47	10	
Domicile	Urban	21	32	173	38	0.31
	Rural	45	68	279	62	
Education	≤8	52	79	105	23	<0.01
	>8	14	21	347	77	
Married	No	8	12	165	63	<0.01
	Yes	58	88	287	37	
Employed	No	42	64	109	24	<0.01
	Yes	24	36	343	76	
Blood transfusion	No	2	3	442	98	<0.01
	Yes	64	97	10	2	
Dialysis (months)	0-24	28	42	-	-	-
	24-48	13	20	-	-	-
	48+	25	38	-	-	-
Pts. always in the same unit	No	5	7	-	-	-
	Yes	61	93	-	-	-
Total		**66**	**100**	**452**	**100**	

Table 2

Seroprevalence of viral hepatitis, HIV, HSV-2, *Treponema pallidum* and HHV-8 of patients in haemodialysis, compared to non-haemodialysed patients

Serology	Haemodialysis				Fisher's exact test p-value	Crude OR (95%CI)	Sex- and age-adjusted OR (95%CI)
	yes		no				
	N	%	N	%			
HAV[1]	66	100	424	94	0.037	NE	NE
HBsAg[2]	14	21	16	3	**<0.0001**	**7.34 (3.39,15.89)**	**5.18 (1.87,14.32)**
HBsAb[3]	5	8	69	15	0.13	0.45 (0.18,1.17)	**0.27 (0.09,0.79)**
HBcAb[4]	50	76	107	24	**<0.0001**	**10.08 (5.51,18.42)**	**6.43 (3.22,12.85)**
HBV vax[5]	2	3	0	0	**0.016**	NE	NE
HDV[6]	1	1	0	0	0.127	NE	NE
HCV[7]	57	86	3	1	**<0.0001**	947.89 (249.39,3602.83)	NE
HIV[8]	0	0	0	0	1	NE	NE
HSV-2[9]	19	29	45	10	**<0.0001**	**3.66 (1.98,6.77)**	**3.2 (1.46,7)**
T. pallidum[10]	2	3	1	0.2	**0.044**	**14.09 (1.26,157.66)**	NE
HHV-8[11]	12	18	-	-	-	-	-

In bold: results significant at an alpha ≤ 0.05. Abbreviations used: OR: odds ratio; NE: not estimable; 1: hepatitis A virus; 2: hepatitis B surface antigen; 3: hepatitis B surface antibody; 4: hepatitis B core antigen: 5: HBV vaccinated subjects; 6: hepatitis delta virus; 7: hepatitis C virus; 8: human immunodeficiency virus: 9: human herpes virus 2; 10: Treponema pallidum; 11: human herpes virus 8

the hospital in the Peja region, supported by the Veneto Regional Health Authority and the Italian Co-operation Agency [12].

Methods

Field work for this cross-sectional study was conducted from 1 January 2005 to 30 March 2005. The association between the prevalence of viral hepatitis and other infections and the haemodialysis status was assessed by comparing the ESRD patients with a group of blood donors and subjects who had been examined for routine laboratory testing. In addition, the scientific literature was reviewed to compare the HBV and HCV prevalence of patients in DC of different Eastern and Western European countries.

Study population

All 66 ESRD patients treated at the DC of Peja regional hospital were enrolled in the study. Candidate blood donors being screened for donation suitability and individuals (18 years of age and older) who had undergone routine check-ups in two clinics in Peja and whose serum was sent for routine testing, were included in the study as a comparison group. In order to approximately randomise the group, patients screened on Monday, Wednesday and Friday were selected. In the three months of the study period, 285 blood donors and 187 subjects examined in clinics were potentially eligible for comparison. Out of the total number of 472 subjects, 20 refused to be tested or to respond to the questionnaire. The final number of 452 subjects was recruited. Approval from the Kosovo Institute of Public Health, the Regional Health Authorities

Table 3

HBsAg prevalence in haemodialysis centres in Western and Eastern European countries. Data on the general population is reported for comparison

Country	General population	Year	Reference	Haemodialysis Centres	Year	Reference
North European countries						
Germany	0.60%	1998	Thierfelder	4.60%	2001	Burdick
UK	<0.5%	2001	Eurohep	<0.5%	2001	Burdick
South European countries						
Italy	1%	2001	Eurohep	4.30%	2001	Burdick
Spain	1.70%	2001	Solà	3.10%	2001	Burdick
Eastern European countries						
Moldovia	9%	2004	Emiroglu	17%	1999	Covic
Romania	6%	2001	Eurohep	22%	1998	Vladutiu
Bulgaria	5%	2001	Eurohep	-	-	-
Serbia	-	-	-	15%	1999	Djukanovic

Table 4

HCV prevalence in haemodialysis centres in Western and Eastern European countries 1997-2001. Data on the general population is reported for comparison

Country	General population	Year	Reference	Haemodialysis centres	Year	Reference
North European countries						
Germany	0.60%	1999	Esteban	3.80%	2003	Fissell
UK	1%	2001	Bird	2.60%	2003	Fissell
South European countries						
Italy	3.50%	1997	Esteban	20.50%	2003	Fissell
Spain	2.50%	2001	Dominguez	22.90%	2003	Fissell
Eastern European countries						
Moldavia	5%	1997	Covic	75%	1999	Covic
Romania	6%	2001	Esteban	73%	1998	Vladutiu
Bulgaria	3%	2001	Esteban	48%	2008	Atanasova
Poland	2%	2001	Esteban	44%	1999	Jadoul
Hungary	0.50%	2001	Müller	15%	1999	Jadoul
Serbia	-	-	-	23%	1999	Djukanovic

and the Ethical Committee of the Peja region was obtained and a signed informed consent form from each participant was requested before entering the study.

Questionnaire

For all study participants information on socio-demographic characteristics and information related to haemodialysis treatment were collected by local physicians and nurses, interviewing patients using a structured questionnaire. The questionnaire included queries on age, sex, occupation, education, area of residence, partner status, length of dialysis treatment, number of transfusion received and if the patient remained always in the same unit of treatment. The serum was collected for laboratory investigations.

Laboratory investigations

The collected serum was tested for the following hepatitis markers: total anti-HAV (IgG and IgM), HBsAg, anti-HBs, total anti-HBc (IgG and IgM), and anti-HCV using AxSYM microparticle enzyme immunoassay (MEIA) (Abbott Diagnostics, North Chicago IL). HBsAg-positive subjects were tested for antibodies to hepatitis delta virus (anti-HDV IgG) using a commercial enzyme-linked immunosorbent assay test (ELISA) (DiaSorin, Saluggia, Italy). A line immunoassay (LIA) (INNO-LIA HIV I/II Score, Innogenetics N.V., Gent, Belgium) was used for detecting antibodies to HIV type 1 and 2, and samples that were reactive were confirmed with Western blot. To detect anti-HSV-2 antibodies, a commercial HSV-2 specific IgG enzyme immunoassay (EIA) (HSV 2 IgG EIA WELL, Radim, Pomezia, Italy) was used. IgG and IgM antibodies to *Treponema pallidum* were detected by a *Treponema pallidum* recombinant EIA (Syphilis Screening Recombinant EIA WELL, Radim, Pomezia, Italy). HHV-8 serum antibodies were detected by a commercially available ELISA assay (HHV-8 IgG Elisa, Advanced Biotechnologies Incorporated, Columbia, MD, Unites States). All tests were performed according to the manufacturer's instructions at the Istituto Superiore di Sanità Laboratory, Rome, Italy, and partner institutions.

Statistical analysis

Prevalence of viral hepatitis and other infectious diseases in haemodialysis patients was estimated and 95% confidence intervals (CI) calculated. We tested whether viral hepatitis and other infectious diseases were associated with haemodialysis by comparing seroprevalence in dialysis patients to seroprevalence in two comparison groups: blood donors and subjects who had been examined in clinics. At a first stage, association was tested separately in dialysis patients vs. blood donors, and in dialysis patients vs. patients who had been examined in clinics. Provided that the estimates were homogeneous in the two analyses, the two groups were pooled together to form a unique comparison group. To account for data sparseness, association was tested by means of Fisher's exact test. Odds ratios (OR) and 95% CI were calculated using logistic regression models. All statistical analyses were performed using R 2.8.0 [13].

Results

Sixty-six haemodialysis patients were recruited (males: 59%, mean age: 55±14 years). The patient characteristics are reported in Table 1. The duration of haemodialysis treatment ranged from 12 to 264 months (median time 48 months). Concerning the aetiology of ESRD, glomerulonephritis was the first cause (20 cases, 30%), followed by diabetes mellitus (12 cases, 18%), pyelonephritis (9 cases, 14%), hypertension (7 cases, 10%), polycystic kidney diseases (4 cases, 6%), and systemic diseases (2 case, 3%). Aetiology was unknown for 12 cases (19%) of haemodialysis patients.

When comparing the distribution of hepatitis status in ESRD patients with subjects not undergoing haemodialysis, we found consistent results. Here we present results to the comparison between haemodialysis patients and the pooled group of comparison subjects. In total, 452 individuals (males: 35%, mean age: 34±11 years) were recruited for comparison. Participants' characteristics were all heterogeneous between haemodialysis and non-haemodialysis patients, except for the domicile (p-value=0.31) (Table 1).

Serological status of dialysed patients

All ESRD patients were HAV positive indicating previous infection (Table 2). Prevalence of HBsAg, HBsAb, and HBcAb was 14 of 66, 21% (95%CI: 12-33%), 5 of 66, 8% (95%CI: 5-22%), and 50 of 66, 76% (95%CI: 64-85%), respectively. Two patients had been vaccinated for HBV. One male patient in his late forties was the only patient positive for HDV: he was also positive for HAV, HBV (HBcAb) and anti-HCV. HCV prevalence was 57 of 66, 86% (95%CI: 76-94%). Concerning the co-occurrence of HBV and HCV in haemodialysis patients, we observed that 45 (70%, 95%CI: 58-81%) were both HBV (HBcAb) and HCV, 10 (16%, 95%CI: 8-27%) had HCV but no HBV, five (8%, 95%CI: 3-17%) had HBV but no HCV, and four (6%, 95%CI: 2-15%) had none (Fisher's exact test p-value=0.096).

No HIV positive case was found. Prevalence of HSV-2 was 19 of 66, 29% (95%CI: 18-41%). Two patients (3%, 95%CI: 0-10%) were positive for *Treponema pallidum* and 12, 18% (95%CI: 10-30%) were HHV-8 positive.

HAV was associated with the haemodialysis status (Fisher's exact test p-value=0.037). Given that all dialysed patients were HAV positive, the estimation of OR was not possible. ESRD patients were at a higher risk of being HBsAg positive than others: sex- and age-adjusted OR was 5.18 (95%CI: 1.87-14.32). When additionally adjusting for the level of education, employment, marital status, and domicile, the OR increased up to 7.92 (95%CI: 2.31-27-12). HBcAb positivity was strongly associated with haemodialysis: the sex- and age-adjusted OR was 6.43 (95%CI: 3.22-12-85); it increased slightly when further adjusting for education, employment, marital status, and domicile as well to OR 6.9 (95%CI: 3.17-15.03). HCV prevalence was 86% and 1% in presence and absence of haemodialysis treatment, respectively. For ESRD patients and the comparison group an OR could not be calculated. However, the Fisher's exact test for association proved a strong association between haemodialysis and HCV (p-value<0.0001). The OR for association between haemodialysis and HSV-2 positivity was 3.20 (95%CI: 1.46-7.00) when adjusting for sex and age, and rising up to 6.44 (95%CI: 2.40-17.27) when further adjusting for education, employment, marital status, and domicile. Significant associations were also observed between haemodialysis status and *Treponema pallidum* status (Fisher's exact test p-value=0.044). Results of the association study are reported in Table 2.

Prevalence of HBV and HCV in DC of Eastern and Western European countries

Table 3 shows the prevalence of serological markers for HBV in DC of Northwestern European countries, Southwestern European countries and Eastern European countries [8,10,14,15]. Table 4

shows the difference for HCV prevalence among DC in Northwestern European countries (UK, Germany) Southwestern European countries (Italy, Spain) and different Eastern European countries [8-11,14,16].

In the majority of the Eastern countries, the prevalence is over 40%, with more than 70% in Romania and Moldavia. Unlike HBV the HCV prevalence in the general population of Eastern countries is in some cases lower than in Western countries (Table 4) [8, 22-25].

Discussion

The prevalence of viral hepatitis and other agents among ESRD patients in the current study indicates a very high level of endemicity. Twenty-one per cent of patients were found to be HBsAg carriers and more than 78% had been exposed to the virus (anti-HBc positive), with a sex- and age-adjusted six-fold risk when compared to non-haemodialysis patients. In analysing the data in the literature, it was found that approximately 20% of dialysis patients are chronic carriers of HBV in Eastern Europe, compared to approximately 4 % in Western countries. On the other hand, the general population carriage is at least three times higher than in Western Europe (Table 3) [17-20].

Kosovo is a country with a low prevalence of HCV infection [12,21]. This was reflected in the group of non-hemodialysis patients, where the prevalence of HCV was as low as 1%. Nevertheless, the prevalence of HCV in dialysed patients was strikingly high (86%). It was not possible to calculate the OR with the observed numbers. However, the great difference should suggest that, even taking into account potential differences between the two groups compared in this study, hemodialysis should be considered a strong risk factor for HCV infection, in line with the results of other studies carried out in Eastern European countries [8-10,15]. The HCV prevalence in DC in Western versus Eastern countries differs: from around 2% in Germany and the United Kingdom (UK) to 20% in Spain and Italy and up to 50-70% in Eastern European countries [8,15]. In the Dialysis Outcomes and Practice Patterns Study (DOPPS), the mean prevalence of HCV infection in five Western European countries (France, Germany, Italy, Spain and the UK), Japan and the United States (US) was 13% [16].

In Europe, the overall prevalence of HBV and HCV in ESRD patients has been decreasing over the years as a result of HBV vaccination, routine screening of blood products, and the use of recombinant human erythropoietin [6,11,16]. Guidelines for universal precautions - 'Recommendations for preventing transmission of infections among chronic hemodialysis patients' - had been initially recommended by the US Centers for Disease Control and Prevention (US, CDC) in 1985 and successively updated [26]. In Kosovo, erythropoietin started to be used in 2004 but with marked differences between centres. The percentage of haemodialysis patients receiving erythropoietin in Kosovo is, to date, less than 50%. The situation appears to be improving slightly, but precise figures are not available. Screening of blood-donors for blood-borne viruses has only been implemented regularly since 2001. No immunisation policy for hepatitis vaccination existed in general in Kosovo before the war. In Kosovo there is the policy for HBV vaccination of haemodialysis patients and medical staff. The lack of available vaccines hampers its implementation; for example, in 2005 the percentage of vaccinated individuals among the 253 health care workers of the Peja hospital was 16.6% [12]. An important measure for the control of hepatitis infection is the segregation of positive patients and their haemodialysis equipment [27]. Until recently, the lack of resources prevented this practice in Kosovo.

In our study the syphilis prevalence (anti-*Treponema pallidum* IgG) among dialysed patients was 3%, much higher than the 0.2% of non-dialysed subjects. There is little data on syphilis seroprevalence in DC patients. Sexual contact is the primary mode of transmission of syphilis, but blood transfusion, blood contact and accidental inoculations are other modes of infection that place ESRD patients at risk. A report from Taiwan showed a prevalence of syphilis among dialysed patients of 5.6% [28]. In a more recent study, the syphilis seroprevalence in 167 ESRD patients was 6.7%, more than two times higher than the overall prevalence reported in the general population [29].

HHV-8 is a gamma-herpes virus, closely related to the Epstein-Barr virus. We do not have data to compare our study population with the Kosovar general population. Nevertheless, in nearby Albania, HHV-8 seroprevalence in the general population is reported to be 20% [30]. Transmission of HHV-8 infection through blood, although suggested, is controversial. A case-control study performed in 97 dialysed patients from Northern Italy found a prevalence of 9.2% (in this geographic area the prevalence of HHV-8 in blood donors was 12.7%) [31]. In Greece, HHV-8 prevalence in 485 dialysed patients was 7.2% [32]. In Southern Italy, the seroprevalence of HHV-8 among ESRD patients was 27% (comparable to 25% as observed in the general population) [33].

In Kosovo, the prevalence of infection from viral hepatitis,HHV-8, HSV-2 and Treponema pallidum among ESRD patients is high, indicating major ongoing nosocomial transmission. Even though this may be a consequence of limited resources available, targeted recommendations could be implemented to improve the current situation:

- rigorous attention should be paid to infection control procedures such as changing gloves between patients and the decontamination of equipment and surfaces after each patient treatment episode;
- all single-use injectable medications and solutions should used on a single patient, and all parenteral medications should be prepared in a clean area separate from potentially contaminated items and surfaces;
- hepatitis B vaccination should be given to all patients and staff [34];
- HBsAg and HCV positive patients and their dialysis equipment should be segregated;
- periodic diagnostic testing of patients and healthcare workers needs to be carried out;
- dialysis providers should be aware of their responsibility to report clusters of infections to the local health authorities, as the failure to report illness clusters can result in delays in the recognition of disease outbreaks; and
- training for health care workers should be implemented periodically.

Our study has several limitations that have to be emphasised. As the data were restricted to one DC, the results presented here cannot be considered indicative for Kosovo as a whole and figures on serological status of the health personnel are not available. Furthermore data on the incidence of infectious diseases after the regular screening of blood transfusion for blood-borne viruses were implemented (2001) are not available; and information on

possible risk factors is also missing. In Kosovo further studies on the prevalence and incidence of blood borne viruses among ESRD patients are needed, involving more than one DC, and exploring possible risk factors in these patients and settings.

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WHO CRITERIA FOR MEASLES ELIMINATION: A CRITIQUE WITH REFERENCE TO CRITERIA FOR POLIO ELIMINATION

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Smallpox was formally declared as eradicated in 1979. Smallpox is the only infectious disease of humans that has ever been eradicated. Poliomyelitis has been eliminated from three of the six World Health Organization (WHO) regions although not all countries within those regions always meet the elimination criteria. Elimination criteria for measles are being discussed. We use poliomyelitis and measles as examples to illustrate our assertion that the current approach to documenting measles elimination relies too heavily on criteria for surveillance quality, disadvantaging countries with long established and relatively inflexible surveillance systems. We propose an alternative approach to documenting measles elimination, with the two key criteria being molecular evidence to confirm the lack of a circulating endemic genotype for at least one year and maintenance of 95% coverage of one dose of measles-containing vaccine, with an opportunity for a second dose. Elimination status should be reviewed annually. We suggest four principles that should guide development of final criteria to document measles elimination: countries that have eliminated measles should be able to meet the elimination criteria; quality surveillance criteria are necessary but not sufficient to define elimination; quality surveillance criteria should be guided by elimination criteria, not the other way around; and elimination criteria should not differ between the WHO regions without good reason.

Introduction

Smallpox is the only infectious disease of humans that has been successfully eradicated, with a formal declaration made in December 1979 [1]. At this time, eradication was defined by the World Health Organization (WHO) as the absence of circulating wild virus, manifested as no cases in a defined geographic area for a period of at least three years after cessation of vaccination.

In 1988, the World Health Assembly resolved to eradicate polio globally by the year 2000. The eradication of poliovirus requires zero cases of poliomyelitis due to wild poliovirus for three years, high quality disease surveillance which meets international standards, and demonstrated capacity of the countries to detect, report and respond to imported polio cases, including those caused by vaccine-derived polioviruses. In addition, laboratory stocks need to be contained and safe management of polio vaccine manufacturing sites assured before the world can be certified as polio-free [2]. Eradication by 2000 was not achieved, but in 2009, polio remained endemic in only four countries. The eradication of polio is now seen as an achievable goal within the next four or five years [3], although some commentators question even this timeline.

More recently, goals for progress towards measles elimination, rather than eradication, have been proposed by a number of WHO regions, including the European and Western Pacific Regions. Member states of the Western Pacific Region, which include Australia, have resolved to eliminate measles by 2012 [4]. The European region aims to eliminate measles by 2010 [5]. Elimination is defined as the sustained interruption of transmission of endemic virus within a defined geographic region. Sustained endemic transmission is defined as an outbreak of more than 100 cases or ongoing transmission with a measles genotype of identical sequence for more than three months [6]. Elimination does not imply that there is no virus within the defined region (this is eradication), but that the *transmission of endemic virus has been eliminated* [6].

We aim to review the criteria used to define polio eradication and measles elimination in the Australian, European and other international context and discuss alternatives to the criteria for the documentation of the elimination of measles.

Australia and polio

As a member state of the Western Pacific Region, Australia was declared free of circulating endemic poliovirus only in October 2000 [7], although the last case of endemic poliovirus infection probably occurred around 30 years earlier [8]. The cornerstone of the documentation of polio-free status is surveillance of patients presenting with acute flaccid paralysis (AFP), the most common clinical presentation of acute poliovirus infection, although such cases represent only between one in 100 and one in 1,000 cases of infection [9].

The WHO criteria for adequate AFP surveillance are

- An annual notification rate of one case presenting with acute flaccid paralysis per 100,000 population aged under 15 years,
- Collection of two stool samples 24 hours apart within 14 days of symptom onset from 80% of notified cases,

- Testing of stool samples in a WHO-accredited laboratory to exclude wild poliovirus as the cause of the patient's symptoms [9].

Although countries where polio had been endemic in the recent past have met these criteria, Australia has consistently failed to do so. Of the 14 years that AFP surveillance has been undertaken in Australia, the targets for case ascertainment have been achieved in only five years (Figure) and the criteria for stool collection have never been met [10].

We have previously shown, at least for the state of Victoria and by inference for other Australian states, that it was not a lack of AFP cases that led to notification rates below the WHO target, but incomplete notification of cases [11]. Despite not meeting the WHO AFP surveillance criteria for the maintenance of the documentation of polio-free status, Australia, as a member state of the polio-free Western Pacific Region, is nonetheless acknowledged to have no circulating wild poliovirus.

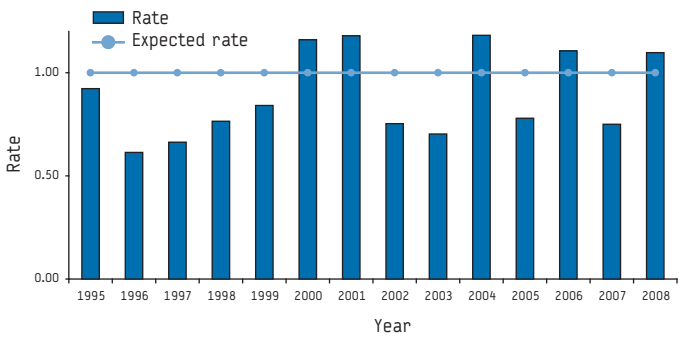
Australia and measles

We have previously reviewed the body of evidence to demonstrate that Australia has eliminated the transmission of endemic measles [12]. Although we acknowledged that measles virus was still detected in Australia, we argued that the transmission of endemic measles virus has been eliminated, based on criteria we compiled using the evidence for Australia [12]:

- Absence of an endemic genotype since 1999,
- High proportion of cases imported or linked to an imported case since 1999,
- Containment of outbreaks without the re-establishment of a specific genotype since 1999,

Figure

Acute flaccid paralysis notification rate per 100,000 population under 15 years of age, Australia 1995-2008



The WHO performance indicator expected rate is >1 notification of AFP per 100,000 population aged <15 years.

Table 1

Australia's documentation of measles elimination compared with the criteria proposed by the WHO Western Pacific Regional Office, September 2007

Western Pacific Regional Office criterion for progress towards measles elimination	Criterion status in Australia
1. Confirmed measles cases <1 per million	Met in 2005 and 2007; not met in 2006 or 2008
2. Reported suspected measles cases >2 per 100,000	Not available at a national level; met in the state of Victoria since 1999
3. At least 80% of districts reporting >1 per 100,00 suspected cases	Data not collected at a national level
4. At least 80% of cases investigated within 48 hours	Data not available at a national level
5. At least 80% of cases with adequate blood samples collected	Data not available at a national level
6. At least 80% of cases with laboratory results within seven days	Data not available at a national level
7. At least 80% of clusters with samples for virus isolation	Data not available at a national level
8. Two-dose MCV coverage >95%	MCV1 >95% and MCV2 >90%
9. At least 80% of clusters with <10 cases	Data not available at a national level
10. Absence of endemic measles virus	No endemic measles virus since 1999

MCV: measles-containing vaccine; WHO: World Health Organization.
Adapted from Heywood et al. [12].

Table 2

Alternative criteria for the documentation of measles elimination

Criterion	Justification
The absence of an endemic measles genotype for at least 12 months	Based on the criterion by which England and Wales declared the re-establishment of endemic viral transmission [18].
One dose MCV coverage >95% with the opportunity for a second dose.	One dose of MCV administered at the age of 12 months with coverage >95% was modelled to be more likely to maintain elimination status than a two-dose regime [19]. The failure to maintain high measles vaccine coverage led to measles becoming again endemic in England and Wales [18].

MCV: measles-containing vaccine.

- Maintenance of an effective reproductive number for measles <1 since 1999,
- Serological evidence of population immunity >90% since 2002,
- Consistently high two-dose vaccination coverage since 2004: >95% for the first dose of measles-containing vaccine (MCV) and >90% for the second dose of MCV,
- <1 notified confirmed endemic case per million population since 2005.

We examined Australia's ability to meet the criteria proposed by the Western Pacific Regional Office (WPRO) in 2007 for the documentation of progress towards measles elimination in member states of the Western Pacific Region (Table 1) [12,13].

The first WPRO criterion requires a national incidence of less than one confirmed measles case per million population. A confirmed case includes laboratory-confirmed cases, cases epidemiologically linked to a laboratory-confirmed case, or clinically confirmed cases; imported cases are excluded. In Australia, national surveillance data are not adequate to demonstrate the proportion of cases that are imported. In both 2005 and 2007, less than one case per million was reported in Australia, inclusive of imported cases. However, an importation leading to a widespread outbreak in 2006 resulted in a notification rate exceeding six cases per million population. Cases in 2008 also exceeded one case per million population. We are unable to quantify the number of confirmed measles cases in 2006 and 2008 that were not imported or directly related to importation [14].

In the first quarter of 2009, 78 cases of measles were notified in Australia, of which 17 were related to importation [15]. Large outbreaks occurred in Queensland and Victoria and smaller outbreaks occurred in other states. In the three months from January to March alone, the number of indigenous cases exceeded an annual notification rate of one per million inhabitants. However, extensive case follow-up and genotyping confirmed that the outbreaks were due to several different genotypes (D4, D8, D9 and H1) and that no one genotype has been circulating for more than 12 months.

The next six WPRO criteria relate to setting surveillance standards for suspected case investigation. Australia is unable to meet any of these criteria (Table 2). The final three criteria refer to vaccine coverage (≥95% two-dose MCV coverage), proving that 80% of outbreaks have fewer than 10 cases and demonstrating the absence of an endemic measles genotype. Australia meets only the third of these criteria. However, in addition to the WPRO criteria, Australia has demonstrated a measles immunity exceeding 90% in the population in serological surveys [12], and a number of disease modelling studies have consistently estimated that the reproductive number for measles was less than one in a number of studies from Australia, indicating that endemic measles transmission cannot be sustained [12].

Measles elimination in other countries

In order of the year of declaration, nine countries – Finland, Cuba, England and Wales, Brazil, Mexico, Canada, the United States (US), South Korea and Australia – have publicly declared measles elimination using a variety of criteria (listed in Table 2 of the paper by Heywood *et al*. [12]). However, unlike the other countries in this list, the Australian government has not formally ratified the declaration of measles elimination in Australia. The mode and median number of the 10 WPRO criteria that these countries satisfied was two (range: one to eight). South Korea, which satisfied eight of the 10 criteria, and Australia, which satisfied only two, are the only two nations in the Western Pacific Region whose declaration might be constrained by WPRO criteria. Finland, which has remained measles-free for 25 years, reports only the two criteria of low incidence and high vaccine coverage [16].

It is clear that disease elimination cannot be declared in the absence of high quality laboratory-enhanced surveillance. Reflecting this, the WPRO criteria for progress towards measles elimination include a number of specific laboratory indicators for high quality surveillance. In countries such as England and Wales, the US and Australia, specific WHO performance indicators for surveillance are difficult to satisfy. These countries were approaching measles elimination prior to the publication of the WHO elimination criteria, and development of national surveillance systems preceded the smallpox and polio eradication programmes. Collating and summarising surveillance data from different state and local sources at a national level is often difficult. Some developed countries such as the US, did not attempt to justify their polio-free status through AFP surveillance [2]. Surveillance systems in these countries were established outside the WHO framework, and do not have routine mechanisms to capture the surveillance process data specified by the WHO and reflected in the WPRO guidelines for the documentation of the eradication of polio or the elimination of measles. England and Wales declared measles elimination in 2003 prior to the establishment of formal elimination criteria [17] The laboratory-enhanced measles surveillance system of England and Wales does not meet all the surveillance benchmarks specified by WPRO criteria. Despite this, the system rapidly detected the re-establishment of endemic measles in England and Wales in 2008 [18]. Furthermore, the experience of England and Wales demonstrates the critical fact that elimination is an ongoing task. While wild virus is circulating elsewhere, vaccine coverage needs to remain high to prevent the re-establishment of sustained transmission of measles virus.

Reviewing the evidence which England and Wales used to declare elimination before acknowledging the re-establishment of endemic measles transmission illustrates the relative importance of elimination criteria [17,18] Measles elimination was declared in England and Wales using the following evidence [17], with the relevant WPRO criteria in brackets:

- MCV1 coverage of over 90% until 1998 (WPRO criterion: two-dose coverage at least 95%),
- Average number of measles cases of 1.8 per million inhabitants per year 1995-2001 (WPRO criterion: <1/million/year),
- Small number of large clusters, four clusters with 10-24 cases and four clusters with 25 or more cases (WPRO criterion: ≥80% of outbreaks or transmission foci with <10 cases),
- 23% of sporadic cases and 43% of clusters linked to a known imported case (no specified WPRO criterion),
- Suspected measles case identification rate ca. 4.4 per 100,000 per year (WPRO criterion: >2/100,000) with 66% tested (WPRO criterion: >80% tested),
- Wide variety of genotypes with absence of previous endemic genotype (WPRO criterion: no endemic genotype),
- Effective measles reproductive number estimated as 0.5-0.7 by a variety of methods (no specified WPRO criterion).

England and Wales, as part of the WHO European region, are not bound by the WPRO criteria for assessing progress towards measles elimination, but other WHO regions are proposing similar criteria. The WPRO criteria are used here to illustrate the comparison

of evidence for elimination with published criteria for assessing progress towards elimination required in one WHO region. Moreover, it is reasonable to expect that a country that has eliminated measles should satisfy criteria assessing the progress towards elimination. The interim criteria from the WHO Regional Office for Europe that would guide member states in declaring elimination [5] include the following:

- Vaccination coverage: achieving and maintaining at least 95% coverage with MCV1 and MCV2 in all districts and nationally;
- Outbreak size: At least 80% of outbreaks should have less than 10 confirmed measles cases;
- Incidence: Achieving a measles incidence of less than one confirmed case per million population per year, excluding cases confirmed as directly imported;
- Endemic measles virus strain(s): zero cases of measles caused by an endemic strain for at least 12 months, i.e. evidence of the absence of endemic transmission by demonstrating zero cases of measles or zero cases with identical genotype sequence over a period of 12 months.

Guidelines for measles elimination criteria in the European region are currently in late draft form, but a recently published review of progress towards measles elimination in Europe confirms the inclusion of the vaccine coverage and measles incidence criteria [5]. A number of surveillance criteria have also been added to the elimination criteria:

- 100% of member states should report monthly to WHO on measles cases;
- 80% of member states should submit at least 80% of case-based reports each month, and submit at least 80% of reports on time.

When declaring measles elimination in 2003, England and Wales did not satisfy the criteria related to vaccine coverage or measles incidence. In addition, the surveillance criteria were not reported at the time.

Measles elimination criteria: an alternative approach

The experience of all countries that have eliminated measles highlights a general problem with WHO criteria for progress towards elimination. It is not possible for most countries that have clearly eliminated measles to meet the criteria for progress towards elimination. This is a strange anomaly.

Since elimination criteria are yet to be finalised, we suggest that consideration be given to documenting measles elimination using only two criteria:

- The absence of an endemic measles genotype for at least 12 months,
- One-dose MCV coverage of at least 95% with an opportunity for a second dose.

In conjunction with suitable surveillance standards, these criteria could also be used for assessing progress towards elimination. Justification for these criteria is presented in Table 2.

Table 3 evaluates the two proposed alternative criteria for measles elimination against evidence presented by the nine countries declaring elimination. All countries reported on measles vaccine coverage targets and all except England and Wales satisfied this criterion. Only Finland and Mexico did not provide evidence of the absence of circulating genotypes, but would without doubt be able to report on these criteria on an annual basis.

Although not absolutely necessary, these criteria could be supported by the demonstration of a reproductive number of less than one for measles and the estimation of at least 90% population immunity. While low measles notification rates are important, we believe that a number of confirmed cases under one per million is

Table 3

Assessment of alternative criteria for measles elimination by countries declaring measles elimination

Country declaring measles elimination and year of declaration	Alternative elimination criteria	
	Absence of an endemic measles genotype for at least 12 months	One-dose MCV* coverage of at least 95% plus opportunity for second dose
Finland, 1994	Not reported	>97% two-dose coverage
Cuba, 1998	Reported absence of circulating virus	One-dose coverage 98% with catch-up campaigns
England and Wales, 2003	Variety of circulating genotypes confirmed	MCV1 coverage >90%; MCV2 introduced in 1996
Brazil, 2003	No endemic genotype	>95% two-dose coverage since 1997
Mexico, 2004	Not reported	>95% coverage at age 1-6 years since 1996; >97% coverage at age 6-10 years since 1999
United States, 2004	No endemic genotype	>90% coverage at age 19-35 months; 98% coverage at school entry; >92% of school children immune
Canada, 2004	No endemic genotype since 1998	MCV1 coverage >95%; MCV2 introduced in 1996
Republic of Korea, 2006	No endemic genotype	>95% two-dose coverage; 93% of school children immune
Australia, 2008 (declaration not endorsed by national authority)	No endemic genotype since 1999	MCV1 coverage >95% MCV2 coverage >90%

MCV: measles-containing vaccine.
Adapted from Heywood et al. [12].

not a necessary requirement for elimination to be declared, because of residual susceptibility in young adults documented in a number of countries [20-22] and because there is an increased risk of transmission within susceptible groups that may have religious or other objections to vaccination. It is, however, necessary to demonstrate that an importation of a specific measles genotype into a susceptible subgroup does not result in transmission of that measles genotype in the wider population over a period of more than 12 months, as has occurred in England and Wales. In Australia, 22 confirmed cases notified in a year will exceed the threshold of one confirmed case per million. Small outbreaks among young adults resulting from importations have regularly resulted in higher numbers of annual cases during the period when there was no endemic measles genotype [23]. These importations have not led to the re-establishment of endemic measles transmission in Australia.

Surveillance criteria are important for the documentation of the elimination of endemic measles transmission. Using the proposed alternative elimination criteria, it is only critical that cases and clusters are identified and that a suitable specimen is sent to a WHO-accredited laboratory for genotype identification. As already recommended by WHO, all suspected cases of measles should have a serum sample sent to an accredited laboratory for testing measles IgM by a commercial enzyme-linked immunosorbent assay. We further suggest that a suitable specimen for genotyping, preferably a nose/throat swab [24], should be collected from all serologically confirmed cases that are not part of clusters and from a minimum of two cases at the start and two cases at the end of any identified cluster. Placing the emphasis on identifying the absence of an endemic genotype over a 12-month period requires efforts to be focussed on genotype capture, rather than performing individual serological tests within a nominated time. If using the alternative criteria suggested here, it would not be necessary to confirm a case within seven days as is specified in the WPRO criteria. However it would still be necessary to collect a specimen suitable for genotype identification not more than two weeks after rash onset [24]. When countries do not have a national laboratory that is able to perform measles genotyping, appropriate specimens could be referred to a regional laboratory for genotyping, with all results reported to the WHO in order to monitor international transmission patterns [25].

The WPRO criteria related to outbreaks (criteria 7 and 9, Table 1) can be subsumed into the single criterion of complete absence of endemic measles genotype (criterion 10). While it may be difficult to find all cases that are not part of a cluster, all countries with an active surveillance system should be able to recognise clusters. In Finland, where measles has been eliminated for 25 years, it is noted that 'some sporadic imported cases may have escaped our attention, but clusters of secondary cases would almost certainly have been detected had they occurred' [16].

Conclusions

Despite best intentions and a considerable amount of effort, Australia has not been able to maintain WHO AFP surveillance criteria for the documentation of polio eradication [26]. However, it is accepted that Australia is free of circulating wild poliovirus, the single most important criterion for eradication. We have provided evidence to support our claim that Australia has eliminated measles transmission, but cannot satisfy the criteria for documenting progress towards elimination promulgated by the WHO WPRO. Neither has this evidence resulted in a formal declaration of measles elimination in Australia. Incidentally, we note that the WHO position on the status of measles elimination in Australia is not completely clear. *The WHO document Global measles and rubella laboratory network – update published in 2005* [27], prior to presentation of evidence for measles elimination in Australia, acknowledged measles elimination in Australia. Map 1 in that document states that *'Measles has been eliminated from the Western Hemisphere and Australia'* [emphasis added] and did not include any countries from the western hemisphere or Australia on the map. The document also noted that multiple genotypes had been detected from imported cases [27]. However, a more recent WHO publication suggests that the Republic of Korea is the first and only country in the Western Pacific Region to have achieved elimination [28].

We believe it is appropriate to separate criteria for the documentation of measles elimination from surveillance performance and laboratory accreditation. We suggest it may be worth considering only two criteria for the documentation of measles elimination with an annual review of elimination status. Finally we suggest there are four principles that should guide the development of formal documentation of measles elimination:

1. Elimination criteria should be able to be met by countries that have eliminated measles;
2. Quality surveillance criteria are necessary but not sufficient to define elimination;
3. Quality surveillance criteria should be guided by elimination criteria, not the other way around;
4. Without good reason, elimination criteria should not differ by WHO region.

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Laboratory support for the diagnosis and surveillance of sexually transmitted infections (STIs) in Eastern Europe

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This report outlines the proceedings of the 4th Annual Meeting of the Eastern European Network for Sexual and Reproductive Health (EE SRH Network) [1,2], which took place at Uppsala University in Uppsala, Sweden between 30 May and 3 June, 2009. The meeting was attended by 65 network participants from 14 Eastern European countries (Armenia, Azerbaijan, Belarus, Bulgaria, Estonia, Georgia, Hungary, Kazakhstan, Kyrgyzstan, Lithuania, Russian Federation, Ukraine, Uzbekistan and Tajikistan), representatives of the World Health Organization (WHO) and invited experts from Sweden, United States, Denmark, and United Kingdom. The plenary session was followed by workshops on: (a) the development of sexually transmitted infections (STI) laboratory diagnosis guidelines, (b) surveillance of antimicrobial resistance of Neisseria gonorrhoeae and (c) epidemiological surveillance systems.

During the conference, it was emphasised, that STIs remain an unrecognised, but significant public health problem in the majority of Eastern European (EE) countries. WHO in its "Global strategy for prevention and control of STIs for 2006-2015" states that it is crucial to increase the commitment of national governments and to use integrated approaches in order to address the problem [3]. The EE SRH Network has endorsed these aims and contributed to the work of WHO by promoting cooperation on both national and regional levels and by developing international consensus approaches for the diagnosis of STIs [4].

It has long been recognised that laboratory testing plays an essential role in patient management and epidemiological surveillance of STIs. However, a survey of laboratory diagnostic methods among the network countries demonstrated that individual tests and approaches used to establish a diagnosis often do not achieve recommended international standards. For example, serological tests are used to diagnose chlamydial infection in up to 70% of clinical laboratories in several EE countries, while screening for gonococcal infections in women is largely conducted by using microscopy of Gram-stained cervical smears. In addition, few laboratories use type-specific herpes simplex virus (HSV) serology for the diagnosis of genital herpes [5].

In order to improve the quality of STI diagnostic services in the region, the EE SRH network has prepared "consensus" guidelines for the laboratory diagnosis of gonorrhoea, syphilis and chlamydial infections. These guidelines were formulated by the network participants during previous meetings, using evidence-based principles. This approach stimulated direct communication between leading experts from "East" and "West", resulting in consensus documents which were first published internationally [6-8] and then subsequently adopted and published at the national level [1,2].

During the meeting reported here, workshop participants reached consensus on further guidelines for the laboratory diagnosis of four specific infections, namely, bacterial vaginosis (BV), infections caused by Mycoplasma genitalium, trichomoniasis and genital herpes. International and national publications of these guidelines are currently in preparation [9].

It is recognised that both the quality of test kits used and the implementation of quality assurance systems contribute to the confidence in results provided and reputation of diagnostic services. STI diagnostic test kits manufactured in EE countries have rarely been internationally validated. The network has conducted a number of studies comparing Russian-manufactured tests for the detection of *N. gonorrhoeae, Chlamydia trachomatis* and *Mycoplasma genitalium* with internationally acknowledged methods, which yielded promising results [10-13]. It is clear that the regional biomedical industry has the potential for producing reliable reagents and tests kits at affordable prices; however, strict quality assurance is crucial [14]. Comprehensive evaluations of locally manufactured tests should be conducted according to internationally accepted guidelines as a prerequisite to marketing products in the region. In addition, other issues related to laboratory quality assurance have emerged as a high priority for many EE countries. The establishment of an extensive external quality assurance programme for the serological diagnosis of syphilis in Russia has revealed a number of difficulties, including lack of willingness to participate and high rates of false-positive/negative

results [15]. Such programmes should be extended to include all laboratory testing, with appropriate sanctions being implemented for those laboratories that consistently fail to provide satisfactory results.

Another factor which is necessary to assure high-quality laboratory practices is the establishment of national or regional reference laboratories for STIs, preferably supported and financed by the state authorities. At present, there are no such institutions in Eastern Europe. Such institutions could provide a source of expertise to support national or regional STI initiatives, perform reference testing and collect surveillance data. In addition, these laboratories could maintain external quality assurance (EQA) programmes, supervise updating of national STI laboratory guidelines and establish international collaborations [16].

The emergence and spread of antimicrobial resistance (AMR) among isolates of *N. gonorrhoeae* is recognised as a major concern globally. However, in the majority of the EE countries AMR testing of *N. gonorrhoeae* isolates is performed only occasionally, because gonococcal culture is rarely undertaken [17]. At the EE SRH meeting, a workshop to establish AMR surveillance of *N. gonorrhoeae* in the network countries was conducted at the Swedish Reference Laboratory for Pathogenic Neisseria (Örebro University Hospital). Representatives from Russia, Belarus, Estonia, Georgia, Ukraine and Kazakhstan adopted WHO protocols regarding culture, identification and AMR testing for *N. gonorrhoeae* and received WHO quality control strains and reagents to enable them to initiate collection of *N. gonorrhoeae* strains for AMR testing. The remaining countries will be supported in order to overcome some technical difficulties before the collection of strains can commence.

Most EE countries inherited complicated and labour-intensive communicable disease surveillance systems. STI surveillance is mostly suboptimal owing to old-fashioned, non-standardised, paper-based surveillance systems and the absence of computer-based statistical tools [18]. Furthermore, legal constraints have shown to be a potential barrier for good STI surveillance [19]. Surveillance systems for STIs differ from one country to another depending on the availability of laboratory services and the accessibility of healthcare-provider institutions. However, all countries should strive to nationwide establish, implement, and maintain as high quality laboratory service as possible considering resource constraints [20].

During a one-day epidemiological surveillance workshop, participants were introduced to a computer-based system for communicable disease surveillance (ULISAS), developed as a result of a joint Lithuanian-Swedish project [21]. Lithuanian and Belarusian epidemiologists presented their recent experiences with this programme. The system is already being used as a national tool in Lithuania; while in Belarus, it has been fully implemented in the capital, Minsk and is currently being introduced in other parts of the country [22]. Discussions on the adaptation and implementation of the system by other EE SRH countries are in progress.

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Rhinoviruses, A(H1N1)v, RSV: The race for hivernal pandemics, France 2009-2010

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To the editor: The A(H1N1)v circulation in France, like in other European countries (Sweden), is still reported as sporadic. The incidence of A(H1N1)v infections monitored in the community by the French National Influenza Centre has remained stable for 6 weeks from week 37 to week 42 (159 cases per 100,000 inhabitants). This is right above the epidemic cut-off of 114 cases per 100,000 inhabitants two months after the start of the new school year. This delay in the A(H1N1)v outbreak expansion is puzzling. At the same time, we report a high rhinovirus activity (34.5 % of samples positive for rhinovirus) in the community and in the hospital (unpublished data).

It has been postulated by A. Linde *et al.* [1] that the viral interaction between the A(H1N1)v and the rhinoviruses may explain partly this delay. This is an interesting hypothesis, indeed it is well known [2,3] that during winter, rhinovirus, respiratory syncytial virus (RSV) and influenza viruses epidemic peaks happen one after the other and occasionally overlap. The seasonal epidemiology of influenza is surely dependent on weather conditions such as low relative humidity and cold temperature [4]. These features were observed in our laboratory last winter.

Indeed, during the 2008-2009 winter, our laboratory analysed samples from the paediatric hospital of Lyon. The laboratory diagnosis was based on cellular culture for RSV and influenza viruses detection and on specific RT-PCR technique for the influenza and the rhinoviruses detection. Between week 31 of 2008 and week 9 of 2009, 6516 respiratory samples (nasal swabs or nasopharyngeal aspirates) were analysed (culture and PCR) in our laboratory. The number of confirmed rhinoviruses, RSV and Influenza A viruses is reported week by week in the Figure.

This year, rhinovirus detection started on week 37, peaked on week 40 and decreased on week 43. At that moment, we can report the first detection of RSV and an increasing activity of A(H1N1)v. Regarding what was observed during last winter on the circulation of rhinovirus, RSV and A(H3N2) virus , it will be of much interest to follow the impact of the A(H1N1)v pandemic on the coming RSV peak. In other words, which respiratory virus between RSV or A(H1N1)v, will win the race for second place ?

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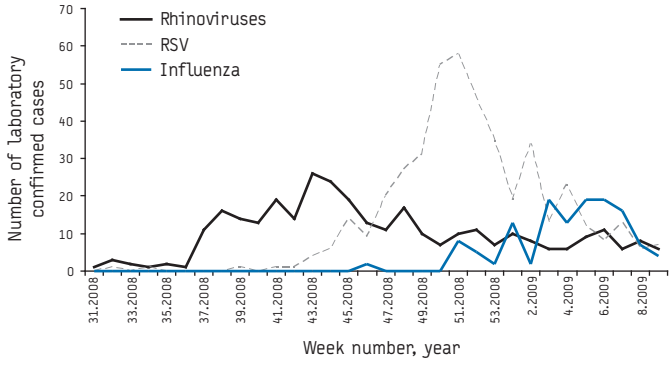
Figure

Number of laboratory confirmed cases of rhinovirus, RSV and influenza A during autumn and winter 2008-2009, Lyon



Letters

Author's reply

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To the editor: It is interesting to see that several other countries have the same experience as Sweden concerning the rhinovirus and its interaction with influenza A(H1N1)v. Further studies will show the impact of co-circulation of viruses and how the weather affects the transmission of viruses

First isolations of KPC-2-carrying ST258 Klebsiella pneumoniae strains in Finland, June and August 2009

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The first two *Klebsiella pneumoniae* carbapenemase-producing (KPC) type 2 strains carrying ST258 were detected in Finland in June and early August 2009. They were found colonising two patients transferred from the Mediterranean; one patient referred from a hospital in Greece where isolates were first found in 2007 and another from Italy where the first isolates have been described only very recently.

Case 1

The first carbapenemase-producing *Klebsiella pneumoniae* (KPC) strains in Finland were detected this summer in two patients transferred to Helsinki University Central Hospital (HUCH) from Crete, Greece, and northwestern Italy, respectively. Case 1 was a patient transferred from Greece at the end of June 2009. In Greece, the patient was initially hospitalised at a ward but later transferred to the intensive care unit, due to pneumonia and acute myocardial ischemia. The clinical history upon referral to our hospital did not mention antibiotic treatment although it is highly probable that antibiotics were used when the patient was first admitted to hospital in Greece.

Since the patient arrived to the HUCH intensive care unit from a high risk epidemic area where carbapenemase-carrying strains are common, a stool sample was tested for extended spectrum beta-lactamase (ESBL) and carbapenem resistance, using ESBL Chrom-ID agar (bioMérieux, Marseille, France) detecting both ESBLs and AmpC at the HUCH laboratory. *Klebsiella pneumoniae* grew on this plate; from this isolate, a direct KPC PCR was done, and sequencing of the PCR product confirmed the gene to be bla_{KPC-2}. Antibiotic susceptibility was tested using Etests (AB Biodisk, Solna, Sweden). The isolate was resistant or intermediately resistant to all antibiotics except trimethoprim-sulphamethoxazole and gentamicin (Table). Case 1 later died of multiorgan failure, not from infection related to the KPC strain.

Case 2

Case 2 was a patient transferred to Finland from north-western Italy in mid- August, after having been hospitalised for ten days during a trip due to seizures, unconsciousness and anaemia caused by an underlying alcohol-induced liver cirrhosis and total red cell aplasia. The clinical history upon referral to our hospital did not mention antibiotic treatment, however, it is highly probable that antibiotics were used at the hospital in Italy. The patient was found to have a chronic sacral wound from which a swab was taken and analysed at the HUCS laboratory.

K. pneumoniae grew on the culture plate and the isolate was further analysed as it showed high level resistance to all β-lactams, including carbapenems. It remained susceptible only to colistin and gentamicin (Table). The isolate was found to be positive for bla_{KPC-2} by PCR and sequencing. Case 2 later died from the multiple underlying conditions unrelated to the KPC strain.

Table

Minimum inhibitory concentration (MIC) profiles of the carbapenem-producing *Klebsiella pneumoniae* isolates*, Finland, June-August 2009 (n=2)

Antibiotic	Case 1	Case 2
Piperacillin/tazobactam	>256	>256
Cefuroxime	>256	>256
Ceftazidime	>256	>256
Cefotaxime	>256	48
Aztreonam	>256	>256
Ertapenem	32	>32
Imipenem	8	>32
Meropenem	32	>32
Colistin	24	0.19
Doxicycline	6	6
Minocycline	4	3
Tigecycline	2	2
Amikacin	48	32
Gentamicin	2	2
Tobramycin	16	12
Trimethoprim/sulphamethoxazole	0.38	>32

*Both isolates were also resistant to levofloxacin, cefpodoxime, cefpodoxime/clavulanic acid, ceftazidime/clavulanic acid and cefotaxime/clavulanic acid, tested using Oxoid disks (Oxoid, Basingstoke, UK).

Results

The isolates were sent for multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) typing to the National Institute for Health and Welfare, where the PCR results were confirmed. Both strains contained bla_{TEM-1}, and pyrosequencing identified an ESBL variant of bla_{SHV} with Gly to Ser and Glu to Lys mutations at positions 238 and 240, respectively. PCR was negative for CTX-M, VIM-, IMP-, OXA-48 and GES-genes. Hydrolysis of imipenem was confirmed by spectrophotometric analysis of crude cell extracts. MLST [1] showed that both isolates belonged to the epidemic clone ST258. PFGE showed the strains to be somewhat similar (80%) to each other, as also found in other studies on this clone [2,3].

Conclusions

KPC-producing *Klebsiella pneumoniae* was first detected in North Carolina, USA, in 1996 [4]. After first only causing local epidemics on the east coast of the USA during the end of the 1990's and at the beginning of the new millennium [5,6], the KPC epidemic now seems to be accelerating [7].

Both Finnish isolates belonged to the clone ST258, which has been shown to account for probably 70% of the KPC-positive *K. pneumoniae* isolates sent to the US Centers for Disease Control and Prevention (CDC) [2]. It has also been found in Norway and Sweden in patients transferred from Greece and Israel in 2007 [3], in an outbreak strain in Israel [2] as well as in isolates in Poland [8] and Italy [9]. No doubt, MLST of KPC strains from around the world will find that many older isolates also belong to this clone. The resistance varies between isolates of this clone; gentamicin is the only antibiotic effective against all isolates. The Norwegian, Swedish and Polish isolates were reported to contain the beta-lactamase TEM-1 and ESBL beta-lactamases SHV-11 or -12, similarly to our strains, although we have not yet confirmed which SHV ESBL our strains contain.

The first Swedish and Norwegian cases were described at the end of 2007 [10,3]. The preparedness level in Finland was also increased at this time, by educating the clinical microbiology laboratories, and establishing reference methods.

Currently there is no compulsory screening programme for carbapenemase-producing pathogens at national level in Finland, but the authors would strongly recommend that patients transferred from abroad should be screened. Chromogenic ESBL-selective plates seem to be a fast, simple and presumably sensitive tool to detect carriers of multiresistant gram-negative bacteria for this purpose. In addition to ESBLs they detect KPC strains, and in our experience possibly also metallo-betalactamase-carrying strains. At least stool/rectal samples should be tested, preferably also swabs from the oropharynx and axillae [11].

Fortunately, the largest tertiary care hospital in Finland stayed alert to the threat of KPC colonised patients and was thus able to detect these two strains. The epidemic spread of carbapenemase-carrying strains from colonised patients is well-documented [12], and should be taken seriously.

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Experience of European intensive care physicians with infections due to antibiotic-resistant bacteria, 2009

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A survey was performed among European intensive care physicians to obtain information about their perception and experience with selected antibiotic-resistant bacteria. Seventy-eight out of 95 (82%) participants considered having to deal with infections due to antibiotic-resistant bacteria in the intensive care unit where they work was a major or significant problem. Methicillin-resistant *Staphylococcus aureus* (MRSA) and third-generation cephalosporin-resistant *Enterobacteriaceae* were the most frequently reported antibiotic-resistant bacteria with 69 (73%) and 67 (71%) participants reporting having treated at least one patient with such an infection during the preceding six months, respectively. Antibiotic-resistant Gram-negative bacteria, including carbapenem-resistant *Enterobacteriaceae*, were more frequently reported than any selected antibiotic-resistant Gram-positive bacteria, with the exception of MRSA. Fifty (53%) participants declared having treated at least one patient infected with a bacterium totally or almost totally resistant to available antibiotics during the past six months, with 8 participants having treated more than 10 such patients and 13 having treated from 3 to 10 such patients.

Introduction

Antibiotic resistance is a threat to public health and compromises appropriate therapy of infected patients, in particular for infections in the most severely ill in hospitals [1,2]. Increasingly, intensive care physicians in Europe and elsewhere are confronted with patients infected by bacteria for which limited or no adequate therapeutic options are available [2-4]. Data on the situation of antibiotic resistance in Europe are provided by the European Antimicrobial Resistance Surveillance System (EARSS) [5], however these data are not specific for patients in intensive care units (ICUs). There are studies on antibiotic resistance in European intensive care patients, but these are limited to only a few ICUs and countries [1,6-7]. Additionally, there is little data on infections with bacteria that are totally or almost totally resistant to antibiotics that are currently emerging in Europe [8]. In an attempt to obtain information on the perception and experience of European intensive care physicians on infections caused by antibiotic-resistant bacteria, a survey was conducted through the European Society of Intensive Care Medicine (ESICM) among its members in 2009. We report here the first results of this survey.

Methods

The survey was designed by the European Centre for Disease Prevention and Control (ECDC) with input from an ECDC/European Medicines Agency (EMEA) Joint Working Group [9] and then proposed to the Scientific Committee of ESICM. The survey included questions about the experience of the respondent with intensive care medicine and antibiotic prescribing, as well as about the ICU in which they work. It also included questions about perception of the respondent of the problem of antibiotic resistance and the number of patients that were treated, during the preceding six months in the ICU where they work, for infections caused by each of the antibiotic-resistant bacteria listed in the table. These antibiotic-resistant bacteria were selected because they are, in most cases, multidrug-resistant.

Participants gave answers on their experience during the past six months following a semi-quantitative scale: “often” (> 10 patients), “sometimes” (3-10 patients), “rarely” (1-2 patients) and never. The survey was endorsed by ESICM through its European Critical Care Network in March 2009. It was then posted on the ESICM website in its section “Survey of the month” in the beginning of April 2009 and was closed on 8 June 2009.

Results

Characteristics of participants

After excluding responses issued from participants from non-European countries or non-ESICM members, 95 responses were analysed. Responses were obtained from European ESICM members from 24 countries: Austria (2 participants), Belgium (5), Croatia (2), Denmark (2), France (4), Germany (8), Greece (3), Hungary (1), Ireland (1), Israel (1, ESICM includes this country among European countries), Italy (14), Lithuania (1), Luxembourg (1), Montenegro (1), Netherlands (1), Portugal (12), Romania (5), Serbia (1), Slovakia (1), Spain (12), Sweden (2), Switzerland (1) and United Kingdom (14).

Among the participants, the median time since graduation as MD was 20 years (25th-75th percentiles: 13-27 years). Seventy-nine (83%) participants were intensive care medicine specialists with a median time since specialisation of 11 years (25th-75th percentiles: 4-18 years). Eleven participants were still in training and five had a different specialty than intensive care medicine. Seventy-five (79%) ICUs were medico-surgical ICUs with a median

size of 10 beds (25th-75th percentiles: 7-16 beds) and a median of 510 admissions per year (25th-75th percentiles: 350-850). To the question "How often do you personally prescribe antibiotic therapy to ICU patients?", 88 (93 %) responded "commonly (> 10 patients per week)" or "often (≥3 patients per week)".

Perception of and experience with antibiotic-resistant bacteria

Having to deal with infections due to antibiotic-resistant bacteria in the ICU where they work was considered as a major or significant problem by 78 (82%) participants. The experience of the participants of treating patients with infections due to the selected antibiotic-resistant bacteria is summarised in the Figure. Among Gram-positive bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) was the most frequently reported with 69 (73%) participants reporting having treated at least one patient with an MRSA infection during the preceding six months. Vancomycin-resistant *Enterococcus* spp. (VRE) and penicillin-resistant *Streptococcus pneumoniae* were much less frequently reported, and vancomycin-resistant or -intermediate *S. aureus* (VRSA/VISA) was the least frequently reported antibiotic-resistant

Table

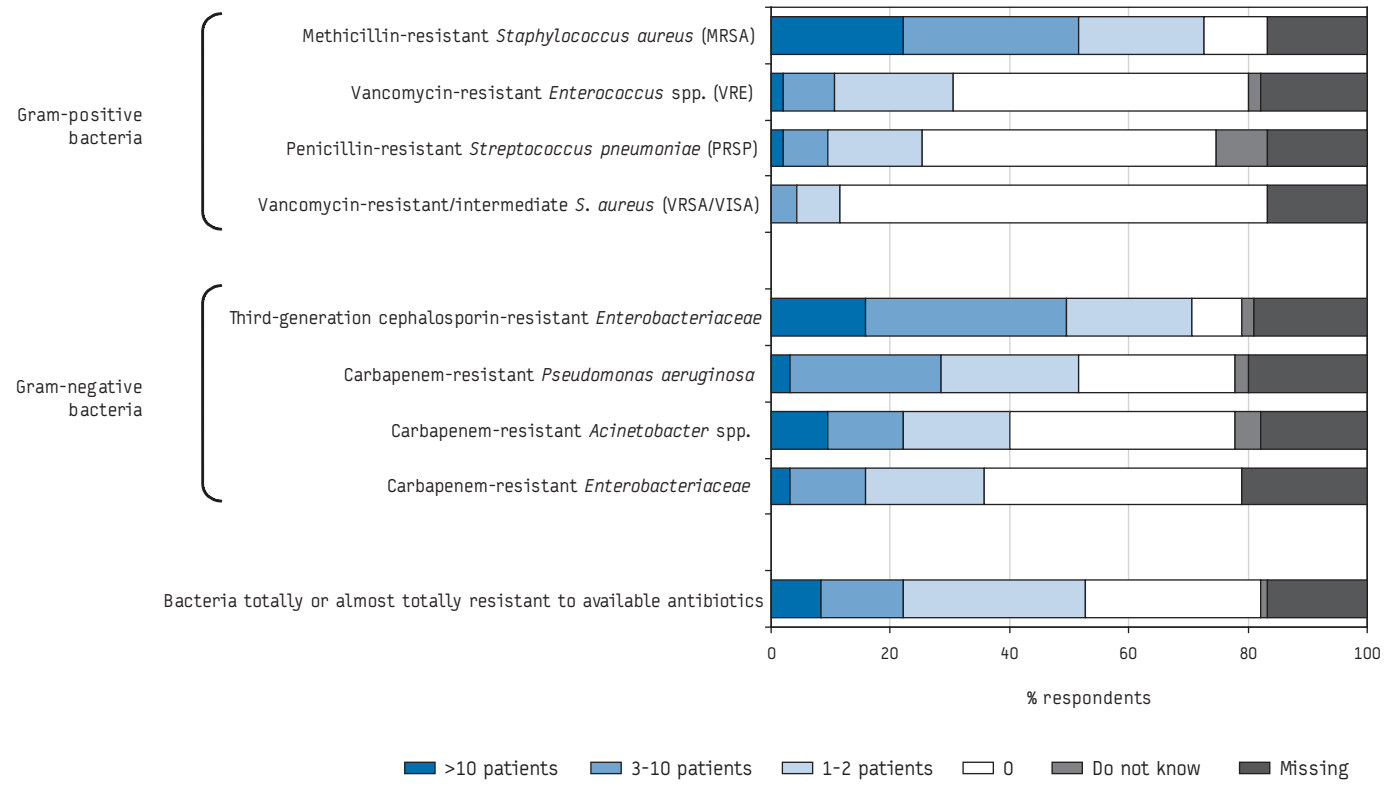
Antibiotic-resistant bacteria selected for the European intensive care physicians survey, 2009

Gram-positive bacteria	Methicillin-resistant *Staphylococcus aureus* (MRSA)
	Vancomycin-resistant/intermediate *S. aureus* (VRSA/VISA)
	Vancomycin-resistant *Enterococcus* spp. (VRE)
	Penicillin-resistant *Streptococcus pneumoniae*
Gram-negative bacteria	Third-generation cephalosporin (cefotaxime or ceftazidime or ceftriaxone)-resistant *Enterobacteriaceae* (e.g. *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp.)
	Carbapenem (imipenem or meropenem)-resistant *Enterobacteriaceae*
	Carbapenem-resistant *Pseudomonas aeruginosa*
	Carbapenem-resistant *Acinetobacter* spp.
Bacteria totally or almost totally resistant to available antibiotics*	

*Any Gram-positive or Gram-negative bacteria considered as totally or almost totally resistant to available antibiotics based upon the appreciation of the participant. In addition, the participant was asked to specify the name of these bacteria.

Figure

Percentage of participants who reported having treated patients with infections due to selected antibiotic-resistant bacteria during the past six months, 2009 (n=95 participants)



Gram-positive bacteria with only 11 (12%) participants reported having treated such patients during the preceding six months. Among Gram-negative bacteria, third-generation cephalosporin-resistant *Enterobacteriaceae* were the most frequently reported with 67 (71%) participants reporting having treated at least one patient with such an infection during the preceding six months. Other selected antibiotic-resistant Gram-negative bacteria were less frequently reported. The least reported antibiotic-resistant Gram-negative bacterium, i.e. carbapenem-resistant *Enterobacteriaceae*, was more frequently reported than any selected antibiotic-resistant Gram-positive bacteria with the exception of MRSA (Figure). Forty-eight (51%) participants reported having treated at least three patients in two or more of the selected categories of antibiotic-resistant bacteria during the preceding six months, thus showing that antibiotic resistance problems faced by the participants in the unit where they work are often not limited to one single antibiotic-resistant bacterium.

Finally, 50 participants declared having treated at least one patient infected with a bacterium totally or almost totally resistant to available antibiotics during the past six months. Moreover, 8 participants declared having treated more than 10 such patients and 13 participants declared having treated from 3 to 10 such patients during the past six months (Figure). Forty-two participants mentioned the names of these bacteria totally or almost totally resistant to available antibiotics or the names of any other antibiotic-resistant bacteria that posed a problem when considering patient therapy in the ICU where they work. Among the 55 bacteria mentioned, most were Gram-negative bacteria: *Pseudomonas* spp. (mentioned 23 times, mostly *P. aeruginosa*), *Acinetobacter* spp. (17 times), *Stenotrophomonas maltophilia* (9 times) and *Enterobacteriaceae* (5 times). *Enterococcus* spp. was only cited once.

Discussion

In hospitals, intensive care units are considered as areas where antibiotic resistance problems are the largest due to the combination of multiple factors. These factors include the concentration of severely ill patients requiring specialised care, the high frequency of use of medical devices and the high frequency of antibiotic treatment [1]. Not surprisingly, most intensive care physicians that participated in the survey felt that antibiotic resistance was a major or significant problem in their practice.

Overall, the picture of antibiotic resistance in Europe provided by this study is similar to that provided by EARSS [5], with MRSA and third-generation cephalosporin-resistant *Enterobacteriaceae* being the most frequently antibiotic-resistant bacteria encountered by European intensive care physicians. The survey also confirmed the observation of a recent joint technical report of ECDC and EMEA which showed that, with the exception of MRSA, the burden of antibiotic resistance in Europe was now mostly due to antibiotic-resistant Gram-negative bacteria [9]. In addition, it showed that many European intensive care physicians are facing patients with infections due to bacteria, mostly Gram-negative, totally or almost totally resistant to available antibiotics. The ECDC/EMEA joint technical report showed that there were very few new antibiotics with a novel mechanism of action in development to meet the challenge of multidrug-resistant bacteria, in particular to treat infections due to Gram-negative bacteria [9]. Patients with infections due to carbapenem-resistant Gram-negative bacteria often require the use of old and toxic antibiotics such as colistin [3, 8].

This study has several limitations. Firstly, it is based on the voluntary declaration of a small fraction of the more than 5,000 ESICM members. This is likely to have resulted in selection bias towards the more concerned ESICM members, in particular from southern Europe. Although the survey instructions explicitly mentioned that only one intensive care physician per ICU should participate in the survey, we cannot exclude duplicate participation from the same ICU. Finally, participants had to answer retrospectively on their experience during the preceding six months, which may have resulted in recall bias and may be the reason for approximately 20 % of missing information. The data presented here, however, are likely to be an underestimate of the situation in the included ICUs since most participants with missing information on specific antibiotic-resistant bacteria considered infections with antibiotic-resistant bacteria in the ICU where they work as a major or significant problem. Despite these limitations, the study provides a first snapshot, based on recalled recent experience, of the current antibiotic resistance problems faced by European intensive care physicians when treating patients. It also highlighted the problem of infections due to totally or almost totally resistant bacteria, which are not covered by existing surveillance systems. More comprehensive studies are now needed to assess the extent of the prevalence of such infections with totally or almost totally resistant bacteria as well as to determine the risk factors for colonization and infection with these bacteria. In the meantime, intensive care and other physicians should be made aware of their current emergence in Europe.

Acknowledgements

The authors thank ESICM members who participated in the survey as well as Professor Jean-Daniel Chiche, President of the ESICM Scientific Committee and Professor Claude Martin, past President of the Infectious Diseases Section of ESICM. They would also like to thank members of the ECDC/EMEA Joint Working Group [9] for their input when designing the survey and preparing the questionnaire.

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Decrease of hypervirulent Clostridium difficile PCR ribotype 027 in the Netherlands

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After the first outbreaks of *Clostridium difficile* PCR ribotype 027 (North American pulsed-field type 1, restriction endonuclease analysis group BI) in the Netherlands in 2005, a national surveillance programme for *C. difficile* infection (CDI) was started. Furthermore, national guidelines were developed to rapidly recognise type 027 infections and prevent further spread. The mean incidence of CDI measured in 14 hospitals remained stable throughout the years: an incidence of 18 per 10,000 admissions was seen in 2007 and 2008. Between April 2005 and June 2009 a total of 2,788 samples were available for PCR ribotyping. A decrease was seen in the number and incidence of type 027 after the second half of 2006. In the first half of 2009, the percentage of type 027 isolates among all CDI decreased to 3.0%, whereas type 001 increased to 27.5%. Type 014 was present in 9.3% of the isolates and *C. difficile* type 078 slightly increased to 9.1%. We conclude that currently there is a significant decrease in type 027-associated CDI in the Netherlands.

Since the new hypervirulent strain of *Clostridium difficile*, PCR ribotype 027, North American pulsed-field type 1 (NAP1), restriction endonuclease analysis (REA) group BI, was found in the United States and Canada in 2001, a large number of countries worldwide reported *C. difficile* infections (CDI) due to this type [1,2]. Several reports indicated that CDI due to type 027 is associated with a higher morbidity and mortality and also has the tendency to relapse more frequently [3-6]. An overview published in July 2008 revealed that type 027 was detected in 16 European countries and was associated with outbreaks in Belgium, Finland, France, Germany, Ireland, Luxembourg, the Netherlands, Switzerland and the United Kingdom [7]. As of July 2008, outbreaks have also been reported in Austria [8] and Denmark [9].

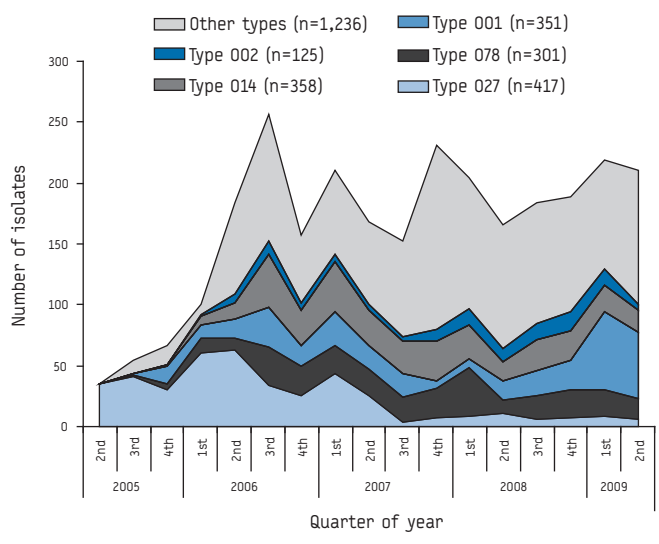
Soon after the first outbreaks in the Netherlands in 2005, a national surveillance programme for *C. difficile* was initiated by the Leiden University Medical Centre (LUMC) and the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment. All medical microbiologists in the Netherlands were requested to send *C. difficile* isolates to the Dutch national reference laboratory at the LUMC for rapid PCR ribotyping and characterisation in case of an outbreak (more than two CDI cases within one week in one department) or when a patient suffered from severe CDI. In addition, a prospective, three year-long surveillance study of the incidence of CDI and the distribution of the *C. difficile* PCR ribotypes was started in 14 Dutch hospitals in June 2006.

In the period between April 2005 and June 2009, a total of 3,137 samples were submitted to the reference laboratory, of which 89% (n=2,788) were available for PCR ribotyping. Of those 2,788 samples, 51% had been submitted by medical microbiologists because of either severe disease or a CDI outbreak, whereas the remaining 49% were part of the national surveillance study. Since no difference in the distribution of various PCR ribotypes was found between the two surveillance systems, we represent the data combined. The reason for this equal distribution is that most hospitals that encountered an outbreak or a case of severe CDI, continued to submit samples on a regular basis thereafter.

The Figure depicts the distribution of the five most common PCR ribotypes in the Netherlands between April 2005 and June 2009. Although the total number of submitted samples increased from 35 in the second quarter of 2005 to a steady number between 150 and 250 after the first quarter of 2006, a decrease in the number of type 027 isolates has been observed since the second half of 2006. In the 14 hospitals participating in the continuous surveillance, a

Figure

***C. difficile* PCR ribotypes in the Netherlands, April 2005 – June 2009 (n=2,788)**



decrease in the quarterly incidence of type 027 (number of isolates per number of admissions) was seen. This decrease was confirmed in linear regression and remained significant after adjustment for the number of samples that we received (p=0.03).

In the first half of 2009, type 027 was found in 3.3% of the 430 submitted samples. Type 001 (n=118; 27.4%) was the most common type, followed by type 014 (n=40; 9.3%), 078 (n=39; 9.1%) and 002 (n=19; 4.4%). We also encountered a number of isolates that did not match a PCR ribotype in our database and belonged to different, yet unknown types (n=49; 11.4%). These are currently subject of further investigation. Finally, of all isolates in the first two quarters of 2009, 35.1% belonged to 41 different PCR ribotypes, which were present in small numbers. Types 015 (n=15; 3.5%), 056 and 087 (both 2.6%), 017 and 046 (both 1.9%) were the five most frequently found types among those. The types that could not be matched in our database and the 41 less common types were combined in the group 'other types', as displayed in the Figure.

To determine the incidence of CDI in the Netherlands, we used the continuous surveillance data only. From the beginning of 2007 to the end of 2008, the mean incidence was 18 per 10,000 hospital admissions, ranging from 8 to 35 per 10,000 admissions among the 14 hospitals. These numbers are in line with a previous study performed in the Netherlands, which showed an incidence of 16 per 10,000 admissions [10]. A nationwide incidence study in neighbouring Belgium revealed a similar (median) incidence of 15 per 10,000 admissions [11].

Discussion and conclusions

To our knowledge, the Netherlands are the first European country with a documented decrease of the hypervirulent type 027. The detection of type 027 in 2005 resulted in a number of measurements taken on a national level. Most hospitals which experienced CDI due to type 027 followed the principles of the infection control guideline supported by the European Centre for Disease Prevention and Control (ECDC) to limit the spread of *C. difficile*, emphasising the importance of responsible use of antimicrobial drugs in conjunction with proper environmental disinfection, compliance with hand hygiene, protective clothing, education of staff and single-room isolation or cohorting of CDI patients [12,13]. Although the role of fluoroquinolones as an important predisposing factor for CDI due to type 027 has been recognised in several outbreaks [13,14], the observed decrease in incidence of type 027 in the Netherlands is not related to a change of nationwide use of fluoroquinolones since this remained stable in hospitals [15].

The relatively high frequency of type 001 in Dutch hospitals is not exceptional and has recently also been reported in southern Germany, Ireland, Luxembourg and the United Kingdom [7,16]. Type 014 is also frequently found in other European countries: it is the most common strain found in Hungary (2002-2004), Norway and Sweden (2008), and the second most common strain in Austria (2006) and Poland (2002-2003) [7,8,17,18]. An increase of type 078 had been noticed previously in the Netherlands [19]. In the quarterly data presented here, the increase is also seen: in the first trimester of 2008 19% of all samples consisted of type 078. After this peak, however, the contribution of type 078 decreased and it became the third most common strain in the Netherlands. Also in several other European countries type 078 is increasingly observed [7]. This type is a predominant strain in some farm animals (especially in pigs and dairy calves) and has recently been found in retail meat in North America [20]. The genetic similarity between animal and human type 078 strains as demonstrated by the highly discriminatory multilocus variable number of tandem repeats analysis (MLVA), also suggests a possible common source of animal and human type 078 strains. Type 078 and type 027 have similar virulence factors (positive for toxin A, B and binary toxin, and a dysfunctional toxin regulator gene). Furthermore, they resemble CDI in their clinical presentation: both cause severe diarrhoea in 40% of cases. A complicated course is seen less often in CDI caused by type 078, possibly because type 078 is observed in a younger population, with a higher frequency of community-associated CDI [19].

In conclusion, CDI caused by the hypervirulent 027 strain is now observed less frequently in the Netherlands, while the 'common' types 001 and 014 remain prominently present in the Dutch hospitals. Type 078 is currently the third most common PCR ribotype in the Netherlands and other European countries, whereas its occurrence before 2005 was very rare. More research is needed on the source of this strain and a possible exchange between animals and humans.

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Surveillance of the first 205 confirmed hospitalised cases of pandemic H1N1 influenza in Ireland, 28 April – 3 October 2009

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From 28 April 2009 to 3 October 2009, 205 cases of confirmed pandemic H1N1 influenza were hospitalised in Ireland. Detailed case-based epidemiological information was gathered on all hospitalised cases. Age-specific hospitalisation rates were highest in the age group of 15 to 19 year-olds and lowest in those aged 65 years and over. Nineteen hospitalised cases (9%) were admitted to intensive care units (ICU) where the median length of stay was 24 days. Four hospitalised cases (2%) died. Fifty-one percent of hospitalised cases and 42% of ICU cases were not in a recognised risk group. Asthma was the most common risk factor among cases; however, people with haemoglobinopathies and immunosuppression were the most over-represented groups.

Introduction

In late April 2009, a novel influenza virus led to human infection in Mexico and the United States (US) and subsequently spread worldwide. On 11 June, 2009, the World Health Organization (WHO) declared a phase 6 pandemic of moderate severity [1].

The first case of pandemic influenza in Ireland was diagnosed on 28 April 2009. Existing surveillance systems were augmented and enhanced case-based surveillance of pandemic H1N1 influenza was commenced. Until mid-July, all cases of influenza were actively followed up; most cases were imported, mainly from the US, the majority were in young adults and there were few hospitalisations [2]. On 16 July Ireland moved to mitigation, and detailed case-based surveillance was confined to hospitalised cases. Influenza-like illness (ILI) presentations to sentinel general practices remained below the seasonal threshold (17.8/100,000) between April and mid-July [3]. By late July, the ILI rate reached 35 per 100,000 population and fluctuated around this level until mid-September. Since then ILI rates have risen, reaching 87.3 per 100,000 population in the week ending 3 October [3].

The WHO has recommended that countries undertake case-based surveillance on the first 200 hospitalised cases, as it is important to identify those most at risk of adverse outcomes, hospitalisation and death due to pandemic H1N1 influenza. To date, there is limited research on risk factors associated with adverse outcomes in Europe [4-11] and therefore, we report on the enhanced case-based surveillance of the first 205 hospitalised cases of confirmed pandemic H1N1 influenza in Ireland.

Methods

Enhanced surveillance was undertaken on all cases of confirmed pandemic H1N1 influenza deemed to require admission to hospital for management of their illness by the treating clinician. Regional Departments of Public Health or the treating hospital clinician completed the enhanced surveillance form. Data collected included demographic details, premorbid medical conditions and pregnancy, antiviral therapy, and complications associated with influenza. Data on asthma and chronic respiratory disease were collected separately, based on findings from elsewhere which indicated that asthma was a significant risk factor [8,10]. These data were checked by the Departments of Public Health for completeness and cases identified with missing or inconsistent data were followed up and the information corrected. Surveillance and laboratory data were collected on the Irish computerised infectious diseases reporting system (CIDR). The Health Protection Surveillance Centre (HPSC) analysed the data. Analysis of variance (ANOVA) was used to test the effect of age on length of stay.

The prevalence of diseases/risk factors in the population was collected using a variety of methods. Data from the Cystic Fibrosis Registry [12], National Cancer Registry [13] and Infectious Disease Registry [14] were extracted to quantify numbers of people in the population with cystic fibrosis, certain malignancies and acquired immunodeficiency syndrome (AIDS). Hospital In-Patient Enquiry (HIPE) data over four years was used to estimate the prevalence of liver, renal disease and asplenia [15]. The prevalence of haemoglobinopathies was based on personal communication from the clinician with national responsibility for this disease group [16]. The number of pregnant women was extrapolated from the number of births in 2008 [17].

Other estimates were calculated by applying disease prevalence estimates from Irish or international data to the Irish population

recorded by the Central Statistics Office (CSO) [17]. Prevalence of asthma and diabetes was estimated extrapolating from prescription data in the Primary Care Re-imbursement Service (PCRS) system (the national scheme providing free medical services to those unable to afford these services without undue hardship; approximately 25% of the population are eligible [18]). Irish and international prevalence studies were used for chronic heart disease [19], respiratory disease [20], obesity [21,22] and neurological disease [23].

Length of stay was calculated as the time from date of admission to date of discharge, or if still in hospital, from date of admission to the date of data extraction from CIDR for analysis (13 October).

Results

During the period from 28 April to 3 October 2009, 205 confirmed cases of pandemic H1N1 influenza were hospitalised, of whom 19 (9.3%) were admitted to ICU.

Of the 205 confirmed hospitalised cases, 106 were female (51.7%). The median age of cases was 21 years (mean age: 25 years; range: five months to 78 years). Seventy-five percent of cases were in persons under 35 years of age (Figure 1).

From mid-July there was an increase in the numbers of people hospitalised and admitted to ICU with pandemic H1N1 influenza (Figure 2).

Age-specific hospitalisation rates have been shifting to younger age groups over time, with the highest age-specific rate initially in teenagers and currently in children under five years (age-specific hospitalisation rate <5 years: 3.4/100,000; age-specific hospitalisation rate total population: 1/100,000) (Figure 3).

Information on medical risk factor and pregnancy were available for 180 (88%) of the 205 hospitalised cases including all those admitted to ICU. Ninety-one cases (51%), including eight ICU cases (42%) were not in a recognised risk group.

Eighty-nine cases (49%), including 11 (58%) admitted to ICU, were in a risk group (medical risk factor or pregnancy). Sixty-seven (37%) had only one risk factor, 14 (7%) had two risk factors, seven (4%) had three risk factors and one case had four risk factors.

Twelve pregnant women with pandemic H1N1 influenza were hospitalised, eleven of whom were admitted due to pandemic H1N1 influenza infection, and one was admitted for a pregnancy-related indication. Period of gestation was available for nine women admitted due to influenza; one was in the first trimester, two in the second trimester and six in the third trimester. Three women had risk factors other than pregnancy, including asthma, obesity, liver disease and immunosuppression.

Being on medication for asthma was the most common risk factor for hospitalisation due to pandemic H1N1 influenza. Chronic respiratory disease and immunosuppression were the next most common risk factors. Chronic respiratory disease was the most common risk factor in ICU admissions, followed by chronic neurological disease, asthma and severe obesity (Table 1).

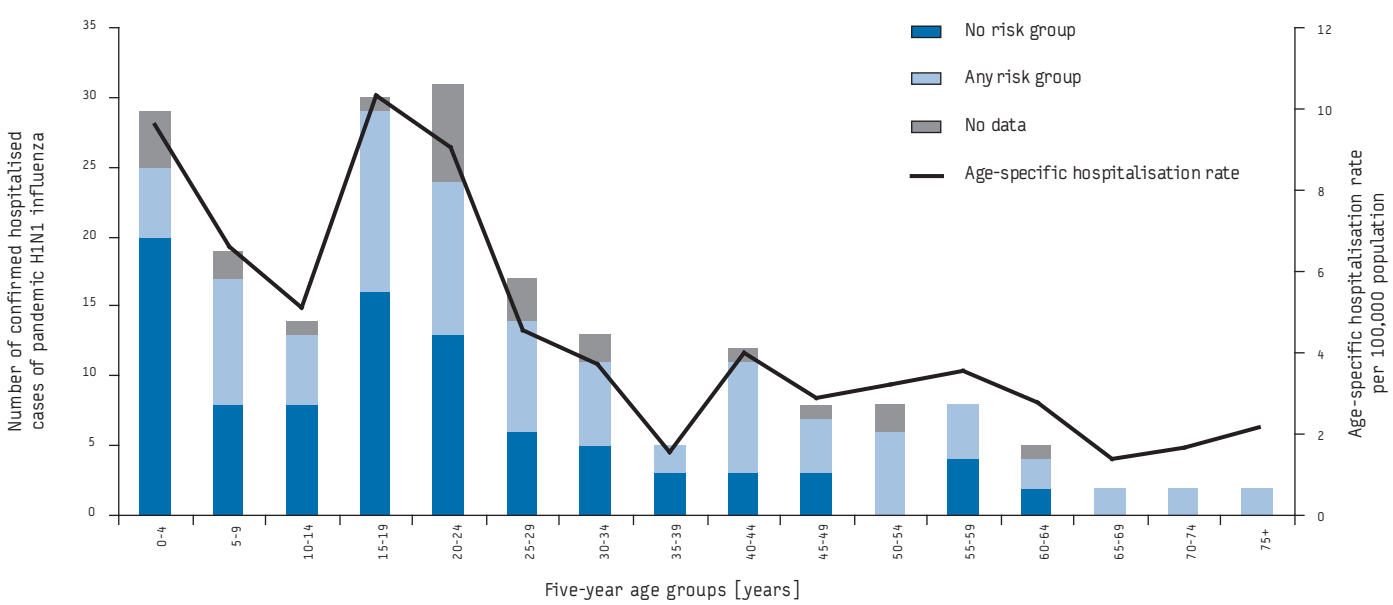
Data on antiviral treatment were available for 196 (96%) of the cases. One hundred and fifty-eight people (79%) received antiviral therapy either prior to admission or in hospital. Oseltamivir was used in 155 people (98%).

Onset date was recorded for 176 (85%) of confirmed cases. Table 2 shows the mean, median and range of the time between date of onset of symptoms and date of diagnosis, date of admission to hospital and date of admission to ICU.

Dates of admission to and discharge from hospital and/or length of stay were recorded for 198 (97%) of cases, including all ICU cases (Table 2). The median length of stay in hospital was two days

Figure 1

Cumulative number and age-specific hospitalisation rate for confirmed hospitalised cases, by presence or absence of risk factors, by five-year age groups, Ireland, 28 April-3 October 2009 (n=205)



for cases under the age of 24 years. While there was a large range of length of stay, there was a significant trend of increasing length of stay for those in the age groups of 25-44 year-olds and 45-64 year-olds, increasing to seven days in adults over 65 years (ANOVA: F=3.16, P<0.001) (Table 3).

Those with chronic neurological disease had the highest mean length of stay, followed by those with severe obesity and then chronic respiratory disease. Asthma, the most frequent risk factor associated with hospitalisation, was associated with a mean length of stay of six days (median: three days). Most risk-groups were associated with a wide range of length of stay (Table 4).

Data on complications were available for 177 (86%) of hospitalised cases. Forty cases (23%) developed pneumonia, 17 (43%) of whom were in a risk group. Ten people (6%) developed adult respiratory distress syndrome (ARDS), of whom six (60%) were in a risk group. Fifteen patients (8%) were ventilated. Fourteen were ventilated in ICU and one received non-invasive ventilation on a general ward.

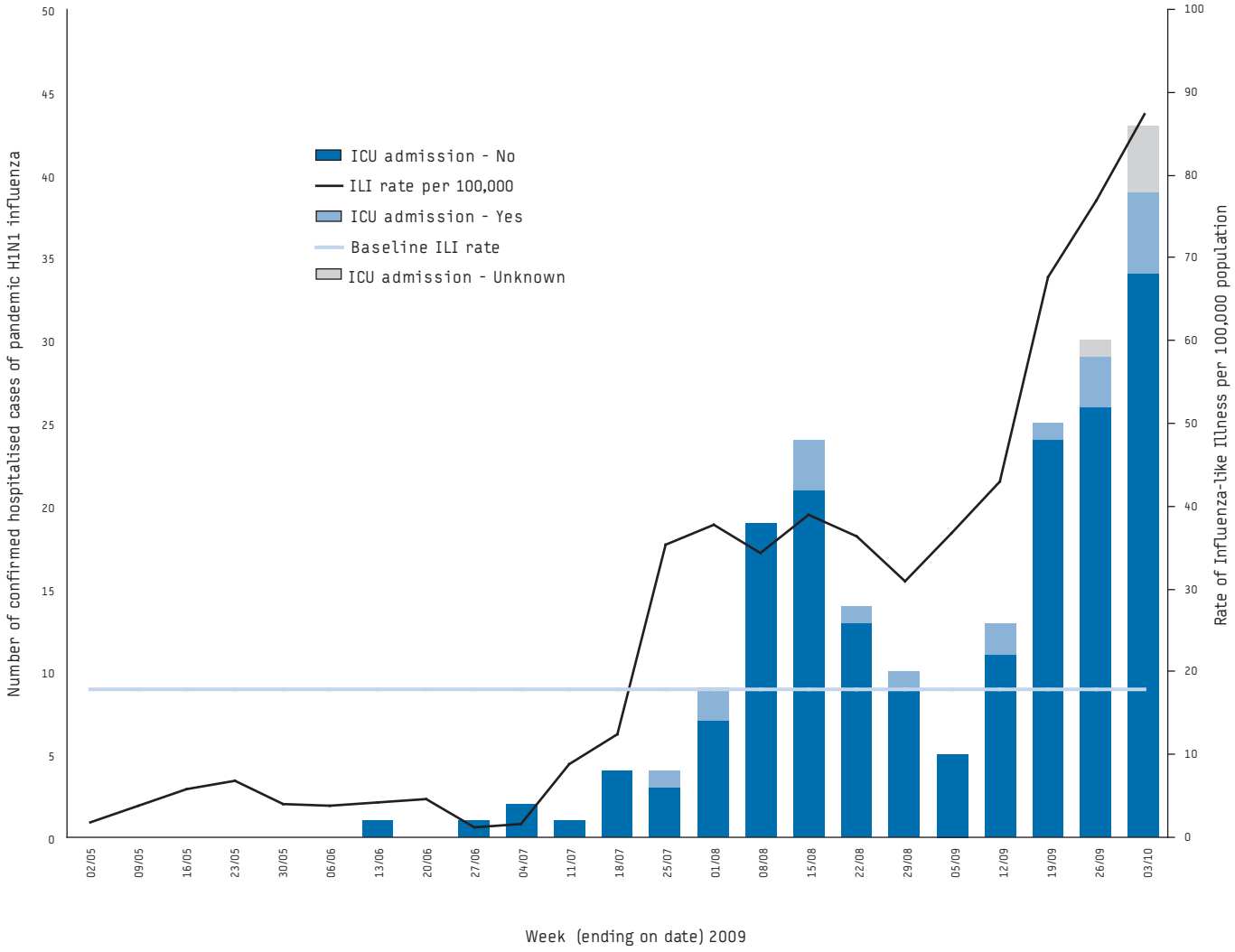
There were four deaths (2% of hospitalised cases) due to pandemic H1N1 influenza, three females and one male. Two were in the 15-24 year age-group, and two in the 50-59 year age-group. Three fatal cases (75%) had underlying risk factors.

Discussion

The epidemiology of hospitalised cases in the first months of the pandemic was similar in Ireland to other countries [24-28]. While younger age groups were more likely to be hospitalised they had shorter lengths of stay than older age groups. The age specific hospitalisation rate for children in Ireland was lower than that reported elsewhere [27,29,30]. Haemoglobinopathies and immunosuppression were the most over-represented risk factors in hospitalised cases. Pregnancy was associated with an increased

Figure 2

Confirmed hospitalised cases (ICU and non-ICU) of pandemic H1N1 influenza and ILI rate per 100,000 population by week, Ireland, 28 April - 3 October 2009 (n=205)*



ICU: intensive care unit; ILI: influenza-like illness.

risk of hospitalisation with the risk highest in the third trimester. Stay in ICU (median: 22 days; range: 0-72 days) was found to be much longer than reported in other countries [11,27].

The under-representation of children under the age of five years among hospitalised cases when compared with other countries [27,29,30] may be a reflection of the stage of the pandemic wave that Ireland is currently in. It is known that the majority of cases before mid-July were imported [2] and therefore more likely to be in young adults who travel. July and August are school holidays in Ireland and this may have further limited spread among children and thus hospitalisations in children. In this time period the majority of

Figure 3

Age-specific hospitalisation rates (per 100,000) of pandemic H1N1 influenza, by week, Ireland, 28 April -3 October 2009 (n=205)

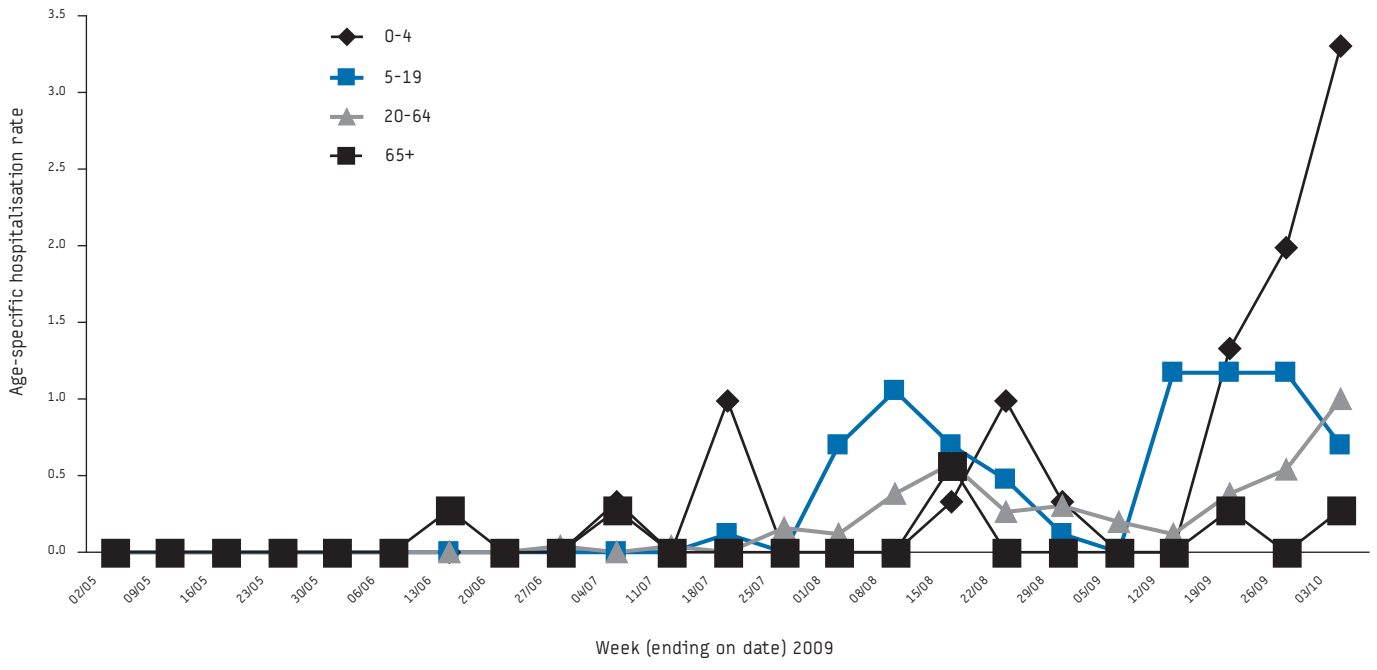


Table 1

Number and percentage of cases of confirmed pandemic H1N1 influenza in risk groups and in the Irish population, Ireland, 28 April – 3 October 2009 (n=180)

Risk Group	N hospitalised cases in risk group*	Risk group as a % of total hospitalised cases n=180** †	N ICU cases in risk group*	Risk group as a % of total ICU cases n=19**	Risk group as a % of total Irish population
No listed risk group	91	50.6	8	42.1	n/a
Chronic respiratory disease	18	10.0	5	26.3	6.00
Chronic neurological disease	9	5.0	2	10.5	8.89
Severely obese (BMI ≥ 40)	4	2.2	2	10.5	1.20
People on medication for asthma	32	17.8	2	10.5	11.50
Chronic liver disease	3	1.7	1	5.3	0.30
Chronic heart disease	9	5.0	0	0.0	7.60
Chronic renal disease	4	2.2	0	0.0	0.30
Immunosuppression	17	9.4	0	0.0	0.08
Haemoglobinopathies	5	2.8	0	0.0	0.01
Pregnancy	12	6.7	0	0.0	1.40
Diabetes mellitus	8	4.4	0	0.0	5.00
Post partum ≤ 6 weeks	0	0.0	0	0.0	0.20

BMI: body mass index; ICU: intensive care unit.
** A case may belong to more than one risk group and so may be counted in more than one row of this table.
** Number of cases with data on risk groups.
† ICU cases are included in the hospitalised case count.

cases in children were associated with residential summer camps [3], suggesting that it is educational settings that are most likely to result in spread in the age groups under 16-year-olds.

The ILI rates exceeded the threshold of seasonal influenza in July, but then reached a plateau in late July and August, and it was only in September (following reopening of schools) that rates started to rise again [3]. International evidence suggests that as the

Table 2

Time from onset of symptoms to admission to hospital, to laboratory confirmation, to admission to ICU, and length of stay associated with pandemic H1N1 influenza, Ireland, 28 April - 3 October 2009

Time period	N Cases	Mean (days)	Median (days)	Min (days)	Max (days)
Onset of symptoms to admission to hospital	176	3	1	0	20
Onset of symptoms to laboratory confirmation†	174	5	4	0	26
Length of stay in hospital* **	198	6	3	0	79
Onset of symptoms to admission to ICU	16	6	5.5	1	20
Length of stay in ICU - all patients	19	25	22	0	72
Patients discharged from ICU	8	21	8	0	72
Patients in ICU on 13 October 2009**	11	28	25	13	62

ICU: intensive care unit.
* ICU and non-ICU cases combined.
** Data extracted on 13 October 2009; only cases confirmed by 3 October 2009 are included.
† Time to laboratory confirmation is longer than time to admission reflecting time taken for laboratory confirmation.

Table 3

Length of hospital stay of pandemic H1N1 influenza cases, by age group, Ireland, 28 April - 3 October 2009 (n=198)

Age group [years]	Mean (days)	Median (days)	Min (days)	Max (days)
0-4	5	2	1	30
5-14	3	2	1	12
15-24	4	2	0	37
25-44	8	3	0	65
45-64	11	5	1	60
65+	8	7	6	11

Table 4

Length of hospital stay by risk group, Ireland, 28 April-3 October 2009 (n=180)

Risk Group		Length of Stay*			
	N Cases**	Mean (days)	Median (days)	Min (days)	Max (days)
Chronic neurological disease	9	15	8	2	62
Severely obese (BMI ≥ 40)	4	13	10	2	28
Chronic respiratory disease	17	12	6	1	60
Chronic liver disease	3	11	11	2	19
Chronic heart disease	9	7	7	1	12
Chronic renal disease	4	6	6	1	12
Immunosuppression	17	6	4	1	22
People on medication for asthma	32	6	3	0	37
No listed risk group	86	5	2	0	79
Haemoglobinopathies	5	5	3	2	11
Pregnancy	12	4	3	1	11
Diabetes mellitus	8	4	3	1	7
Post partum ≤ 6 weeks	0	-	-	-	-

BMI: body mass index.
*Length of stay based on date of analysis for those still in hospital
** A case may belong to more than one risk group and so may be counted in more than one row of this table. Only cases where length of stay is available are included

overall number of cases of pandemic H1N1 influenza increases, the age-specific incidence of hospitalisations will shift to younger age groups [10,31]; the same shift is being seen in cases in Ireland. However, in other countries, highest hospitalisation rates were seen in the children under the age of five years at all stages of the pandemic wave, although the magnitude of this difference varied over time [27,31,32].

The under-representation of young children may reflect an ascertainment bias, as children may not present with typical ILI symptoms, and the diagnosis might not have been considered. This has been reported for seasonal influenza [33].

Some patients have an extremely protracted ICU stay, with a number of current patients in ICU in excess of 60 days. There were limited numbers of patients in ICU initially and hence it is difficult to draw conclusions. However, long stays may reflect the availability of suitable step-down facilities, with for instance some smaller acute hospitals having no high dependency units (HDU) to move patients who no longer require full ICU care to. This will impact on ICU and HDU resources as the pandemic progresses.

Length of stay increased with age. One third of children under the age of 15 years had a length of stay of less than two days compared to 25% of the age group of 15-24 year-olds, 17% in the group of 25-64 year-olds and none of the over 65 year-olds. Short lengths of stay in children have been noted elsewhere [8].

Time from onset of symptoms to admission to hospital was shorter than seen in the US [10]. This may be accounted for by the stage of the pandemic in Ireland. Early in the pandemic there was a high level of uncertainty in relation to clinical presentation and likely progression of the disease, which may have led to a lower threshold for early admission to hospital. As clinicians became more experienced in treating pandemic influenza they may have been more confident in advising homecare.

Data gathered in this paper were for surveillance purposes and provide epidemiological information on the early hospitalised cases of pandemic H1N1 influenza. They also provide information on clinical details but it must be borne in mind that the data were not collected primarily for this purpose and so there are a number of limitations. Enhanced surveillance forms were completed at an early stage of hospitalisation and were updated where possible later during the stay and at discharge. A retrospective chart review was not carried out. This may impact on data validity, particularly recording of influenza complications and on time of onset of symptoms. Data on risk factors were gathered from the treating clinician and may have been subject to bias such as misclassification bias. The proportion of hospitalised cases with risk factors was lower than that reported in studies elsewhere. This may be due to the fact that a detailed case review of each patient's notes was not carried out, as was done elsewhere [10,11]. However, efforts were made to ensure that data collected from clinicians was complete, and incomplete data was rechecked at a later stage, with over 90% of data completed. Also, as data were collected on all hospitalised cases in Ireland, there was less potential for bias than would have occurred if the cases were a sample presenting at one or a few sites.

The prevalence of risk groups in the general population were derived by a number of different methods based on data of varying quality. Information on pregnancy and diseases for which registries are available can be considered reliable. Other data are of varying quality and should be interpreted with caution. Nevertheless, they provide some indication of the representation of different risk groups among hospitalised cases compared with their distribution within the general population as well as some indication as to whether they are over- or underrepresented.

Surveillance data continue to be very important in understanding the pandemic in Ireland. However, information needs have changed. It is now important to identify those at highest risk of complications and to understand what those complications are in order to plan both prevention strategies and hospital surge capacity. Hospital surveillance will continue to be important. In addition, enhanced surveillance of ICU cases has been implemented in Ireland and will provide more information on complications in these patients, particularly severe complications

Acknowledgements

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*Erratum: The x-axis in Figure 2 indicated the wrong dates and this was corrected on 6 November 2009.

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Measures against transmission of pandemic H1N1 influenza in Japan in 2009: simulation model

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The first outbreak of pandemic H1N1 influenza in Japan was contained in the Kansai region in May 2009 by social distancing measures. Modelling methods are needed to estimate the validity of these measures before their implementation on a large scale. We estimated the transmission coefficient from outbreaks of pandemic H1N1 influenza among school children in Japan in summer 2009; using this transmission coefficient, we simulated the spread of pandemic H1N1 influenza in a virtual community called the virtual Chuo Line which models an area to the west of metropolitan Tokyo. Measures evaluated in our simulation included: isolation at home, school closure, post-exposure prophylaxis and mass vaccinations of school children. We showed that post-exposure prophylaxis combined with isolation at home and school closure significantly decreases the total number of cases in the community and can mitigate the spread of pandemic H1N1 influenza, even when there is a delay in the availability of vaccine.

Introduction

Cases of pandemic H1N1 influenza were first reported in Mexico in April 2009 [1]. Subsequently, the virus spread rapidly across the United States and Canada, and then became a global concern [2]. Initial countermeasures, including rigorous fever screening at ports of entry, were introduced by the Japanese government in response to the elevated pandemic alert level of the World Health Organization [3].

In May 2009, an outbreak of pandemic H1N1 influenza occurred in the Kansai region of Japan in Hyogo and Osaka prefectures and was contained by the end of the month [4]. After early July, the virus emerged again and spread throughout Japan [5].

Urgent implementation of measures against pandemic H1N1 influenza is required.

Vaccination against pandemic H1N1 influenza was started in Japan on 19 October 2009, targeting first the healthcare workers. As there may not be enough vaccines to cover all needs, and it is already November, the effectiveness of other measures, such as the use of antiviral drugs and social distancing, must also be considered.

To implement these measures effectively in order to contain the spread of the disease and decrease the associated costs to society, we must first estimate the impact of these measures. Simulation is a useful method for this purpose. We have developed an individual-based Monte Carlo simulation code by constructing a virtual regional community called the virtual Chuo Line, based on the real Chuo Line area west of Tokyo [6].

In the present study, we use the virtual Chuo Line model for the simulation of pandemic H1N1 influenza and propose measures to be implemented. To estimate the impact of these measures in Japan, we decided to base the parameters on the simulation of Japanese pandemic H1N1 influenza cases.

Methods

Simulation of the spread of pandemic H1N1 influenza in virtual communities

We have developed a Monte Carlo simulation code using an individual-based model [6]. We constructed a virtual regional community called the virtual Chuo Line, based on statistical data of the real Chuo Line area west of Tokyo. In the virtual Chuo Line scenario, the total number of people involved was 8,800, including 2,000 in Hachoji City, 2,600 in Tachikawa City, 2,800 in the Kichijoji area of Musashino City next to Suginami ward in Tokyo metropolitan area, and the rest were in Shinjuku and Tokyo. In our model 8,800 people were sufficient for Monte Carlo simulation in the preliminary estimation. These people were connected to many different types of families: singles, couples, fathers, mothers, and children. We also constructed "compartments" consisting of 4,040 homes, 60 schools, 658 companies, and 117 shops. The size of the families ranged from single to eight persons. The proportion of different size families was determined by Japanese census data. For schools we modelled using local government statistics one class or two classes per school; the numbers of students were 30-40 or 70-100 per school. The size of workplace was from 3 to 30 persons and the various size workplaces were determined using local government statistics. We operated trains that moved between stations in the cities according to a virtual railroad timetable. Twelve percent of the people in the model commuted to Tokyo. We gave people event histories, consisting of movement from one compartment to another. Event histories were constructed using statistical data of the daily life of about 30,000 Japanese people. In these compartments, people contacted each other stochastically and were occasionally infected. When measures, such as school closure or prohibition of traffic, were implemented, students or commuters were assumed to stay in their households. The results

of simulations were obtained by an average of 100 runs. We showed other two numbers in parentheses, one is the sum of the lower values of 95% CI and the other is the sum of the upper values of 95% CI.

Real data in public health centres

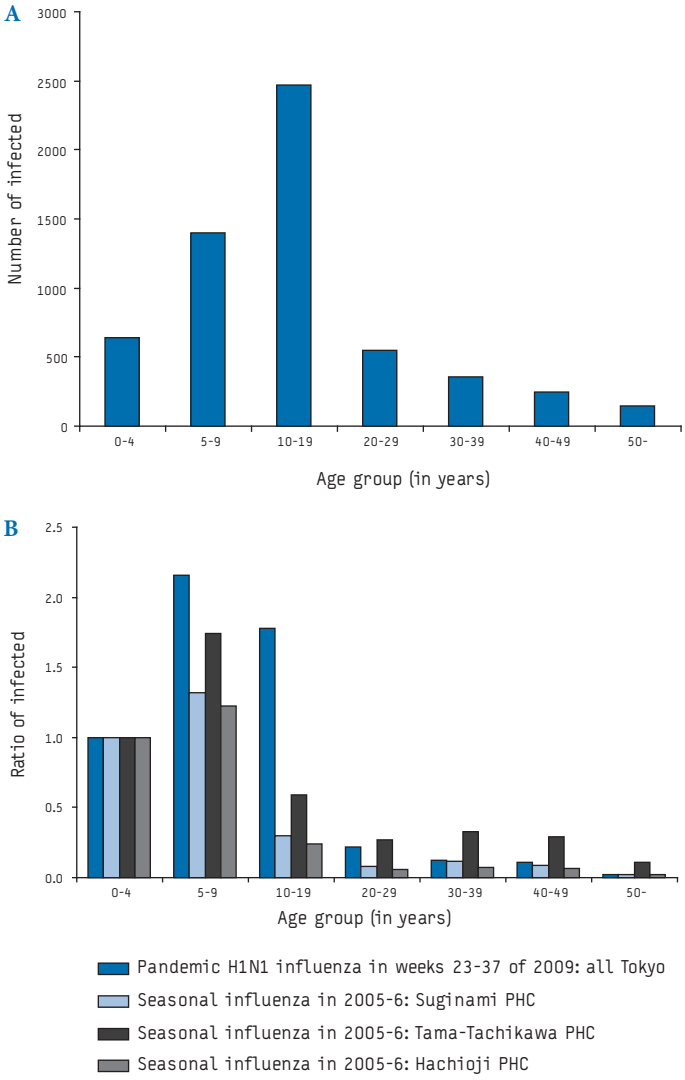
The spread of pandemic H1N1 influenza was reported to be prominent among young people. In order to confirm this, we compared the data on age distribution of cases of pandemic H1N1 influenza in Tokyo in the summer 2009 [7] with the data on cases of seasonal influenza in 2005-6 in three public health centres (PHC): Hachioji, Tama-Tachikawa and Suginami which are in the Chuo Line area [6]. We used surveillance data of infectious diseases including influenza collected by the National Institute of Infectious Diseases (NIID) to which every PHC reports the number of newly infected persons every week. The data reported to PHCs come from 3,000 paediatricians and 2,000 general practitioners. With the permission of NIID, we analysed the number of notifications in the winter of 2005-6 from the Hachioji, Tama-Tachikawa and Suginami PHCs [6]. As for the data of summer 2009, the number and age of patients in the greater Tokyo was published weekly by the Tokyo Metropolitan Institute of Public Health through the notifications from PHCs in the greater Tokyo [7]. The influenza data reported after July 2009 were considered to be mostly the pandemic influenza data, because seasonal influenza is rare in summer in Japan. We also estimated the transmission coefficient of pandemic H1N1 influenza among school children using data on outbreaks among small groups of students during summer vacation 2009.

FIGURE 1

Real data on reported numbers of cases of pandemic H1N1 influenza in Tokyo in 2009 and of seasonal influenza in three public health centres (PHCs) along a real railway, the JR Chuo Line in Tokyo in 2005-6:

A. Age distribution of reported cases of pandemic H1N1 influenza in greater Tokyo from week 28 to week 37 of 2009;

B. Ratio of persons infected by seasonal influenza per unit population normalised by the age group of zero to four, Tokyo and three PHCs along the Chuo Line, 2005-6.



Results

Age distribution of cases of pandemic H1N1 influenza

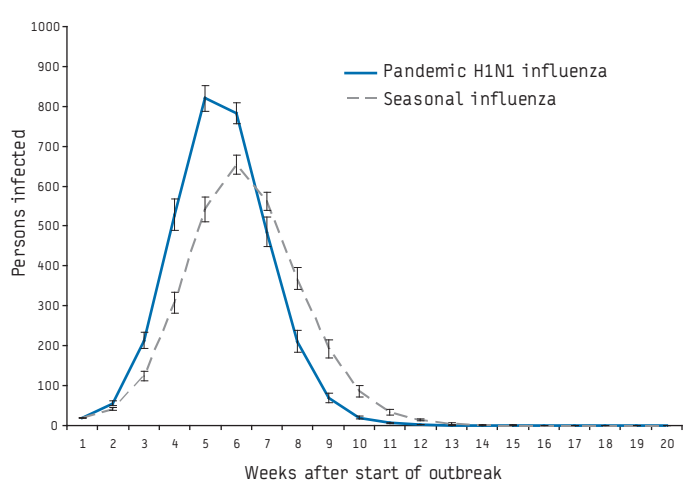
Data on age of persons infected with pandemic H1N1 influenza from week 28 to week 37 (6 July to 13 September) 2009 in Tokyo was obtained from the PHC reports [7] (Figure 1A). We calculated the ratio of the number of persons infected divided by the population of each age group in Tokyo from the Japanese census data and normalised it by age group from 0 to 4 years (Figure 1B). As shown in Figure 1B, the number of cases among school children, especially among teenagers, was significantly higher in comparison to seasonal influenza in the three PHCs in Tokyo: Kichijoji PHC, Tama-Tachikawa PHC, and Hachioji PHC in the Chuo Line area during the 2005-6 season [6].

Transmission coefficient

We searched the national newspapers for information on outbreaks of influenza among children during the summer vacation 2009. During the summer holidays, outbreaks of seasonal influenza are rare in Japan therefore we assumed these outbreaks had been due to the pandemic. We analysed the cases if the size of the group was specified. After 24 July, the policy of the Japanese government

FIGURE 2

Simulation model results for the number of persons infected with pandemic H1N1 influenza and seasonal influenza



has changed from testing all suspected cases to sample testing by PCR. If H1N1 is confirmed by sampling, we assume all cases to be infected with H1N1. After 25 August, no laboratory testing by PCR has been required to confirm an outbreak of H1N1 in a school setting.

The following outbreaks were identified:

Outbreak 1: Health recovery camp for asthmatic children, 29-31 July 2009. Approximately 70 people attended the camp: 43 children and 27 staff members. Of these, 22 children and four staff members showed symptoms, and one child was confirmed to have H1N1.

Outbreak 2: A university tennis club, 30-31 July 2009. Approximately 100 persons attended. The university announced that 12 were infected with H1N1.

Outbreak 3: Residential high school training camp, 1-4 August 2009. Enrolment was 47 people: 38 students, two teachers and seven former students. Of these, 26 were shown by a simple test to be infected with influenza A, and one was confirmed with the pandemic H1N1 influenza.

Outbreak 4: Regional basketball camp on 6 August 2009. Approximately 150 attended, including elementary and junior high school students and coaches. Simple test indicated nine junior high school students were infected with influenza A, and three of them were confirmed with the pandemic H1N1 influenza by PCR.

We estimated the transmission coefficient β by $\beta=\ln(I(T))/(S_0*T)$, I(T): the number of persons infected; S_0: the size of the group; T: the period of the event. To derive the formula, we integrated the following equation from time 0 to T, assuming the number of the initially infected children to be one:

$$\frac{dI(t)}{dt} = \beta S_0 I(t),\ I(0) = 1.$$

The pandemic H1N1 influenza did not prevail during summer vacation in Japan and seasonal influenza is rare in summer, therefore we could assume that susceptible children who attended the event were not exposed to other sources of infection except at the event. Then, I(T) is the number of children infected during the event. The estimated values per day are 0.016, 0.011, 0.017 and 0.012. The settings where the above outbreaks occurred were different from schools. However, from the point of view of the behaviour of a group of children, there are many similarities regarding the contacts among children during class room or physical activities. It is therefore expected that the transmission coefficients calculated from the above outbreaks can be applied to school outbreaks as well.

For probability of infection by seasonal influenza, we used P = 0.005 per hour for homes, P = 0.0016 for schools, P = 0.0125 for trains, and P = 0.00001 for companies and shops. For the probability of becoming infected on the train, we assumed passengers are densely crowded, as during the rush hour peak. The probability of infection by pandemic H1N1 influenza is within the range of seasonal influenza, except for school children. We used the probabilities of seasonal influenza, except for schools. We estimated the probability of infection among school children to be P = 0.0023, assuming 5-8 hours of activity per day in these cases. The medical conditions of simulation were specified by scenario of infection. We specified the latent time to be two days and the period of infection five days.

Simulation in model cities along the virtual Chuo Line

The average number of infected people in 100 simulation runs is shown in Figure 2. No social distancing measures were implemented in the runs. The peak of pandemic H1N1 influenza was higher than that of seasonal influenza and occurred one week earlier. The total number of persons infected with pandemic H1N1 influenza was 3,211 (range: 3,001-3,421), whereas the total number of people with seasonal influenza was 2,945 (2,756-3,152).

Home isolation of school children (HIS)

When one in three adults and 70%, 80%, 90%, or 100% of children stayed home 48 hours after the appearance of symptoms, the total number of persons infected in the community was 2,729 (2,443-3,015), 2,561 (2,298-2,824), 2,425 (2,167-2,683) and 2,121 (1,853-2,389), respectively. When all of the children and 0%, 66% and 100% of adults stayed home 48 hours after the appearance of symptoms, the total number of persons infected was 2,288 (2,089-2,487), 2,001 (1,760-2,242) and 1,779 (1,514-2,044). Figure 3A (simulation with no SC) illustrates a situation where all of the children and one-third of the adults stayed home 48 hours after the appearance of symptoms

School closure (SC)

We implemented SC in a situation where all students/pupils and one-third of adults stayed home 48 hours after onset of symptoms. We simulated seven-day SC for one and two weeks after the outbreak (Figure 3A), and then compared the results with the option without SC. The total number of persons infected was 1,812 (1,532-2,092), 1,766 (1,461-2,071) and 2,121 (1,853-2,389), respectively. Next, we simulated SC for four, five and six days, one week after the outbreak (Figure 3B). The total number of persons infected was 2,136 (1,845-2,427), 1,997 (1,714-2,280) and 1,927(1,662-2,192), respectively. The spread lasted approximately 20 weeks, averaging the results of 100 runs. However, in some cases, the spread ended before 10 weeks. Four of 100 runs in situations without SC ended before 10 weeks. Three, nine, 12 and 17 runs ended before 10 weeks in case of four-, five-, six- and seven-day SC.

Post-exposure prophylaxis with antiviral drugs (MED)

We assumed antiviral drugs were used only for household contacts of cases. When all children and one-third of adults stayed home 48 hours after symptoms appeared, we simulated the situations where all families used MED but the proportion of family members who were administered the antiviral drugs at any time within 48 hours after appearance of symptoms was 20%, 40%, 60%, 80%, and 100%. Then, the total number of persons infected was 1,903 (1,682-2,124), 1,654 (1,397-1,911), 1,412 (1,180-1,644), 1,082 (889-1,275) and 883 (666-1,000), respectively (Figure 3C). In these runs, we assumed the efficiency of antiviral drugs to be 80%, i.e. to prevent infection in eight out of ten contacts of the infected persons.

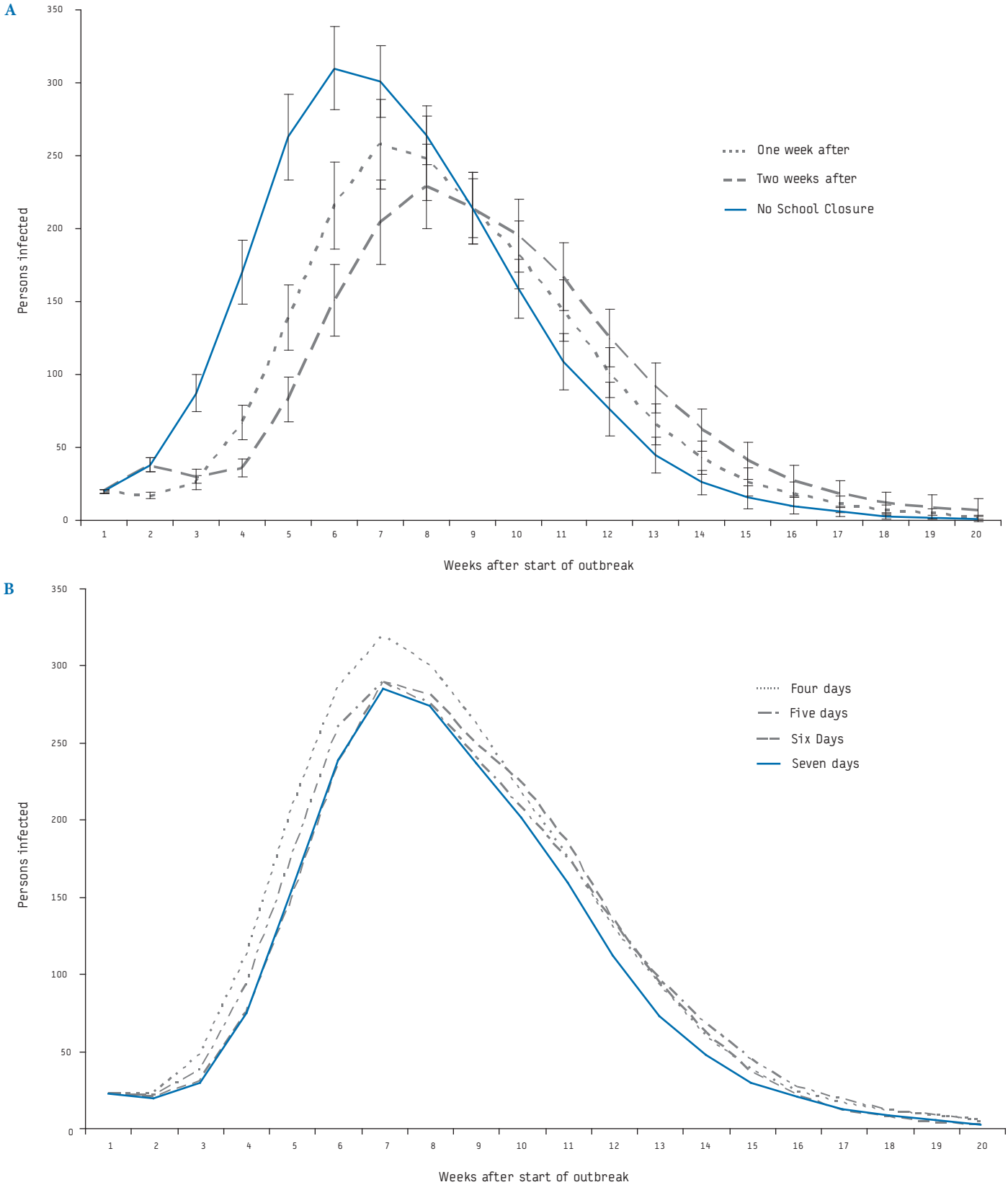
In the situation where 40% of families were administered the drug with an efficiency of drugs 60%, 70%, and 90%, the total number of persons infected was 1,815 (1,560-2,070), 1,761 (1,519-2,003), and 1,574 (1,336-1,812), respectively.

Mass vaccination of school children (VSC)

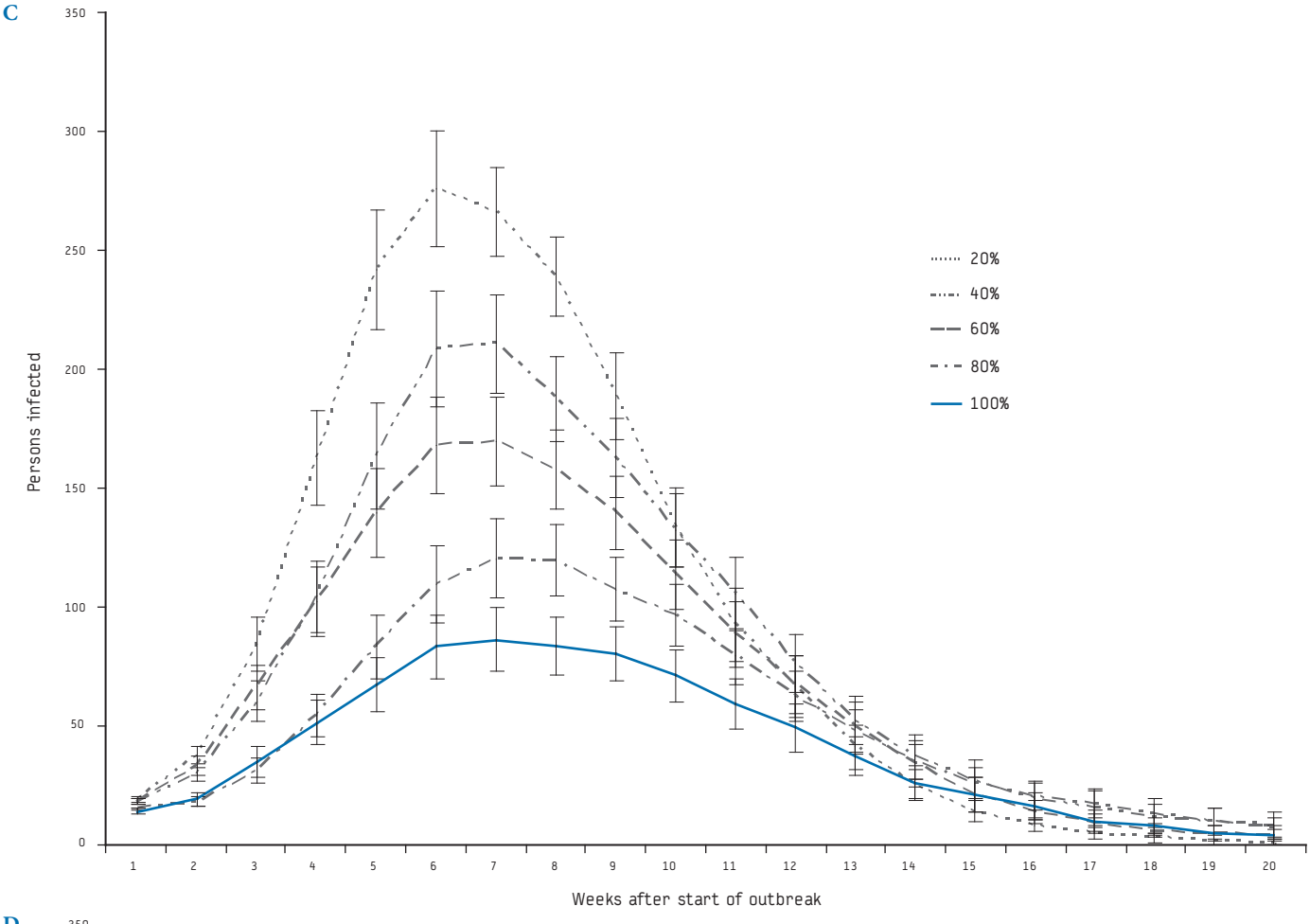
Children were assumed to be vaccinated and become immune before the influenza season. When the efficiency of vaccine is X%, X persons in 100 were assumed to become immune. We also

Figure 3

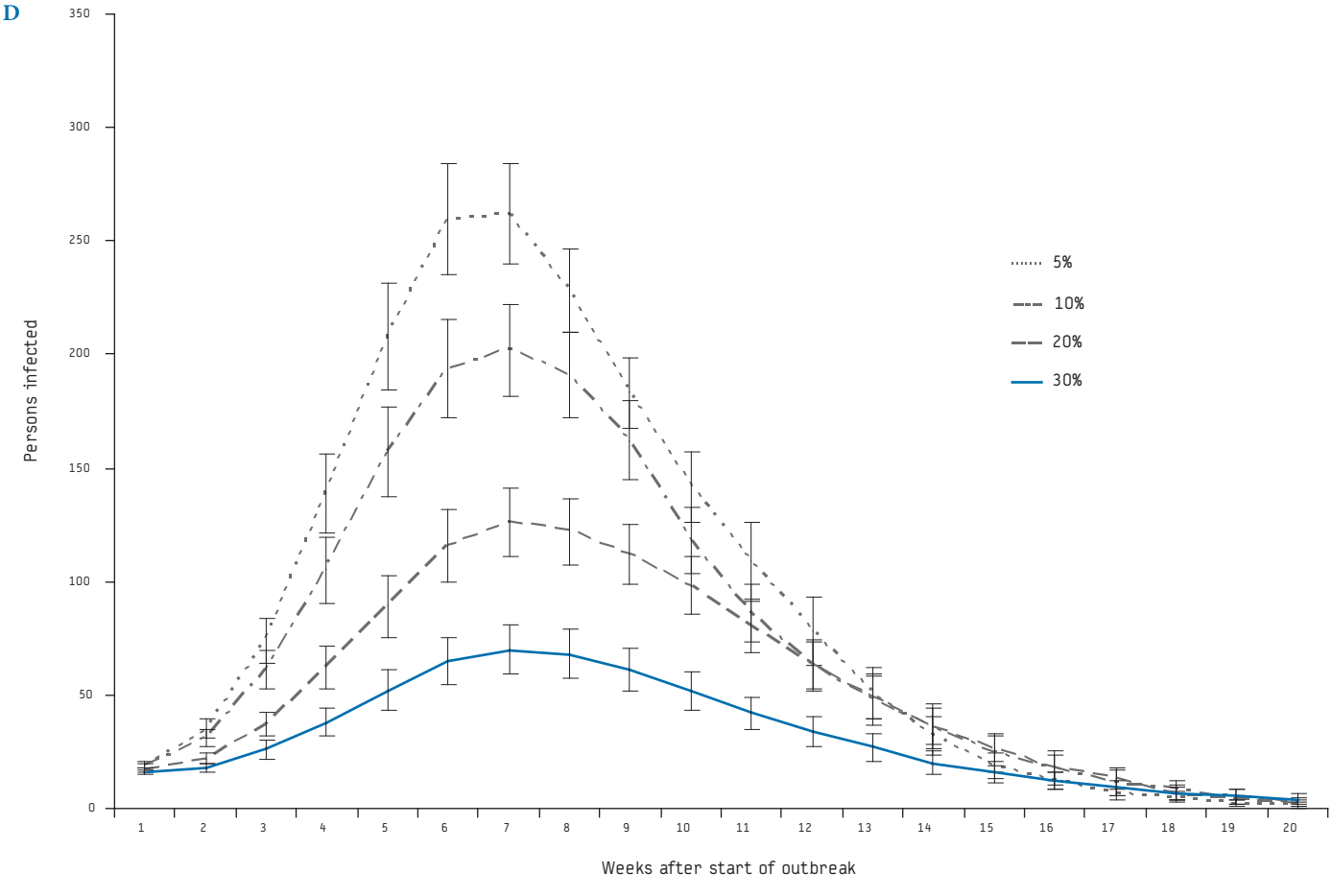
The number of persons infected with pandemic H1N1 influenza, simulation model results for different scenarios:
A. Seven-day school closure one or two weeks after the outbreak and no school closure;
B. School closure for four, five, six and seven days one week after the outbreak;
C. Post-exposure prophylaxis with antiviral drugs administered to 20%, 40%, 60%, 80%, and 100% of the family members;
D. Mass vaccination of school children, assuming the efficiency of vaccinating children was 5%, 10%, 20%, and 30%.



C


Persons infected
Weeks after start of outbreak
20%
40%
60%
80%
100%

D


Persons infected
Weeks after start of outbreak
5%
10%
20%
30%

assumed all children and one-third of adults stayed home 48 hours after symptoms appeared. The total number of persons infected in the community was 1,879 (1,624-2,134), 1,546 (1,324-1,768), 1,094 (932-1,270) and 645 (528-780) when the efficiency of the vaccine to children was 5%, 10%, 20%, and 30%, respectively (Figure 3D). The number of infected children was 975 (838-1,112), 793 (676-910), 538 (451-625) and 291 (229-353), respectively. When the vaccine was delayed, children became immune 1, 2, or 3 weeks after the spread of pandemic H1N1 influenza, and the total number of persons infected was 762 (628-896), 881 (744-1,018) and 1,011 (872-1,150) in case of 30% efficiency.

Combination of measures

We performed a simulation of measures according to the following possible scenario: all children and one of three adults were isolated 48 hours after the appearance of symptoms. Four-day SC one week after the outbreak was implemented. Thirty percent of children became immune by vaccination only eight weeks after the outbreak. Forty percent of families of persons infected were administered the antiviral drugs with efficiency 80%. It is shown that the number of persons infected, indicating the major venues where they became infected, was 1027(860-1194) (Figure 4), strongly suggesting measures to mitigate the spread of pandemic H1N1 influenza even if the vaccine is delayed.

Discussion

In the present study, it was shown that the spread of pandemic H1N1 influenza in Japan is more severe among school children than seasonal influenza. Nishiura et al. [8] estimated the average number of secondary cases in children generated by a single primary child case in Japan to be 2.8. Meanwhile, transmission among other age groups is comparable to that of seasonal influenza. It was thus confirmed that children play an especially important role in the spread of pandemic H1N1 influenza [4].

Home isolation

School principals have the authority to suspend children infected by influenza according to Japanese school health laws. Our simulation shows the total number of persons infected decreased to approximately two-thirds when all children and one-third of adults were isolated at home compared with the scenario of no measures taken. When all children and two-thirds of adults were isolated at home the additional decrease was not so significant, indicating that the impact of HIS is mainly through preventing infection in schools. Children in the household could infect their family members. However the family members were fewer than their classmates.

School closure

In May 2009, an outbreak of pandemic H1N1 influenza, the first in Japan, occurred in Hyogo and Osaka prefectures. In the beginning of the outbreak, primarily high school students were infected. After peaking on 17 May 2009, the outbreak decreased [4]. All junior high and high schools in Osaka prefecture were closed for 1-2 weeks after 16 May, and elementary schools and kindergartens in the cities where cases occurred were closed. Schools were also closed in Kobe city [4].

SC was implemented in our present simulation in addition to HIS, resulting in a lower peak and a decrease of the total number of infected persons in comparison to the scenario without SC. SC without HIS slows only the transmission of spread; peak becomes lower, but the decrease of the total number of persons infected is small [6]. SC mainly slows down the spread and HIS decreases the number of persons infected by pandemic H1N1 influenza in the present simulation.

For the scenario of SC implemented one week later, our simulation shows that SC for four days was not sufficient, although it did delay the peak. The total number of infected decreased with longer SC. However, infected children may be expected to recover at home during SC for four days due to its latency for two days. In large infected families (i.e. 5-8 members) children would be infected newly during SC.

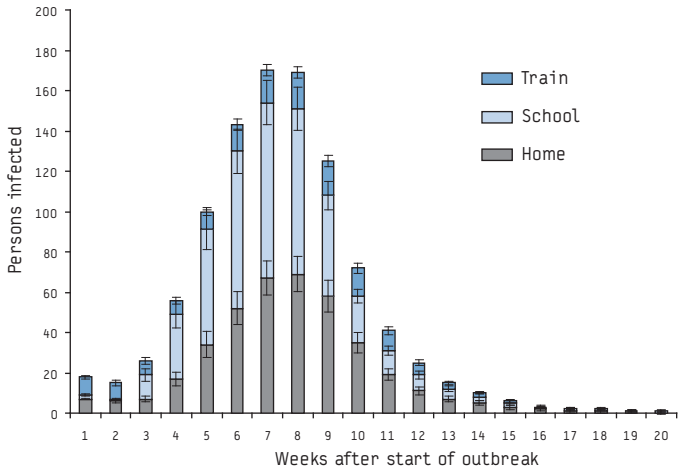
Our simulation shows it is not easy to affect outbreaks using SC in the commuter towns of Tokyo after an epidemic. Although in some cases the spread of disease in three cities ended soon after implementation of SC, in other cases, commuters mitigated the effect of SC. For example, in Hachioji and Tachikawa, the spread ended, but in Kichijoji, it persisted. Influenza was introduced into the cities and began to spread again by commuters in Hachioji and Tachikawa, who were infected in trains or businesses. If we prohibited traffic between cities in the case of seven-day SC, 83 of 100 runs ended before 10 weeks. Indeed, the first outbreak for a short period in Osaka spread among high school students, not adult commuters.

Post-exposure prophylaxis

Post-exposure prophylaxis by administration of antiviral drugs is not officially permitted in Japan. However, antiviral drugs, for example oseltamivir, are the first prescription of choice in cases of seasonal influenza. The use of neuraminidase inhibitors has been reported to decrease the incidence of influenza by 68-89% [9]. Our results show the total number of persons infected in the community decreased significantly when the number of families who received antiviral drugs increased. Hence MED is an effective method that blocks infections in households.

Figure 4

The number of persons infected with pandemic H1N1 influenza in a scenario with combination of measures: traffic prohibition, school closure, and isolation at home



Vaccination of school children

The supply of vaccine for pandemic H1N1 influenza in Japan is estimated to be insufficient and therefore priority of vaccination will have to be scheduled, but to date no decision has been taken as to whether children, except those in the lower grade of elementary school, would be included among the priority groups. Even if the vaccine is closely matched, we cannot expect high efficiency. However, simulations show that vaccines are highly effective in protecting communities; this also holds true for seasonal influenza [6].

We considered mass vaccination of school children, because systematic vaccination of adults seems difficult due to lifestyle differences. In Japan, children were mass-vaccinated by law against seasonal influenza from 1962 to 1987. In 1987, the law was relaxed and then repealed in 1994, but the effectiveness of VSC against seasonal influenza is still under discussion. A study on deaths from pneumonia and influenza from the 1950s to the 1990s demonstrated mortality of the elderly decreased when school children were vaccinated [10].

When children were mass-vaccinated against seasonal influenza, not only did the number of infected children decrease, but also that of infected adults [6]. Mass vaccination of children is therefore effective in protecting the whole community. However, our simulations showed that when children did not become immune due to the delay of vaccine the number of persons infected increased. Our simulation strongly suggests vaccination is effective; however, delay of distribution of vaccine mitigates the effectiveness. After the end of October 2009, the effectiveness of vaccine in preventing the spread of disease is questionable.

Combination of measures

In the present study, the spread of influenza is decreased, even when the delivery of the vaccine is delayed. The mechanism of spread also shows that infected commuters introduce influenza into cities, then infections occur in the homes, children spread influenza in the schools and, in turn, infected children infect their families in the households, similar to seasonal influenza [6].

Conclusions

Home isolation of infected children greatly decreases the number of persons infected. In Osaka in May 2009, SC slowed down the outbreak. However, our simulation shows it is not easy for the commuter towns of Tokyo to slow down outbreaks after the beginning of an epidemic, even if long SC with HIS is implemented. Post-exposure prophylaxis combined with HIS greatly decreases the total number of infected people in the community. Also mass vaccination of school children combined with HIS greatly decreases the total number of persons infected, even if the efficiency is low. However, the delay of VSC decreases the efficiency. Our simulation shows that a combination of measures can mitigate the spread of pandemic H1N1 influenza, even when vaccines are delayed.

Acknowledgements

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Interpreting "Google Flu Trends" data for pandemic H1N1 influenza: The New Zealand experience

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For the period of the spread of pandemic H1N1 influenza in New Zealand during 2009, we compared results from *Google Flu Trends* with data from existing surveillance systems. The patterns from *Google Flu Trends* were closely aligned with (peaking a week before and a week after) two independent national surveillance systems for influenza-like illness (ILI) cases. It was much less congruent with (delayed by three weeks) data from ILI-related calls to a national free-phone Healthline and with media coverage of pandemic influenza. Some patterns were unique to *Google Flu Trends* and may not have reflected the actual ILI burden in the community. Overall, *Google Flu Trends* appears to provide a useful free surveillance system but it should probably be seen as supplementary rather than as an alternative.

The website *Google Flu Trends*, developed by Google.org, uses aggregated Google search data on influenza-like illness (ILI) symptoms to estimate influenza activity "up to two weeks faster than traditional systems" [1]. As of mid-October 2009, the site graphically presents data for Australia, New Zealand, Mexico (selected regions only), the United States (US) and 14 European countries [2]. An analysis of this surveillance system for seasonal influenza data in the US indicated that it was able to "accurately estimate the current level of weekly influenza activity in each region of the United States, with a reporting lag of about one day" [3]. For the Australian state of Victoria, the data from *Google Flu Trends* showed a "remarkable correlation" with ILI surveillance data from sentinel practices and the Melbourne Medical Deputising Service [4]. This was for data from May and June 2009 – the time of the spread of new pandemic H1N1 influenza in that state. In fact, the Google data showed an increase in ILI activity five to six weeks prior to the actual increase in reported ILI cases.

As New Zealand has a number of different influenza surveillance systems in operation [5-7], we aimed to further explore the possible utility of *Google Flu Trends* in the setting of an influenza pandemic.

Methods

We downloaded the freely available data for New Zealand in 2009 from the *Google Flu Trends* website [1] from the week beginning 29 March (week 14) to the week beginning 4 October 2009. Data were for the 'Google search ratio', a metric developed by Google and based on Google searches for ILI symptoms that were calibrated against past seasonal influenza data reported through the specific surveillance system(s) in a given country. These data were then compared graphically with ILI data from a national network of sentinel general practices (Sentinel GP system) and another much larger national network of computerised general practices (HealthStat). A comparison was also made with ILI data from a national free-phone Healthline. These systems have all previously been described in *Eurosurveillance* [5]. Of note is that in the graphs the 'weeks' are shifted by one day against those used for *Google Flu Trends:* the reporting week in *Google Flu Trends* starts on Sunday, while the HealthStat week starts on the day before (Saturday) and the reporting weeks in the Sentinel GP system and Healthline start on the day after (Monday).

In addition we obtained a weekly tally of media reports relating to the H1N1 influenza pandemic in New Zealand in 2009 by searching the news archive of 'Google news (New Zealand)' [8]. The search used all the following terms together: 'swine' AND 'flu' AND 'Zealand' AND (the phrase) 'Ministry of Health'. Less specific search strategies (e.g. without the phrase 'Ministry of Health') did not return results that were sufficiently specific for local news media reports from New Zealand because there was extensive international media reporting of some early events relating to New Zealand, such as the arrival of a group of symptomatic students in Auckland on a flight from Mexico in late April 2009.

Results

The initial increase in the weekly rate of ILI cases reported from the Sentinel GP system and the increase in the Google search ratio (representing internet searches for ILI symptoms) were very similar and were noted between week 19 (starting 3 May) and week 24, 2009 (Figure 1). However, the Google search ratio peaked a week earlier, in week 28 (starting 5 July) versus week 29.

The comparison with computerised general practice (HealthStat) ILI data gave some indication that the Google search ratio increased initially before the increase in the ILI data (Figure 2). After that, it seemed to lag behind and peaked a week later, in week 28 versus week 27 for HealthStat data.

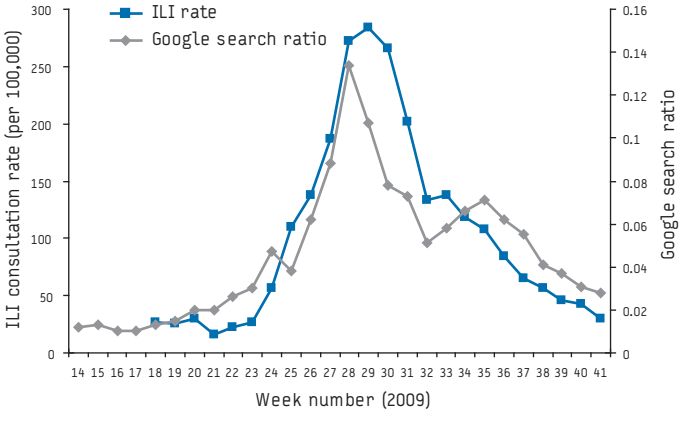
When compared to the ILI calls to the Healthline, there was a similar pattern initially and then a growing gap with the Google search ratio following behind (Figure 3). Indeed, the latter peaked 3 weeks after the peak in ILI Healthline calls (which peaked in week 25 [starting 14 June]).

The comparison with news item media coverage is shown in Figure 4. There appears to be little congruence, especially around the massive peak in media coverage associated with week 18 (starting 26 April) when a group of symptomatic school students returned to New Zealand on a plane from Mexico, the first confirmed cases in New Zealand. There was some similarity in the pattern of increase in week 24 when official reports were of cases first exceeding a total of 1000. But there was no similarity after that point except where both levels declined from week 29 onward.

While a second, smaller peak appears in the Google search ratio in week 35 (starting 23 August), no such peak was seen in the Sentinel GP and HealthStat systems, in the Healthline calls data, or in media items (Figures 1–4).

Figure 1

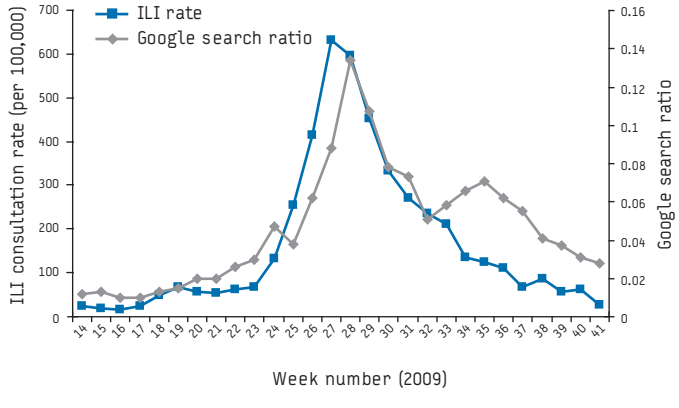
Weekly rate of ILI per 100,000 registered population from the national Sentinel General Practice Surveillance System, compared to the Google search ratio, New Zealand, 29 March – 4 October 2009



ILI: influenza-like illness.

Figure 2

Weekly rate of ILI per 100,000 registered population from the national computerised general practice (HealthStat) surveillance system, compared to the Google search ratio, New Zealand, 29 March – 4 October 2009



ILI: influenza-like illness.

Discussion

Key findings and interpretation

These results suggest that the patterns from the G*oogle Flu Trends* system are fairly congruent with actual surveillance systems for ILI cases in New Zealand. For 2009, these ILI cases were representative of mainly pandemic H1N1 influenza activity, albeit with some minor contribution of seasonal influenza [5]. Furthermore, the week in which the Google search ratio peaked (week 28, starting 5 July) was also the peak week for hospitalisations and admissions for pandemic H1N1 influenza to intensive care units in New Zealand (as detailed elsewhere [5]). Nevertheless, *Google Flu Trends* would not have provided any advance warning of ILI cases compared to the weekly reporting of HealthStat data (neither of the major increase nor the timing of the peak).

The overall similar results with primary care data on ILI are not surprising in that *Google Flu Trends* for New Zealand was initially calibrated on the Sentinel GP surveillance data for seasonal influenza in previous years. But of course the congruence of the two systems with regards to pandemic influenza, has never before been examined for New Zealand.

The fact that *Google Flu Trends* data lagged behind the increase in Healthline ILI-related call levels may reflect the design of the former, being originally calibrated on Sentinel GP surveillance. Another contributing factor could be that symptomatic people used the Healthline before thinking of performing Google searches. This could reflect Ministry of Health promotion (e.g. in media statements) of this national free service as an alternative to people consulting their general practitioner. It might also reflect social patterning of disease spread: If lower-income New Zealanders were at increased risk of influenza early in the pandemic (e.g. household crowding and family size are influenced by socio-economic status), then this group may prefer using Healthline as they have better telephone access than internet access. Healthline callers may also represent individuals who were influenced more by media coverage, but in fact, the major increase in Healthline calls occurred several weeks before the week when the first death attributed to pandemic H1N1 influenza in New Zealand was officially announced (in week 27, starting 28 June) [9]. In the same week, the regular (at least daily) Ministry of Health media release first referred to hospitalised cases of pandemic H1N1 influenza.

Google Flu Trends data might also produce spurious minor patterns that are not mirrored by other systems e.g. the second peak identified in week 35, starting 23 August. This second peak was probably not due to the return to school, as this appears to have occurred earlier during the holiday period and was identified through increased HealthStat consultation rates for school age groups (5–14 years) in weeks 30–32 (the weeks starting 19 June to 2 August) [5].

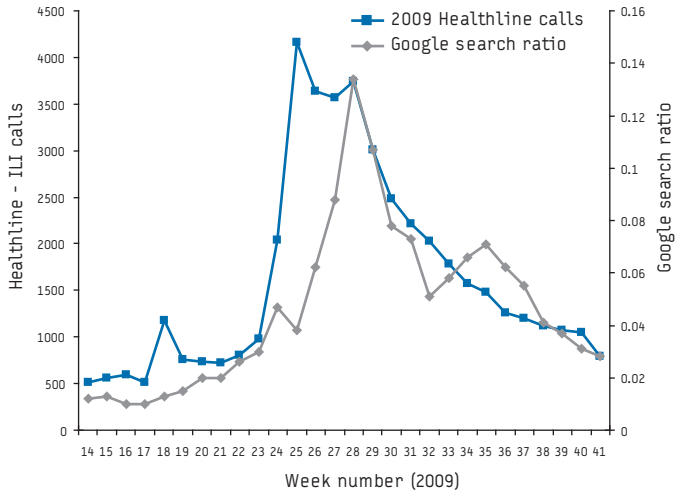
Implications for surveillance and research

A major benefit of *Google Flu Trends* is that it is free and that it is likely to provide some indication of when the incidence of ILI has started to increase in the community and is likely to have peaked. This system also provides daily graphical data and weekly total data that are immediately available to download at the end of each reporting week. This contrasts with an average delay of four days for the GP Sentinel system and four days for HealthStat data (the time for national health authorities to report these data to the rest of the health sector at the end of the data collection week).

Google Flu Trends could be particularly useful for countries where other influenza surveillance systems are poorly developed, though it would probably be less reliable if it had not been calibrated with a robust existing surveillance system for the country in question. Countries with well-established surveillance systems can also potentially profit from *Google Flu Trends* as a supplementary and partial backup surveillance system. In particular, it could assume an important role if the normal systems were disrupted (e.g. in a particularly severe pandemic where health systems are overburdened), or when people with mild illness are discouraged from visiting doctors. *Google Flu Trends* should therefore continue to be closely studied. One question to be addressed is, for example: Does the area under the *Google Flu Trends* epidemic curve reflect the total disease burden in the community (as validated by sero-surveys) better than other surveillance systems?

FIGURE 3

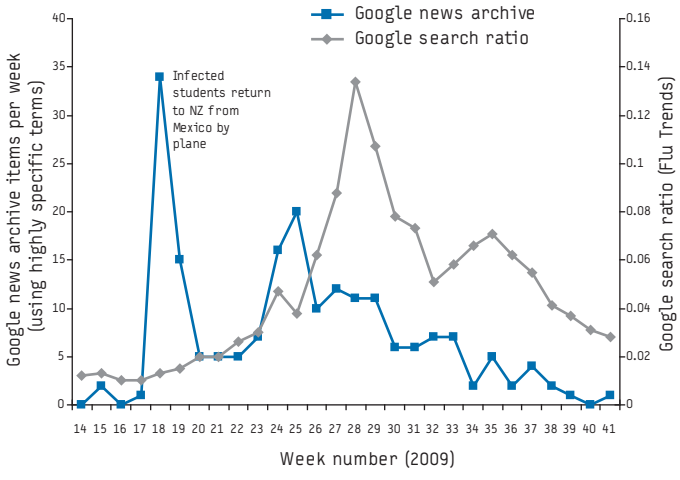
Weekly ILI-related calls to the national Healthline, compared to the Google search ratio, New Zealand, 29 March – 4 October 2009



ILI: influenza-like illness.

FIGURE 4

Weekly news items from the Google news archive related to pandemic H1N1 influenza in New Zealand*, compared to the Google search ratio, New Zealand, 29 March – 4 October 2009



* Retrieved in a search for 'swine AND flu AND Zealand AND "Ministry of Health" '.

Acknowledgements

We thank the many health workers in New Zealand who collect ILI data via the different surveillance systems referred to in this study.

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A simple mathematical approach to deciding the dosage of vaccine against pandemic H1N1 influenza

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Results from early clinical trials have shown that a single dose of pandemic H1N1 influenza vaccine may generate sufficient antibody response, but the relevance of this fact to public health decision making has yet to be clarified. The present study compares the risk of clinical attack (i.e. clinical attack rate) between one- and two-dose vaccination schemes. If the efficacies do not greatly vary between one- and two -dose schemes, one-dose vaccination may well be supported. Nevertheless, two-dose vaccination is shown to result in less morbidity if the vaccine efficacies are greatly diminished by reducing the dose. As long as the detailed efficacy estimates rest on theoretical assumptions, single-dose vaccination may only be sufficiently justified in a specific setting where the number of vaccines is extremely limited.

Introduction

As the world has experienced the global spread of the pandemic H1N1 influenza since April 2009, various pandemic vaccines have been manufactured around the world to reduce the incidence of the disease and to prevent severe illness and death. Since the number of vaccines that can be produced in parallel with a growing pandemic wave is limited, optimal timing of vaccination and prioritisation strategies have been sought to minimise the potential impact [1-3]. Results from early clinical trials have shown that a single dose of H1N1 vaccines probably generates antibody response at a sufficient level [4,5]. Following this early evidence, in United States it has been suggested that individuals aged ≥ 10 years receive a single dose [6]. However, although the early studies report immunogenicity (expressed as antibody titres) and safety of vaccination [4,5], their relevance to public health decision making has yet to be clarified. Taking into consideration that vaccines produced by various manufacturers differ in composition (e.g. adjuvanted and unadjuvanted vaccines), and optimal route of administration (i.e. intramuscular and subcutaneous injections), policymakers have faced the difficult choice whether to choose a one- or a two-dose regimen. The present study proposes a simple mathematical approach to deciding the optimal dosage of a pandemic vaccine by clarifying the population level implications of choosing either the one- or the two-dose vaccination scheme.

Methods

Theoretical basis

The number of doses of vaccine to use against the pandemic H1N1 influenza has not been established to date. Given that the antibody response to single-dose vaccination is not significantly different from that to a two-dose regimen (i.e. one dose on day 0 and another dose typically on day 21 or 28), the practical implication is that with one-dose alone we can vaccinate a population twice as large as that vaccinated with a two-dose regimen. In other words, given that the limited number of vaccines covers a proportion f of the population with a two-dose regimen, a one-dose regimen is expected to cover a proportion $2f$ with the similar efficacy. Nevertheless, the expected risk of clinical attack (i.e. which is equivalent to the so-called clinical attack rate or illness attack rate) at the end of an epidemic is influenced by herd immunity (which is non-linear), and most importantly, the actual protective effects of vaccination are unknown for both one- and two-dose schemes. Accordingly, we formulated our study question as follows: "Which should we implement, one- or two-dose vaccination, to minimise the risk of contracting influenza?" Whereas the optimal dosing of a pandemic vaccine against H5N1, accounting for continuous dose-response phenomena [7,8] has been discussed, our approach is different from previous studies in that we solely focus on two discrete doses, i.e., one- or two-dose regimens alone, analysing a wide range of relative efficacies for the one-dose regimen compared to two-dose scheme specifically against the pandemic H1N1 influenza virus.

Epidemiological model

Our arguments rest on a type of Kermack and McKendrick epidemic model. For mathematical convenience, and to offer simple arguments which are not case-specific (i.e. arguments which are independent of the ongoing pandemic waves), we assume that vaccination takes place sufficiently in advance of a pandemic. The numbers of unvaccinated and vaccinated new cases at calendar time t, $j_u(t)$ and $j_v(t)$, respectively, are described by the following renewal equations [9]:

(1)

$$j_u(t) = R_{uu}(t)\int_0^\infty j_u(t-s)g(s)ds + R_{uv}(t)\int_0^\infty j_v(t-s)g(s)ds,$$

$$j_v(t) = R_{vu}(t)\int_0^\infty j_u(t-s)g(s)ds + R_{vv}(t)\int_0^\infty j_v(t-s)g(s)ds,$$

where $R_{ij}(t)$ represents the average number of secondary cases in sub-population i generated by a single primary case in sub-population j at calendar time t, and $g(s)$ is the density function of the generation time. Linearising the system (1) near the disease-free equilibrium, we get the next-generation matrix:

(2)

$$K = \begin{pmatrix} R_{uu}(0) & R_{uv}(0) \\ R_{vu}(0) & R_{vv}(0) \end{pmatrix}$$

Let p_i be the vaccination coverage under an i-dose vaccination scheme ($i = 1$ or 2), $p_1 = 2p_2$ for $p_2 \leq 0.5$. There are two different types of efficacy which directly influence the transmission dynamics; i.e., reductions in susceptibility and in infectiousness, denoted by α_S and α_I, respectively. We assess the risk of a clinical attack in a homogeneously mixing population in which the next-generation matrix is simplified as

(3)

$$K = R\begin{pmatrix} (1-p_2) & (1-p_2)(1-\alpha_I) \\ p_2(1-\alpha_S) & p_2(1-\alpha_S)(1-\alpha_I) \end{pmatrix}$$

for a two-dose regimen, and

(4)

$$K = R\begin{pmatrix} (1-p_1) & (1-p_1)(1-k_I\alpha_I) \\ p_1(1-k_S\alpha_S) & p_1(1-k_S\alpha_S)(1-k_I\alpha_I) \end{pmatrix}$$

for one-dose regimen where R is referred to as the reproduction number, i.e., the average number of secondary cases generated by a typical infected individual at the initial growth phase of an epidemic. It should be noted that we do not use more widely known notation, the basic reproduction number, R_0 in light of the potential presence of immune adults before the pandemic. k_S and k_I, respectively, represent the relative efficacies of α_S and α_I for a one-dose regimen compared to a two-dose scheme (k_S, $k_I \leq 1$). The reproduction number under vaccination R_v is expressed as $R\{1-p_2+p_2(1-\alpha_S)(1-\alpha_I)\}$ for a two-dose scheme and $R\{1-p1+p1(1-k_S\alpha_S)(1-k_I\alpha_I)\}$ for a one-dose scheme.

Assuming that everyone without vaccination is susceptible before the epidemic, the proportions of those who have experienced infection by the end of the epidemic (i.e. final sizes) among unvaccinated and vaccinated individuals, z_u and z_v, are given by [10]:

(5)

$$z_u = 1-\exp\left[-\left(R_{uu}(0)z_u + R_{uv}(0)z_v\right)\right],$$
$$z_v = 1-\exp\left[-\left(R_{vu}(0)z_u + R_{vv}(0)z_v\right)\right].$$

Let b be the conditional probability of symptomatic disease given infection. The expected risk of clinical attack is expressed as $b[(1-p_i)z_u+p_i(1-\alpha_P)z_v]$ where α_P is the efficacy of reducing the probability of symptomatic disease, assumed to be independent of the transmission dynamics. We examine the sensitivity of the expected risk of clinical attack for different values of α_S, α_I and α_P by iteratively solving z_u and z_v in equations (5), where $R_{ij}(0)$ are dependent on the reproduction number (R), susceptibility effect (α_S), vaccine-induced reduction in infectiousness (α_I) and vaccination coverage (p_i).

Vaccine efficacy and other parameter values

The Table summarises parameter values that we extracted from literature. Although the reproduction number may vary across time and place as the subpopulations involved tend to vary greatly [11-17], we assume $R = 1.5$ as a common estimate in different settings [1,11,12]. The conditional probability, b, of developing symptomatic disease (given infection) has been suggested to be 66.7% [18]. Since vaccine efficacy estimates for the pandemic H1N1 influenza have yet to be reported, we adopt the estimates for seasonal influenza vaccines from an epidemiological analysis of metadata [19]. Conservatively, we assume that α_I and α_P following

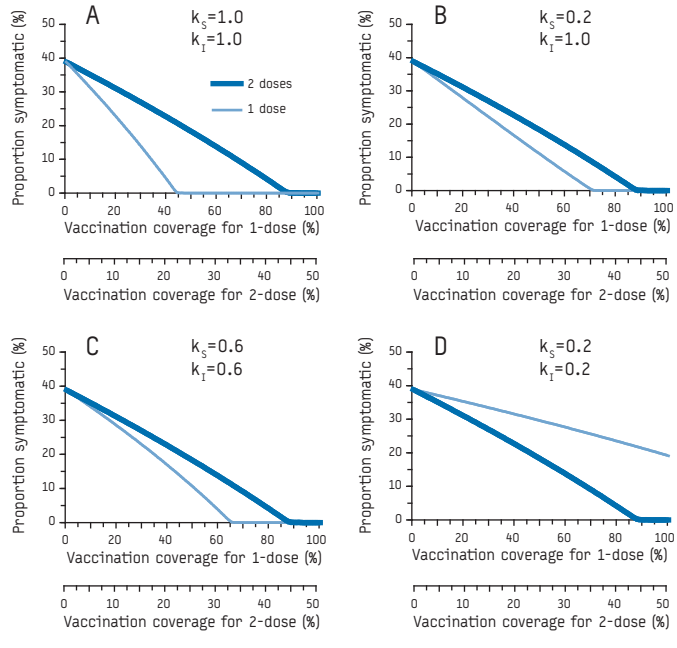
Table

Parameter values used for comparative risk assessment of vaccination against pandemic H1N1 influenza

Parameter	Value	References
Reproduction number (R)	1.5	[1,11,12]
Conditional probability of symptomatic disease given infection (b)	66.7 %	[18]
Reduction in susceptibility (α_S)	40.0%, 60.0%, 80.0%	Assumption and [19]
Reduction in infectiousness (α_I)	40.0 %	[19]
Reduction in the risk of contracting clinical disease (α_P)	67.0%	[19]

Figure 1

The expected risk of clinical attack as a function of vaccination coverage



Panels A-D compare the expected risks of contracting clinical disease between one- and two-dose vaccination schemes with different dose-related protective effects. The vaccination coverage (horizontal axis) for a one-dose regimen is twice as large as that for a two-dose scheme. k_S represents the relative efficacy (of one dose as compared to two doses) for reducing susceptibility, while k_I represents the relative efficacy of reducing infectiousness by the same dose reduction. The relative reduction in reducing the conditional probability of symptomatic disease (given infection) is assumed to be equal to that of infectiousness. The baseline parameters for a two-dose vaccination scheme are shown in Table, and the reduction in susceptibility α_S is assumed to be 0.6 for two-dose regimen.

a two-dose regimen are the same as those reported in [19] for inactivated vaccine (the estimates in literature are based on a one-dose regimen). We allowed α_S following two-dose vaccination to vary from 40% to 80% where the lower bound is equivalent to an estimate of meta-analysis based on one-dose scheme [19]. For a one-dose scheme, we assume that the susceptibility effect is reduced to $k_S\alpha_S$ where $k_S \leq 1$. Similarly, the reduction in infectiousness and the conditional probability of clinical disease given infection are reduced to $k_I\alpha_I$ and $k_I\alpha_P$ where $k_I \leq 1$; for simplicity we use the identical reduction factor for these two different types of efficacy.

Results

Figure 1A shows the baseline results of the risk of clinical attack as a function of vaccination coverage, assuming that the efficacies are identical between one- and two-dose vaccinations. In the absence of vaccination, 38.9% of the population is expected to experience clinical attack. If the efficacy estimates were identical, a one-dose vaccination could limit the impact using only half of the vaccine doses which are required for a two-dose scheme.

The superiority of a one-dose regimen is maintained even when k_S is reduced to 0.2 (with k_I = 1.0; Figure 1B), though the vaccination coverage needs to be higher to achieve the similar reduction of the risk of clinical attacks to that in Figure 1A. Even when both k_S and k_I are reduced (Figure 1C), this relationship (i.e. one-dose being superior) is still maintained. Nevertheless, when both k_S and k_I are greatly reduced (to 0.2; Figure 1D), a two-dose scheme becomes more efficient.

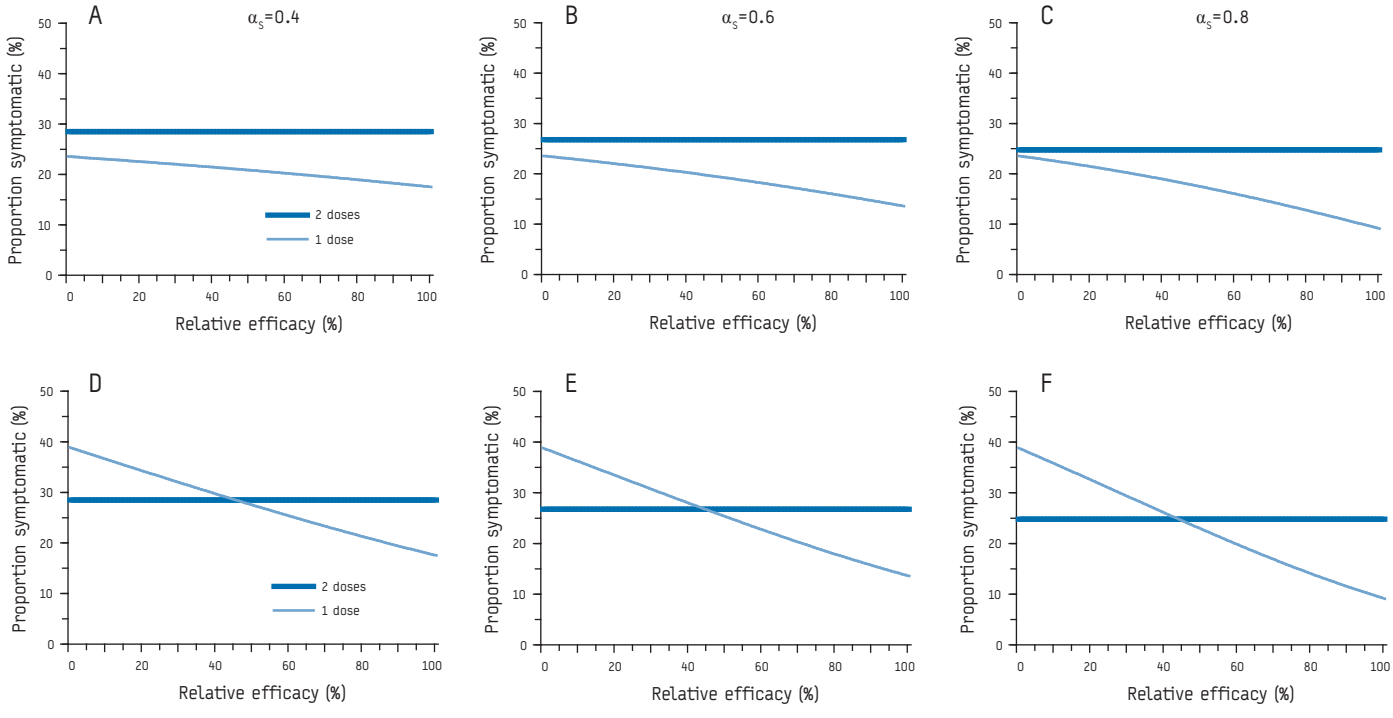
Figure 2 examines the sensitivity of the expected risk of clinical attack to different relative efficacy estimates (i.e. k_S and k_I) due to dose-reductions with fixed vaccination coverage under a one-dose scheme (30%). Figures 2A-2C compare the risk between one- and two-dose vaccinations, assuming that k_S alone varies with dose and k_I is fixed at 1.0. The expected risk with a one-dose scheme is more sensitive to k_S with a higher α_S estimate, but in general the superiority of a one-dose scheme is commonly seen. Figures 2D-2F compare the risks, varying both k_S and k_I simultaneously. If the dose-related relative reduction in efficacy is > 50%, a two-dose scheme yields a smaller risk of clinical attacks than a one-dose regimen. In addition, even when we discard the herd immunity effect (so that α_S and α_P alone would directly inform the frequency of clinical attack by $1-(1-\alpha_S)(1-\alpha_P)$), a two-dose scheme yields smaller risk than that of a one-dose scheme for the large dose-related relative reduction in efficacy. For instance, if α_S= 0.400 and α_P= 0.667, making $k_I < 0.42$ shows the two-dose regimen to be superior to the one-dose scheme.

Discussion

The present study compared the risk of clinical attack in pandemic H1N1 influenza under one- and two-dose vaccination

Figure 2

The expected risk of clinical attack as a function of the relative efficacy of vaccination as a result of a reduction in vaccine dosage



All panels compare the expected risks of contracting clinical disease between one- and two-dose vaccination schemes. In panels A-C, we assume that only the reduction in susceptibility is altered by reduction in the dosage of the vaccine. In panels D-F, all the efficacies (i.e. reductions in susceptibility, infectiousness and probability of symptomatic disease) are assumed to be equally reduced due to reduction in the vaccine dose. The baseline parameters for a two-dose vaccination scheme are shown in Table, and the reduction in susceptibility α_S is assumed to be 0.4 (A and D), 0.6 (B and E) and 0.8 (C and F) under a two-dose regimen. The vaccination coverage is fixed at 30% for one dose and 15% for two doses.

regimens, with an intention to assist relevant public health decision making. Instead of studying the impact of vaccination on reducing the probability of death among high risk groups (e.g. reducing the risk of death among those with underlying medical conditions), we employed a simple transmission model to find the optimal vaccination strategy which reduces the transmission itself. A single dose enables us to vaccinate twice as many people as a two-dose scheme can cover. Under the circumstances of an extremely limited number of vaccines, one-dose vaccination may well be supported if the efficacies do not greatly vary between one- and two-dose schemes. Although the dose-reduction for such a purpose (i.e. decrease doses to increase vaccination coverage) has not been recommended in the present pandemic because the number of vaccines is expected to increase over time [20], similar suggestions were given prior to the emergence of the H1N1 pandemic [7,8]. Moreover, exploring a wide range of relative efficacies for a one-dose regimen, the present study has also shown that a two-dose scheme may result in less morbidity if the vaccine efficacies are greatly diminished by reducing the dose.

An important technical message from the present study is that the relevant decision cannot be made by measuring antibody titres alone. Interpreting antibody titre usually forces us to adopt a well-known criterion, i.e. the haemagglutination inhibition titre > 1/40, as a correlate for individual protection [21], but this criterion itself has yet to be validated for the pandemic H1N1 influenza virus. Moreover, even if we can gain some practical insights into actual protection from the antibody titre, the validity of individual protection does not directly extend to the validity of herd immunity, which is more pertinent in respect to population level protection from infection. To understand the population level implications it is necessary to study in more detail the multidimensional protective effects of vaccination based on epidemiological studies [7,22], because an assessment of any infectious disease risks at the population level requires vaccine efficacy estimates which influence the transmission dynamics. Such efficacies include reductions in susceptibility, infectiousness and probability of symptomatic disease, as described in the present study.

The most difficult aspect of the ongoing pandemic H1N1 influenza is that we do not have an opportunity to analyse the abovementioned estimates in advance of vaccination practice. Moreover, the decision making for vaccination in the ongoing pandemic has to be done during the course of the pandemic waves [12]. In particular, one may prefer a one-dose to a two-dose scheme near the peak incidence of any pandemic wave to immunise as many susceptible individuals as possible. Nevertheless, as a practical implication of the present study, and as long as the detailed efficacy estimates rest on theoretical assumptions, one may consider that single-dose vaccination may be sufficiently justified only in a specific setting where the number of vaccines is extremely limited. At the same time, any observation of dose-related reduction in any biological action of vaccine efficacy (i.e. dose-related effects of reducing susceptibility and infectiousness) needs to be reported as soon as such an insight is gained during the course of the pandemic.

It should be noted that there are several limitations in the arguments we make here. First, parameter values in Table rest on theoretical assumptions, as the empirical estimates for H1N1 vaccines have yet to be clarified. Second, potential heterogeneity in vaccine efficacy must be noted as relevant. Efficacy estimates may differ between age- and risk- groups, as is the case for antibody responses [6,20], and this in turn may greatly influence decisions related to dosage for different age- and risk-groups. Third, we ignored heterogeneous patterns of transmission. In a heterogeneously mixing population, a one-dose regimen may not yield as large community benefit as presented in the present study, because the residual number of vaccines which were generated by reducing dosage from two-dose to one-dose may well be distributed to those with small risks of secondary transmission and severe manifestations.

There are several different pandemic vaccines (including those adjuvanted and unadjuvanted) with different routes of administration [23], and the efficacies of these are likely to be different. Thus, the decision on dosage cannot be made in a uniform theoretical fashion. Nevertheless, we believe that our simple approach satisfies the need to offer a basic insight into the question of vaccine dosage based on firm theoretical understanding.

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Pandemic influenza A(H1N1)v: Human to pig transmission in Norway?

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In Norway there is an ongoing outbreak in pigs of infections with pandemic influenza A(H1N1)v virus. The first herd was confirmed positive on 10 October 2009. As of 26 October, a total of 23 herds have been diagnosed as positive. The majority of the herds seem to have been infected by humans. Sequence analysis of pig viruses from the index farm shows that they are identical or virtually identical to human viruses from the same geographical region.

Introduction

The Norwegian pig herds have been considered free of swine influenza (the classical strain H1N1 and H3N2) as documented by a serological surveillance programme running since 1997 [1]. The pig industry in Norway is relatively small with approximately 2,700 herds and a little less than 1.5 million slaughtered animals in 2008.

Responding to the emergence of a novel influenza A(H1N1) strain (hereafter called pandemic influenza) affecting humans in April 2009, the surveillance of pandemic influenza in humans was initiated in Norway in late April, as a continuation and enhancement of the seasonal influenza surveillance systems. After the first detections of the pandemic influenza virus in Norway in early May, sporadic infections, mostly in travellers from abroad, increased gradually through the summer. After a peak in late July, the numbers declined while an increasingly larger proportion of cases were infected in Norway. A new increase has been seen through October and the cumulative number of laboratory verified cases by 26 October exceeded 3,300 [2].

There are reports from the World Organisation for Animal Health (OIE), on ProMED-mail [3] and in general media from other countries (Argentina, Canada, Australia, North Ireland, Ireland, United States) that human to animal transmission has occurred with the new pandemic influenza.

This paper describes an ongoing outbreak in pigs of infections with the pandemic influenza virus in Norway, providing insights on the source of infection and on the control strategies put into force for its control.

Detection of outbreak

On 9 October 2009, the Norwegian Food Safety Authority (NFSA) was contacted by a local veterinarian who informed about a possible outbreak of influenza in a pig herd of 85 sows and 850 growers and fattening pigs in Nord-Trøndelag County. In the period from 4 to 9 October a sow in the farrowing unit had been observed coughing. No other clinical signs of infection were observed in the rest of the herd and no animal had died. The NFSA was informed that a farm staff member had been ill with influenza-like symptoms (ILI) since 1 October, and tested positive for pandemic influenza virus on 8 October. The NFSA therefore decided to take nasal swabs from 20 pigs in the herd, and the samples were sent to the National Veterinary Institute (NVI) for analyses. On 10 October a total of 18 of the sampled pigs tested positive for influenza A and for 12 of these pandemic influenza viruses was confirmed.

An epidemiological investigation performed by the NFSA began on 11 October, and samples were collected from six additional herds located in close proximity to the index herd or with a history of close human/animal contacts. One of these, a herd with about 500 slaughter pigs, tested positive for pandemic influenza virus. This herd was owned by the infected animal handler of the index herd. This second herd positive for pandemic influenza was situated in an area with very intensive pig farming. Based on the possibility of a potential further airborne spread to neighbouring farms and with the aim to keep the Norwegian pig population free from swine influenza, it was decided to eradicate the second infected herd quickly. For animal welfare reasons and in spite of potential hazard for airborne spread during transport, all the pigs from this herd were transported to a nearby slaughterhouse and put down.

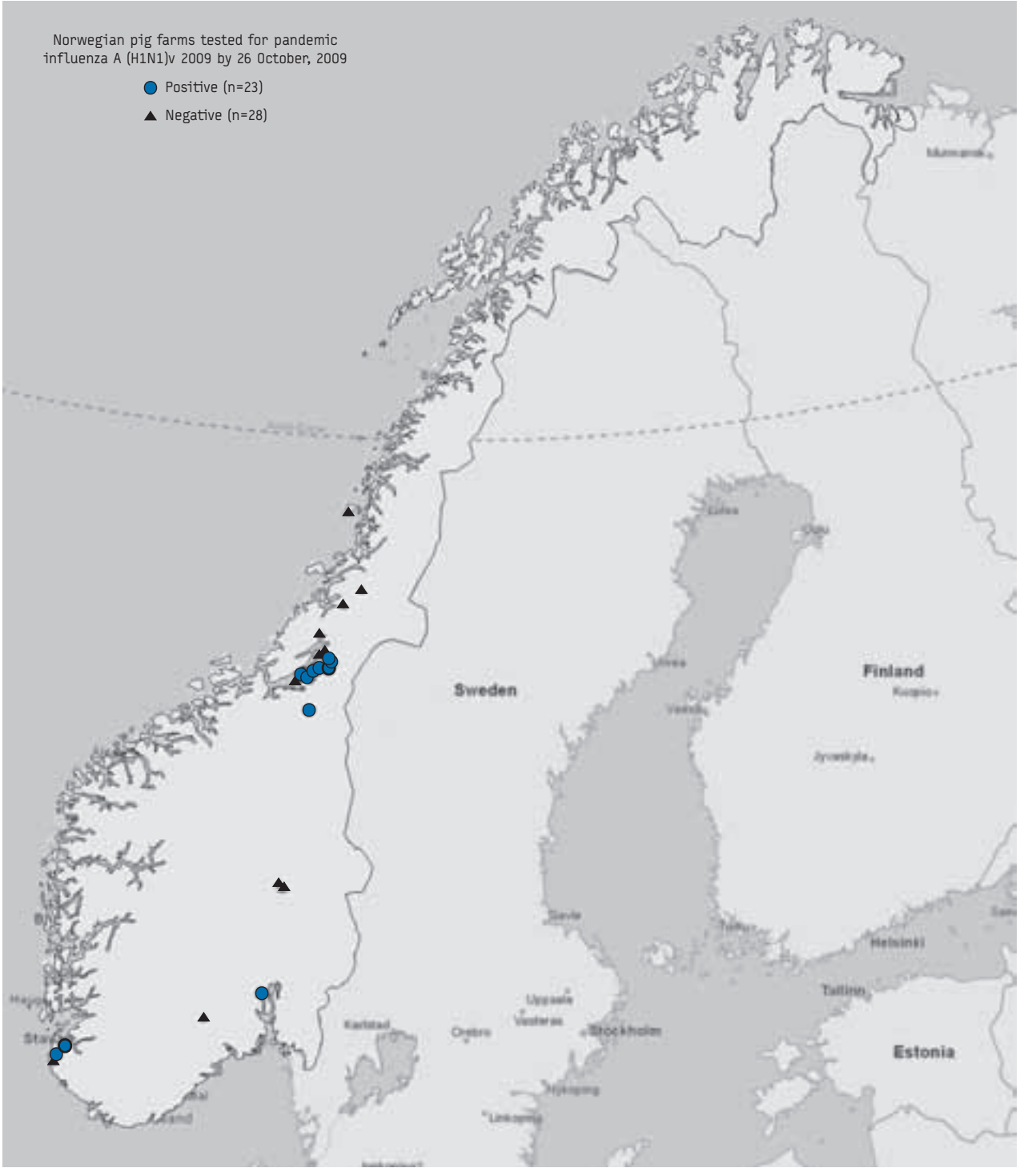
The plan was to slaughter the index herd during the same week. However, the eradication strategy was abandoned when four more herds in the area tested positive the next few days. It soon became clear that all the herds tested positive so far had been in contact with humans with ILI symptoms or with verified infection with pandemic influenza virus. At the same time, there was no evidence indicating there had been contact (pigs, staff, vehicles, etc.) between the new positive herds, and the possibility of airborne transmission was also ruled out due to long distances between the positive herds. Thus the sampling strategy was revised to include pig herds throughout Norway, having staff members with ILI or confirmed pandemic influenza, should be sampled. Later on, a revised surveillance programme for the Norwegian pig herds will be implemented.

Materials and methods

All herds except two, have been sampled by 20 nasal swabs. These swabs have been tested at the NVI by real-time RT-PCR to detect influenza A [4]. Samples positive in this test have also been tested for the pandemic influenza A(H1N1)v virus subtype [5]. The remaining two herds were sampled by 20 blood samples and

Figure

Pig herds tested for pandemic influenza A(H1N1)v virus, 10 October – 26 October, Norway, 2009 (n=51)



tested by enzyme-linked immunosorbent assay (ELISA, ID Screen® Influenza A Antibody Competition test, IDVET) and for subtype A(H1N1)v by haemagglutination inhibition test.

Results

In Nord-Trøndelag County, in the period between 10 October and 26 October, a total of 39 herds were tested and 18 of these were positive for pandemic influenza. Of these 18 positive herds, a total of 15 herds were in contact with people diagnosed with pandemic influenza (n=10) or with people with ILI symptoms (n=5). For the three remaining herds, there is no available information on such contact.

So far, in six of the 18 positive herds in Nord-Trøndelag County the clinical status of the herd has been recorded. Moderate clinical signs of influenza (coughing, fever) were recorded in four herds, while signs were mild to non-existing in two herds. In five of these six herds, the clinical signs in the pigs occurred after humans in contact with the pigs became ill.

In addition, during the period 12 October to 26 October, a total of 12 herds from six other counties were tested and five herds from three counties were positive for pandemic influenza virus. Also in these counties, the majority of positive herds are suspected of having contracted the virus from infected people.

The influenza virus in specimens taken from the index herd in Nord-Trøndelag has been sequenced at the Norwegian Institute of Public Health and compared to human strains from Norway and elsewhere, including the virus from the initial human case associated to the outbreak on this farm. The virus from two individual animals showed full identity in the two genome segments analysed for both pigs (full length H1 and 727 nt partial N1). There was also full identity to the 1,744 nt H1 gene of the virus from the farm staff member. Very high similarity was also observed to some of the viruses isolated from other humans in Norway, in particular to a virus found in the same geographical region. Within the entire 1,744 nt H1 and 727 nt N1 sequences compared, a difference in only one nucleotide in H1 was observed (99.9 and 100% identity, respectively). Full genome sequencing of the virus from one of the swine specimens confirms a very high similarity throughout the viral genome to the pandemic virus circulating in humans.

Discussion

In this investigation, humans infected with the pandemic influenza virus seem to be the most likely source for the spread of the infection to the pigs, even though additional routes, like airborne transmission or transmission by vehicles cannot be ruled out at the moment. So far, no evidence has suggested that animals play any particular role in the epidemiology or the spread of the pandemic influenza among humans. [6].

The Norwegian pig population has until this outbreak been free of classical swine influenza. The current situation thus presents an acute challenge for the pig industry and the NFSA. This has major long term implications for both the pig industry and for the public in terms of zoonotic potential. Transmission from humans to pigs and the possible vice versa is especially worrying. In addition pigs could potentially be effective multipliers for the virus, and might act as reservoirs of the virus during the out-of-season periods when the virus does not circulate in humans. Also, the virus could possibly further re-assort in case of swine or avian influenza viruses co-circulation, or mutate within the pigs to produce a more virulent strain [8]. The Norwegian authorities have taken several measures to control the outbreak such as monitoring the situation and the affected farms closely and restricting movements of animals from affected farms. Furthermore Norway follows the European Union working document [7] which recommends not slaughtering animals before at least seven days after the termination of clinical signs.

Further investigations are being carried out to clarify the extent of the outbreaks in the rest of Norway. Studies are also underway to evaluate risk factors for the infection at farm level. Farmers claim to maintain proper biosecurity as change of clothes and the use of face mask (surgical mask, gauze mask) before any contact with the pigs. However, due to lack of extra hands, on several occasions it had been necessary for the farmers to attend the pigs in spite of having influenza symptoms.

To further test the hypothesis that the pigs are infected by humans, follow up investigations should gather detailed information on directionality of transmission, such as what time point the farmer and the pigs showed signs of illness. To assist in such investigations, the results from nasal swabs taken initially and additional serological results should be further studied. Virus isolates from possible human and pig "pairs" are also available and can be further characterized.

Acknowledgements

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Assessing the impact of the 2009 H1N1 influenza pandemic on reporting of other threats through the Early Warning and Response System

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Since the start of 2009 H1N1 influenza pandemic, a notable surge in messages communicated through the Early Warning and Response System (EWRS) for the prevention and control of communicable diseases in the European Union has been recorded. In order to measure the impact of this increase on the reporting of other events, we compared the messages posted in the EWRS since April 2009 with those posted in the previous years (2004-2008). The analysis revealed that a ten-fold increase in messages was recorded during the pandemic period, from April to September 2009, and that the reporting of other threats dropped to a significantly low rate. These results suggest an important impact on the notification process of events in case of a situation requiring extensive mobilisation of public health resources. It emphasises the importance keeping an appropriate balancing of resources during sustained emergencies, in particular in view of a possible second wave of pandemic influenza cases, to ensure prompt detection and reporting of potential concomitant emerging threats.

Introduction

The Early Warning and Response System (EWRS) was created in 1998 under Decision No 2119/98/EC of the European Parliament and of the Council with the aim of establishing a permanent communication between the public health authorities of the European Union (EU) Member States (MS) responsible for planning and taking measures to control the spread of communicable diseases in the European Community. Under this decision, the MS are required to inform each other and the European Commission (EC) in order to coordinate public health measures to control events caused by communicable diseases of relevance for the European Union [1]. In addition, specific planning for pandemic influenza by the EC designates the EWRS system as the primary network used by the MS for exchange of information and coordination of measures during an influenza pandemic [2]. Since its establishment in March 2005, the ECDC has been supporting the EC by operating the EWRS.

Since the first cases of pandemic 2009 H1N1 influenza reported in the United States on 24 April 2009 [3], the MS, the EC and the ECDC have relied heavily on EWRS to communicate messages related to the pandemic, with a significant increase in the number of messages posted on EWRS compared with the same period of the previous years. The objective of this study was to analyse the use of EWRS from April to end of September 2009 and to assess the impact of the ongoing H1N1 influenza pandemic on reporting of other events to be notified through the EWRS under the EU legislation on communicable diseases.

Methods

The MS, the EC and the ECDC exchange information through EWRS using three types of communications: messages sent to all users, selective exchanges between two or more users, and comments to existing messages. For this study, EWRS activity was quantified using the term "new event" defined as a message posted for all users by any user. Selective exchange messages and comments were excluded.

New events were aggregated on monthly intervals from May 2004 through September 2009. Data prior to May 2004 were not included in the review because of a major change in the reporting system preventing historical comparisons. A descriptive analysis of the 65-month series was performed in order to observe reporting trends. Monthly reporting activity in 2009 was compared with averages of corresponding months over the five previous years (2004-2008). Events related to pandemic H1N1 influenza were then removed from the data set in order to focus the analysis on the reporting pattern of non pandemic-related events.

A Poisson test was used to quantify the decrease in notification of non-pandemic events as compared with the average notification for the same period in previous years. Averages were compared for months before and during the pandemic. A $p<0.05$ was considered statistically significant.

Results

The analysis of the 65-month series, totalling 917 new events, indicates a very sharp increase in recent months corresponding with the start of the pandemic H1N1 influenza. In addition, a smaller increase can be noticed during the first six months of 2006, corresponding to events related to the introduction of avian influenza (H5N1) to Europe. The average number of new events posted per month during the pandemic period of April to September 2009 was 68.0 versus 8.6 during the preceding five years, indicating an unprecedented increase in reporting during the pandemic period.

The average number of new events from 2004 to 2008 shows a seasonal pattern, with more new events being posted between June and October, on average. The maximum value observed in February 2006, 23 new events, corresponds to the avian influenza (H5N1) situation (Figure 1).

Significant deviation from expected seasonal reporting trends was observed during pandemic H1N1 influenza. Numbers indicated a dramatic increase in the total number of new events (n=120 in April 2009) and a historical low in the number of new events unrelated to pandemic H1N1 influenza (Figure 2).

The results of the Poisson probability test indicate that monthly event posting decreased significantly during March, June and July 2009 compared with the 2004-2008 averages (Table). These three months deviated from expected values with a $p<0.05$, indicating a significant decrease. Among the 48 new events reported in July, from 2004 to 2008, averaging 9.6 per month, 16 new events reported were related to food and water borne outbreaks (on average 3.2 per month), 8 were related to legionellosis cases (1.6 per month), 8 were related to vaccine preventable diseases (1.6 per month) and 16 were related to other conditions (3.2 per month).

Discussion

EWRS is known to be a very specific and reliable system, used to report confirmed events of European Community relevance requiring coordinated actions between the EU MS. EWRS has confirmed its value during the current pandemic, facilitating the necessary communications between MS, EC and ECDC to support implementation of rapid measures. However, on the basis of the results of our review, the dramatic increase in messages related to the current pandemic has masked a significant decrease in the reporting of other events. In July 2009, the number of non-pandemic related threads posted to EWRS dropped to zero. In August and September 2009, the number of new threads regained consistency with historical baseline values.

The decrease in March 2009 might be considered in the light of the high values reported in February and March 2006, related to avian influenza, which may have increased the historical baseline average value for these months. The significant decrease in the two consecutive months of June and July 2009 is extremely unlikely to be explained by chance alone.

In June and July 2009 several Member States were confronted with a dramatic increase in influenza cases. In this early stage of the H1N1 influenza pandemic, most Member States implemented a containment strategy aimed at preventing the introduction and community spread of the novel influenza virus. This strategy placed a tremendous strain on public health resources. During summer months, as cases tended to decrease during school holidays, most Member States discontinued active containment activities such as screening passengers and switched to a mitigation approach [4].

The concomitance of the dramatic decrease in notification of non-pandemic related threats during this period of extreme activity by national public health authorities suggests that the strain on

Figure 1

New events per month in the Early Warning and Response System (EWRS), May 2004 to September 2009 (n=917)

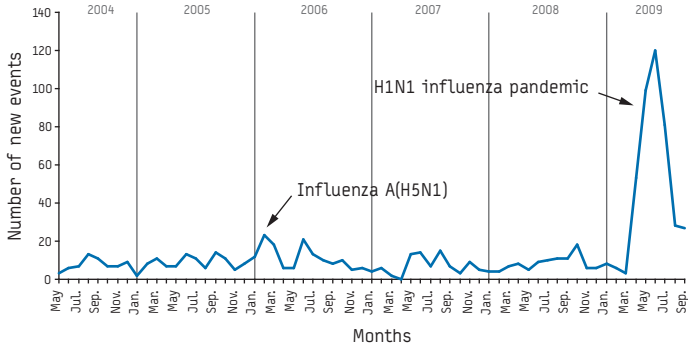
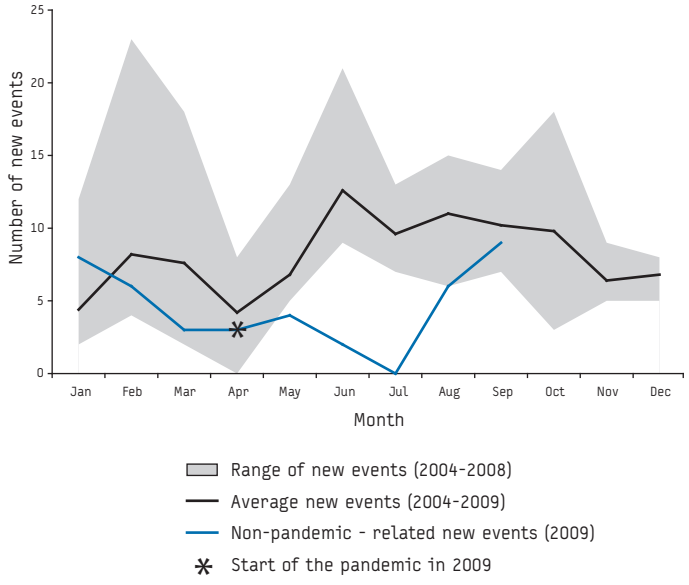


Figure 2

Monthly average and range of non-pandemic related new events in the Early Warning and Response System (EWRS) in 2004-2008 vs. number of new events in 2009



Table

Poisson probability test indicating significance of decrease in monthly threat reporting in the Early Warning and Response System (EWRS) during 2009 compared with 2004-2008 averages

Month	2004-2008 Average	2009 Number of new events (H1N1 influenza pandemic - related events excluded)	p-value
January	5.5	8	0.89
February	10.3	6	0.12
March	9.5	3	0.01*
April	5.3	3	0.23
May	6.8	4	0.19
June	12.6	2	0.0003*
July	9.6	0	0.00007*
August	11.0	6	0.08
September	10.2	9	0.43

* $p < 0.05$: significant value

public health resources had an impact on the notification process of other events. However, other factors may have contributed to the decrease. It is possible that a public health crisis such as the pandemic H1N1 influenza would result in a decrease of non-essential reporting of new events. Even if not thoroughly evaluated, the review of new events posted during historical baseline period does not indicate a significant reporting of non-essential events, such as events not fulfilling the criteria for notification through EWRS. In addition, it is unlikely that the pandemic H1N1 influenza would result in a true reduction of other threats at a time where relatively few cases were occurring in the EU.

In March and April 2003, a five-fold increase in reporting of new events was noted, in relation with the emergence of the severe acute respiratory syndrome (SARS) epidemic. However, no significant decrease in reporting of other new events was noticed in the EWRS (unpublished data). This could be due to the fact that SARS only affected several MS and responded well to control measures.

Conclusions

These findings highlight the need to maintain awareness of potential emerging threats, especially in the context of an ongoing pandemic. The sustained nature of a pandemic necessitates that those in charge of threat detection and response keep a high level of vigilance. In preparation for the expected second wave of pandemic H1N1 influenza in the European Union, it is important to consider the consequences of possible concomitant events, should they occur. This is an ideal opportunity to revisit current pandemic plans, taking into account appropriate allocation of resources to ensure an optimal level of vigilance.

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Public perceptions in relation to intention to receive pandemic influenza vaccination in a random population sample: evidence from a cross-sectional telephone survey

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A cross-sectional telephone survey on a nationally representative sample of 1,000 Greek households was performed to assess the acceptability of the pandemic influenza A(H1N1)v vaccine, factors associated with intention to decline and stated reasons for declining vaccination. The survey was initiated the last week of August 2009 (week 35) and is still ongoing (analysis up to week 44). The percentage of participants answering they would "probably not/definitely not" accept the vaccine increased from 47.1% in week 35 to 63.1% in week 44 (test for trend: p<0.001). More than half of the people which chronic illnesses (53.3%) indicated "probably not/definitely not". Factors associated with intention to decline vaccination were female sex, age between 30-64 years, perception of low likelihood of getting infected or of low risk associated with influenza, and absence of household members suffering from chronic illnesses. For the majority of the respondents (59.8%), the main reason for intending to decline vaccination was the belief that the vaccine might not be safe. Promotion of vaccination programmes should be designed taking into account the attitudinal barriers to the pandemic vaccine.

Introduction

One of the first priority actions following the declaration of influenza A(H1N1)v as the first pandemic of the 21st century was the timely development of a safe and effective vaccine. Although vaccination is an effective measure to reduce the number of infections, hospitalisations and deaths, modelling studies have shown that the impact of vaccination depends strongly on the time when it is initiated as well as on the coverage of the target populations [1-3]. Until the beginning of November 2009, the European Commission had granted authorisation for three specific influenza A(H1N1)v vaccines and vaccination has already started in several European countries. However, there is a major concern about the acceptability of the pandemic vaccine among target populations in several European countries. In the present study, we analysed the data from a weekly telephone survey carried out in the Greek population in order to assess the levels of acceptance of the vaccine and the related attitudinal barriers.

Methods

Telephone survey

A telephone survey on 1,000 households has been carried out in Greece on a weekly basis starting from the last week of August 2009 (week 35) and was still ongoing until the time of this analysis (week 44). One of the aims of the study was to assess perceptions in relation to risks of pandemic influenza A(H1N1) and the attitude towards immunisation. Proportional quota sampling was used to ensure that selected households were representative of the total of Greek households, with quotas based on household size and urban/rural location. The average household size in the selected households was 2.9 persons. The mean age of the respondents was 51.9 (standard deviation ± 17.0) years and 65.8% of them were female.

One participant per household was asked to provide answers to questions about the age and sex of the household members, knowledge and perceptions about influenza A(H1N1)v, the presence of members with chronic illnesses etc. Chronic illnesses included chronic respiratory diseases (including asthma), chronic cardiovascular diseases (except hypertension), chronic metabolic disorders (including diabetes mellitus), chronic renal and hepatic diseases, haematological disorders (including sickle cell disease), immunosuppression and chronic neurological/neuromuscular diseases. A specific question was asked concerning the willingness of the participants to accept vaccination once the pandemic vaccine becomes available: "Do you consider getting vaccinated against the novel influenza (you or the other members of your household) once the vaccine becomes available?" with five possible answers ("definitely yes", "probably yes", "probably not", "definitely not", "don't know").

Statistical methods

The presence of trend in the intentions of the population sampled every week was evaluated using the chi-squared test for trend. The data from week 44 were further used to identify associations between questionnaire-related variables and the

reported vaccination intentions using one-way analysis of variance and the chi-squared test. A multiple logistic regression model was used to evaluate independent predictors of intention to decline the vaccine (where the answers were grouped as "definitely not/probably not" versus "definitely yes/probably yes"). A similar model was used to identify the profile of a non-negligible proportion of the sample answering "don't know" (versus "definitely yes/probably yes").

Results

Overall, according to the most recent data of week 44, 63.1% of the sample indicated "probably not/definitely not" as their intention to get vaccinated. The trends from week 35 through week 44 in the willingness of the respondents to get the pandemic vaccine are depicted in Figure 1 (1,000 persons per week). The percentage of participants answering "definitely not" increased from 32.3% on week 35 to 45.8% in week 44 (test for trend: p<0.001). The proportion of individuals responding "definitely yes" decreased from 22.9% in week 35 to 9.1% in week 44 (test for trend: p<0.001).

Respondents' age, sex and educational attainment, the presence of chronic illness and history of seasonal influenza vaccination in the past year (of the respondents and of members of their household), presence in the household of children aged 0-12 years or of individuals aged 65 or older, and respondents' perceptions concerning the risk related to infection were associated with the reported intention towards getting vaccinated (Table 1). Women intended to decline vaccination at higher rates (67.6%) compared with men (54.4%) and were more determined in their answer (51.8% answered "definitely not"). Persons with a history of previous seasonal influenza vaccine reported intention to decline vaccination at lower rates compared with those who have not

Figure 1

Trends in respondents' intention to receive pandemic vaccine, Greece, 2009 (1,000 respondents per week)

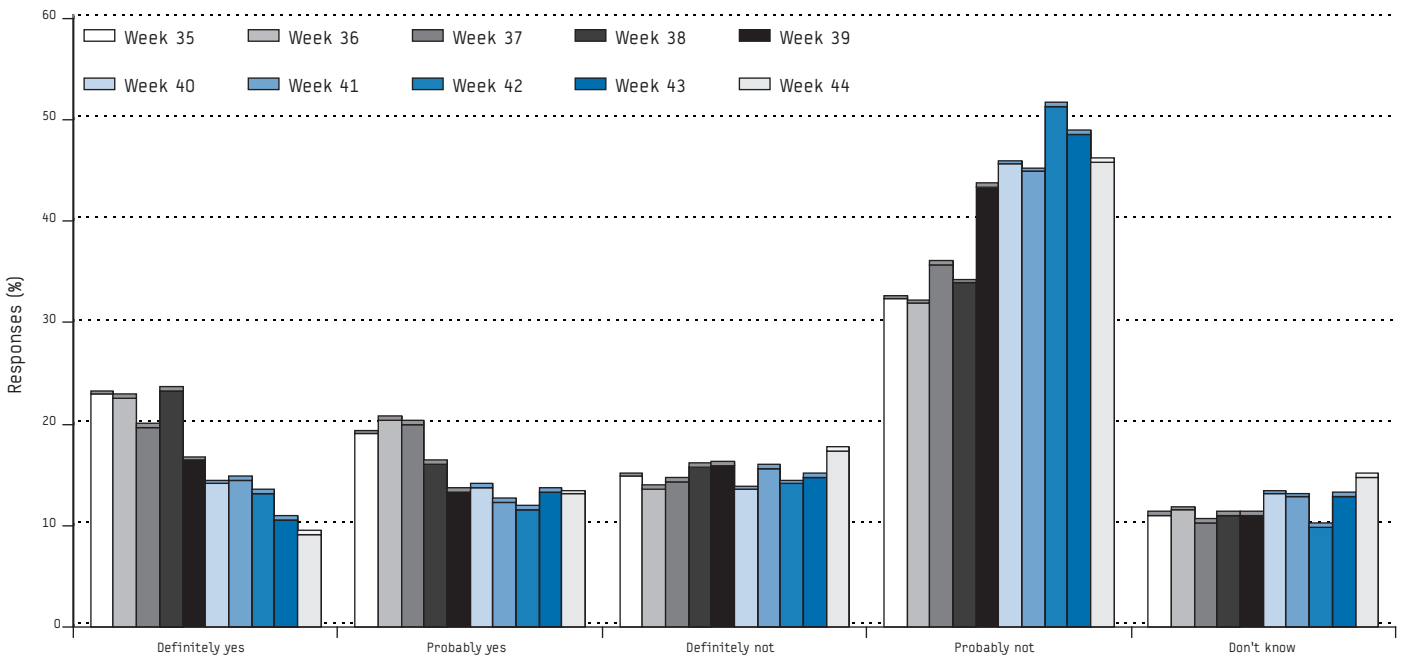


Table 1

Univariate association of variables potentially affecting respondents' intentions concerning vaccination, Greece, 2009

	Intention to accept vaccination					
	Definitely yes	Probably yes	Probably not	Definitely not	Don't know	P value
Age, mean	56.7 (17.0)	49.3 (19.3)	52.4 (16.9)	49.8 (15.8)	56.8 (17.3)	<0.001
Sex						
Male	50 (14.6)	62 (18.1)	60 (20.2)	117 (34.2)	44 (12.9)	<0.001
Female	41 (6.2)	69 (10.5)	104 (15.8)	341 (51.8)	103 (15.7)	
Urban/rural location						
Athens/Thessaloniki	41 (8.2)	61 (12.2)	84 (16.8)	250 (50.0)	64 (12.8)	0.196
Other urban	21 (8.4)	36 (14.4)	49 (19.6)	106 (42.4)	38 (15.2)	
Semi-rural/rural	29 (11.6)	34 (13.6)	40 (16.0)	102 (40.8)	45 (18.0)	

Educational attainment						
Primary school	20 (7.4)	34 (12.6)	53 (19.6)	101 (37.4)	62 (23.0)	
High school (3 years)	11 (10.8)	16 (15.7)	16 (15.7)	36 (35.3)	23 (22.6)	<0.001
High school (6 years)	32 (10.2)	39 (12.4)	47 (14.9)	162 (51.4)	35 (11.1)	
University/postgraduate studies	28 (9.0)	42 (13.4)	57 (18.2)	159 (50.8)	27 (8.6)	
Presence of chronic illness (respondent)						
No	65 (8.3)	97 (12.3)	138 (17.6)	379 (48.2)	107 (13.6)	0.016
Yes	26 (12.2)	34 (15.9)	35 (16.4)	79 (36.9)	40 (18.7)	
Presence of chronic illness (household)						
No	54 (8.2)	75 (11.4)	116 (17.7)	325 (49.5)	87 (13.2)	0.005
Yes	37 (10.4)	56 (16.4)	56 (16.4)	132 (38.7)	60 (17.6)	
Children aged 0-12 years in the household						
No	77 (9.9)	110 (14.1)	139 (17.9)	332 (42.7)	120 (15.4)	0.005
Yes	14 (6.3)	21 (9.5)	34 (15.3)	126 (56.8)	27 (12.2)	
Persons ≥65 years in the household						0.003
No	45 (7.2)	81 (13.0)	113 (18.1)	306 (49.1)	78 (12.5)	
Yes	46 (12.2)	50 (13.3)	60 (15.9)	152 (40.3)	69 (18.3)	
Pregnant women (respondent)						
No	90 (9.1)	130 (13.1)	173 (17.5)	451 (45.6)	146 (14.8)	0.513
Yes	1 (10.0)	1 (10.0)	0 (0.0)	7 (70.0)	1 (10.0)	
Pregnant women (household)						
No	90 (9.2)	129 (13.2)	171 (17.4)	445 (45.4)	146 (14.9)	0.372
Yes	1 (5.3)	2 (10.5)	2 (10.5)	13 (68.4)	10 (5.3)	
Seasonal vaccination (respondent)						
No	61 (7.5)	102 (12.6)	146 (18.0)	399 (49.2)	103 (12.7)	<0.001
Yes	30 (15.9)	29 (15.3)	27 (14.3)	59 (31.2)	44 (23.3)	
Seasonal vaccination (household)						
No	56 (7.6)	97 (13.1)	138 (18.6)	359 (48.5)	91 (12.3)	<0.001
Yes	35 (13.5)	34 (13.1)	35 (13.5)	99 (38.2)	56 (21.6)	
Self-reported level of knowledge about pandemic influenza						
Very much	16 (10.1)	18 (11.3)	26 (16.4)	78 (49.1)	21 (13.2)	
Quite enough	46 (7.7)	76 (12.8)	103 (17.3)	284 (47.8)	85 (14.3)	0.705
Little	24 (12.2)	29 (14.7)	35 (17.8)	76 (38.9)	33 (16.8)	
Not at all	5 (12.2)	6 (14.6)	7 (17.1)	17 (41.5)	6 (14.6)	
Likelihood of getting infected						
Very likely	9 (10.0)	10 (11.1)	16 (17.8)	44 (48.9)	11 (12.2)	
Quite likely	16 (9.1)	26 (14.8)	32 (18.2)	83 (47.2)	19 (10.8)	0.472
Not very likely	26 (8.3)	47 (14.9)	59 (18.7)	145 (46.0)	38 (12.1)	
Not likely at all	13 (7.0)	17 (9.1)	24 (12.8)	110 (58.8)	23 (12.3)	
If likely to become infected, perceptions related to severity						
High risk	15 (19.2)	9 (11.5)	9 (11.5)	30 (38.5)	15 (19.2)	
Moderate risk	19 (11.2)	31 (18.3)	35 (20.7)	58 (34.3)	25 (15.4)	<0.001
Little risk	10 (4.3)	30 (12.8)	43 (18.4)	131 (56.0)	20 (8.6)	
No risk	2 (3.6)	3 (5.4)	12 (21.4)	37 (66.1)	2 (3.6)	

Values express number of respondents and brackets indicate the corresponding percentage with the exception of age where mean (standard deviation) are provided.

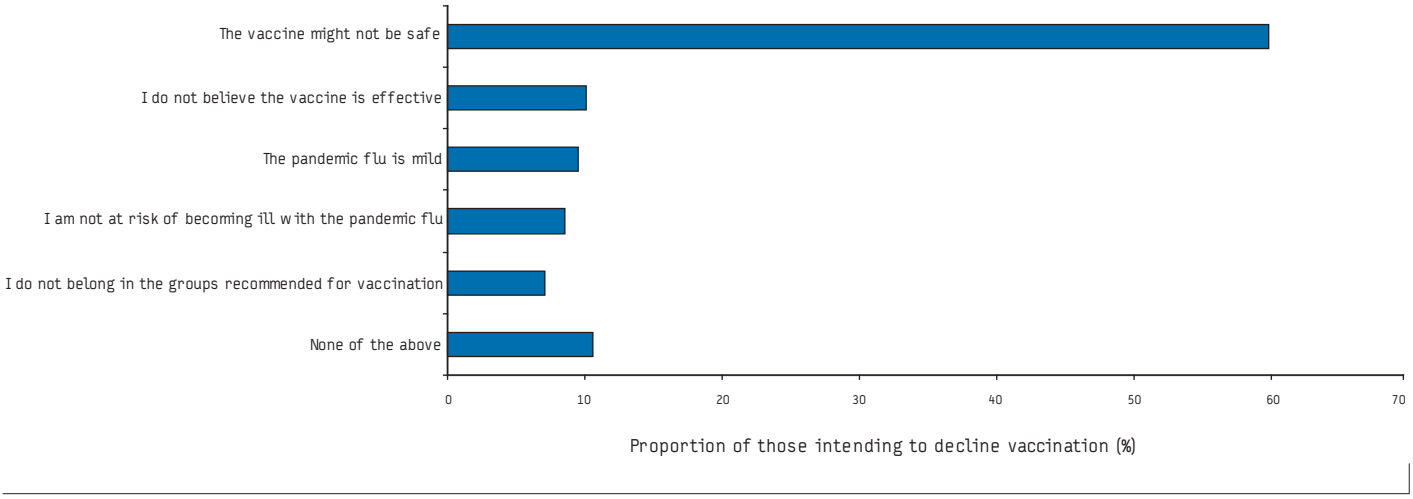
Table 2

Multiple logistic regression models for intention to decline pandemic vaccination (versus those intending to accept), Greece, 2009

Variable	odds ratio	95% confidence interval	P value
Sex			
Male	1		
Female	2.75	(1.94 to 3.90)	<0.001
Age (years)			
15-29	1		
30-64	1.85	(1.07 to 3.22)	0.029
65+	1.24	(0.54 to 2.81)	0.612
Educational attainment			
Nine-year high school/university	1		
Primary/three-year high school	1.07	(0.71 to 1.60)	0.751
Urban/rural location			
Semi-rural/rural	1		
Athens/Thessaloniki	1.56	(1.02 to 2.38)	0.040
Other urban	1.22	(0.75 to 1.96)	0.424
Presence of chronic illness (household)			
Yes	1		
No	1.60	(1.10 to 2.33)	0.013
Presence of child aged 0-12 years in household			
Yes	1		
No	1.47	(0.91 to 2.38)	0.118
Vaccination for seasonal influenza in the household			
Yes	1		
No	1.46	(0.96 to 2.23)	0.077
Person ≥65 years in the household			
Yes	1		
No	1.17	(0.65 to 2.09)	0.600
Self-reported level of knowledge about pandemic influenza			
Not at all/little	1		
Quite enough/very much	1.30	(0.87 to 1.93)	0.196
Likely to get infected and perceived severity			
Likely and dangerous	1		
Likely but not dangerous	2.72	(1.73 to 4.27)	<0.001
Not likely at all	3.26	(1.92 to 5.53)	<0.001
Don't know if likely	1.36	(0.84 to 2.18)	0.210

Figure 2

Reasons for intention to decline pandemic vaccination as reported by 631 participants in week 44/2009 (multiple answers were allowed), Greece



received that vaccine before (45.5% versus 67.2%). It is of note that more than half of the respondents with chronic conditions (53.3%) did not intend to accept pandemic vaccination ("probably not/definitely not") and seven of the 10 pregnant women in the sample provided "definitely not" as an answer.

According to multiple logistic regression analysis, respondents who did not intend to get vaccinated were more often found among females (odds ratio (OR) versus males: 2.75, 95% confidence interval (CI): 1.94 to 3.90, p<0.001), among individuals aged 30-64 years (OR versus 15-29 year-olds: 1.85, 95% CI: 1.07 to 3.22, p=0.029), among those with a perception of low likelihood of getting infected or low risk associated with it (OR (95% CI) compared to those reporting "likely of getting infected and dangerous": 2.72 (1.73 to 4.27) for those answering "likely but not dangerous" and 3.26 (1.92 to 5.53) for those reporting "not likely at all", p<0.001) (Table 2). Additionally, participants from households where no member suffered from chronic illnesses were more likely to provide negative answers concerning vaccination (OR 1.60 versus households with members suffering from chronic illness, 95% CI: 1.10 to 2.13, p=0.013). A multiple logistic regression model was used to identify factors associated with higher probability of answering "I don't know" compared to "probably yes/definitely yes". Females and individuals reporting a low educational status of the head of their household were more likely to be undecided whether to get vaccinated or not (females versus males: OR=2.42, 95% CI: 1.50 to 3.93, p<0.001 and primary/three-year high school versus nine-year high school/university: OR=2.24, 95% CI: 1.35 to 3.73, p=0.002).

In week 44, 631 participants who indicated "probably not" or "definitely not" as their intention to get vaccinated were further asked to indicate their reasons among a pre-defined set of possible answers (multiple answers were allowed) (Figure 2). For the vast majority of the respondents (59.8%), the main reason was their belief that the vaccine might not be safe.

Discussion

According to our findings, the intention to decline vaccination against pandemic influenza A(H1N1) showed increasing trends since the end of August 2009 and reached 63% in week 44 (26 October-1 November 2009). The corresponding rate of likely acceptance in week 44 was 22.2%, whereas a considerable proportion of the population (15%) had not decided yet. Vaccination had not started in Greece at that time. The most frequently reported barrier against the uptake of vaccination was the fear that the vaccine might not be safe. It is noteworthy that the rates of intention to decline among individuals belonging to vaccination target groups were high: 53.3% among people with chronic conditions and 70.0% in a small sample of pregnant women. Factors independently associated with intention to decline vaccination were female sex, age between 30 and 64 years, perception of low likelihood of getting infected or of low risk associated with it, and absence of household members suffering from chronic illnesses.

To our knowledge, this is the only study conducted so far in a European population during the ongoing influenza A(H1N1) pandemic that assesses perceptions towards influenza, willingness to accept vaccination and related barriers in vaccine uptake. The sample was large (1,000 households per week) and representative of Greek households with quotas based on household size and urban/rural location. Data was collected on numerous items that allowed identifying the profile of the population that will be less likely to accept vaccination. It should be taken into account that as an epidemic unfolds in a population, intentions may change. Other factors, such as media attention or vaccine promotion programmes, may also play a role in shaping perceptions and attitudes. These may differ from country to country and as a result, our estimates concerning willingness to accept vaccination might not strictly apply in the case of other populations. However, as those who do not wish to get vaccinated may have similar characteristics in all countries, qualitative results concerning attitudinal barriers could be used to explain negative intentions towards vaccine uptake in other countries too.

Low rates of intention to accept vaccination have also been reported by other studies on the current pandemic or pre-pandemic vaccines [4-7]. As in our study, perceptions concerning the risk associated with infection were consistently found to affect the intention to accept or decline vaccination and the fear of side-effects was the most frequently reported barrier [6,7]. Even in the case of seasonal influenza, concerns about side effects were reported at high rates (43%) as a reason for avoiding immunization [8].

Overall, this study has identified high rates of intention to decline pandemic vaccination in the Greek population, even among vaccination target groups, mainly due to the perception that the vaccine might not be safe. Vaccination promotion programmes should be carefully designed in order to achieve timely vaccination of the target populations at satisfactory levels of coverage.

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Behaviours regarding preventive measures against pandemic H1N1 influenza among Italian healthcare workers, October 2009

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A survey on attitudes and behaviours towards preventive measures against pandemic H1N1 influenza 2009 was carried out during the month of October 2009 in Italy through an online questionnaire adapted to the Italian situation from a similar survey of the Harvard School of Public Health in the United States (US). Results show that the intention to get vaccinated against pandemic H1N1 influenza 2009 is generally low and that there are differences in attitudes and behaviours towards preventive measures against pandemic H1N1 influenza 2009 between physicians and nurses, especially concerning vaccination. Differences relate also to sex, region of residence and marital status.

Introduction

One of the main concerns related to the present pandemic H1N1 influenza 2009 is the overwhelming burden on medical structures and resources that it poses and the consequent negative impact on mortality and morbidity. This situation puts healthcare workers (HCW) in the unusual position of being both the main actors and one of the main targets of the prevention strategies against the pandemic H1N1 influenza 2009, and considering also their usual unavoidable risk of being an important vector for transmission [1,2]. That is why it is so important to understand the behaviour and attitudes of HCW in relation to the spreading pandemic [3].The importance of this understanding is also demonstrated by studies carried out worldwide [4,5].

The aim of our survey was to gather information about attitudes and behaviours towards preventive measures against pandemic influenza among Italian HCW, taking into account the characteristics of the Italian health care setting. The survey was carried out by means of a questionnaire distributed to and collected from physicians and nurses.

Materials and methods

The questionnaire was designed by the Clinical Medicine and Public Health section of the Sapienza University of Rome, adapted to the Italian situation on the basis of a similar one used in a telephone survey in the US by the Harvard School of Public Health [6]. The adaptation consisted in changing some questions, i.e. concerning health insurance (Italy has a National Health System) or referring to pandemic H1N1 influenza 2009 instead of swine influenza as in the original version of the questionnaire.

The questionnaire was made available through the Italian Journal of Public Health website (www.ijph.it) and a remote recording

Table 1

Socio-demographical characteristics of the survey participants, Italy, October 2009 (n=1,960)

Socio-demographical characteristics (number of responders)	Total
Age group (n=1,960)	
18-29 years	82 (4.2%)
30-49 years	1,444 (73.7%)
50-64 years	422 (21.5%)
≥ 65 years	12 (0.6%)
Sex (n=1,960)	
Female	1,360 (69.4%)
Male	600 (30.6%)
Civil status (n=1,908)	
Married/cohabitant	1,480 (78%)
Single	264 (13.7%)
Separated/divorced	144 (7.3%)
Widow	20 (1%)
Children < 18 years in your home (n=1,955)	
Yes	1,007 (51.5%)
No	948 (48.5%)
Job (n=1,960)	
Physicians	249 (12.7%)
Nurses	1,711 (87.3%)
Regions of residence (n=1,955)	
Northern Italy	1,101 (56.2%)
Central Italy	598 (30.5%)
Southern Italy and islands	256 (13.1%)
Health status (n=1,960)	
Excellent, very good, good	1,874 (95.6%)
Poor	86 (4.4%)

system collected the anonymous answers given by physicians and nurses [8]. The survey was advertised through an email sent to addresses in databases of Public Health professionals and nurses, owned by the Italian National Society of Public Health. Access to the online questionnaire was permitted from 1 to 31 October 2009, including week-ends when the website was accessed more often.

In order to perform an inferential analysis, we considered the following dependent variables:

a) willingness to get vaccinated against pandemic H1N1 influenza 2009;

b) washing hands and using hand sanitisers more frequently than before the beginning of the pandemic.

A univariate analysis was then carried out using a chi-squared test in order to investigate the association between the dependent variables and socio-demographic characteristics, as well as occupation. Moreover, two multiple logistic regression analyses were performed, using the backward elimination procedure as described by Hosmer and Lemeshow [7]. The goodness of fit of the regression model was tested using the Hosmer-Lemeshow test. The following were considered as potential explanatory variables: age group (18-29 years as the reference group), sex (reference modality male),

Table 2

Univariate analysis to investigate the association between the dependent variables and socio-demographic characteristics, as well as occupation, Italy, October 2009 (n=1,960)

	Would you get vaccinated against pandemic influenza ?			Did you wash your hand or use hand sanitiser more frequently ?		
	Yes	No	p	Yes	No	p
Age group						
18-29	26 (41.3%)	37 (58.7%)		56 (68.3%)	26 (31.7%)	
30-49	359 (31.1%)	797 (68.9%)	<0.001	1,112 (77.5%)	323 (22.5%)	0.068
≥ 50	179 (50.1%)	178 (49.9%)		345 (79.9%)	87 (20.1%)	
Sex						
Male	244 (49.2%)	252 (50.8%)		429 (72.2%)	165 (27.8%)	
Female	320 (29.6%)	760 (70.4%)	<0.001	1084 (80%)	271 (20%)	<0.001
Residence						
Northern Italy	261 (29.1%)	637 (70.9%)		837 (76.5%)	257 (23.5%)	
Central Italy	187 (39.1%)	291 (60.9%)	<0.001	471 (78.9%)	126 (21.1%)	0.267
Southern Italy and islands	115 (58.4%)	82 (41.6%)		204 (80.6%)	49 (19.4%)	
Marital status						
Married/cohabitant	438 (36.4%)	765 (63.6%)		1,169 (79.4%)	303 (20.6%)	
Single/divorced/ separated/ widow	126 (33.8%)	247 (66.2%)	0.355	344 (72.1%)	133 (27.9%)	0.001
Occupation						
Physicians	141 (67.1%)	69 (32.9%)		161 (64.7%)	88 (35.3%)	
Nurses	423 (31%)	943 (69%)	<0.001	1,352 (79.5%)	348 (20.5%)	<0.001

Table 3

Multivariate analysis, Italy, October 2009 (n=1,908)

	Yes, I would get vaccinated		Yes, I washed my hands or used hand sanitisers more frequently	
	Crude OR (IC95%)	Adjusted OR (IC95%)	Crude OR (IC95%)	Adjusted OR (IC95%)
Age group				
18-29 (reference)	1	1	1	1
30-49	0.71 (0.44-1.15)	0.66 (0.52-0.83)	1.6 (0.99-2.59)	-
≥ 50	1.51 (0.91-2.5)		1.84 (1.09-3.1)	1.56 (1.17-2.08)
Sex				
Male (reference)	1	1	1	1
Female	0.45 (0.37-0.55)	0.64 (0.51-0.8)	1.54 (1.23-1.92)	1.59 (1.24-2.03)
Region of residence				
Northern Italy (reference)	1	1	1	1
Central Italy	1.47 (1.17-1.83)	-	1.16(0.92-1.48)	1.36 (1.06-1.76)
Southern Italy and islands	2.63 (1.98-3.49)	1.81 (1.36-2.41)	1.3 (0.92-1.82)	1.76 (1.23-2.53)
Marital status				
Single/divorced/separated/widow (reference)	1		1	1
Married/cohabitant	1.18 (0.94-1.49)		1.49 (1.18-1.89)	1.54 (1.21-1.96)
Occupation				
Nurses (reference)	1	1	1	1
Physicians	3.98 (3.02-5.23)	2.87 (2.14-3.85)	0.47 (0.35-0.63)	0.42 (0.3-0.57)
p-value from Hosmer-Lemeshow test		0.52		0.58

OR: odds ratio; CI: confidence interval

region of residence (reference modality Northern Italy), marital status (single/divorced/separated/widow as the reference group), occupation (physicians vs. nurses, with the latter as the reference group). The level of statistical significance was set at a p-value of ≤ 0.05.

The statistical analysis was performed using the statistical software SPSS 13.0 for Windows.

Results

One thousand nine hundred and sixty individuals participated in the survey (249 physicians, 12.7%, and 1,711 nurses, 87.3%). The socio-demographical characteristics of the sample are shown in Table 1.

We found that 70.4% of the 1,360 females of our sample would not get vaccinated against pandemic H1N1 influenza 2009, while 49.2% of the 600 males would get vaccinated (p<0.001) (Table 2). The main difference for the same question was related to occupation: 67% of physicians and 31% of nurses would get vaccinated against pandemic H1N1 influenza 2009 (p<0.001). In contrast, nurses were more prone (79.5%) than physicians (64.7%) to wash their hands or use hand sanitisers more frequently in response to reports of pandemic influenza (p<0.001).

Results from the multivariate analysis (Table 3) show that respondents aged 30-49 years are less likely to get vaccinated in comparison to young adults (18-29 years old) (adjusted odds ratio (AOR)=0.66; 95% confidence interval (CI): 0.52-0.83). Females also are less likely to get vaccinated (AOR=0.64; 95%CI: 0.51-0.8), confirming the results from the univariate analysis. Health professionals who are more likely to get vaccinated live in Southern Italy or on the islands (AOR=1.81; 95%CI: 1.36-2.41) and are physicians (AOR=2.87; 95%CI: 2.14-3.85).

As far as concerns the variable "Yes, I washed my hands or used hand sanitisers more frequently", there is a statistically significant association with: age (≥50 years: AOR=1.56; 95%CI: 1.17-2.08), sex (female: AOR=1.59; 95% CI: 1.24-2.03), region of residence (Central Italy: AOR=1.36; 95%CI: 1.06-1.76; Southern Italy and islands: AOR=1.76; 95%CI: 1.23-2.53), marital status (married/cohabitant: AOR=1.54; 95%CI: 1.21-1.96) and occupation (physicians: AOR=0.42; 95%CI: 0.3-0.57).

Conclusions

HCW are a strategic target for pandemic H1N1 influenza 2009 prevention such as vaccination and frequent hand-washing, since they are at higher risk themselves of contracting influenza, can place their patients at risk and are critical for a functioning health care system. Our online survey demonstrated that pandemic H1N1 influenza 2009 modified the behaviour of HCW, but a high percentage may still not realise that vaccination is a fundamental means of prevention and how important it is that they get vaccinated. This finding is surprising, as many studies worldwide present different attitudes among HCW [1,2].

The present study has some limitations, and the results must be interpreted with caution. First of all, a possible selection bias could have occurred, since healthcare professionals with internet skills would have been more likely to participate in the online survey. Moreover, it is likely that participants are mainly representative of younger HCW and this is supported by the age of responders (almost half of the participants should have been over 50 years old, according to the information included in the databases). Concerning possible information bias, we are convinced of the validity of the self-report answers, since it is unlikely that participants spent time giving unreliable and biased views of their attitudes and behaviours.

Despite some limitations, our survey could be a useful tool for Italian decision makers to promote and launch programmes and campaigns aimed at informing and educating HCW. The results could also be used to motivate HCW to adopt attitudes and decisions which correspond to public health policies, since at the end of November 2009, only 14% of healthcare professionals had been vaccinated against pandemic H1N1 influenza 2009 at the national level [8]. Finally, this study could also help tailor vaccination campaigns by concentrating on groups (nurses, females, adults ≥30 years) or regions (Northern Italy) where the intended vaccine uptake is lower.

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Behaviour of the pandemic H1N1 influenza virus in Andalusia, Spain, at the onset of the 2009-10 season

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In Andalusia, Spain, the pandemic influenza A(H1N1)v virus has spread throughout the community, being the dominant influenza strain in the season so far. The current objective of the Andalusia Health Service is focussed on the mitigation of the health and social impact by appropriate care of the patients at home or in health centres. The 2009-10 seasonal influenza epidemic started early compared with to previous seasons. This article analyses the influenza A(H1N1)v situation in Andalusia until the week 39/2009.

Introduction

In Spain, first suspected cases of pandemic H1N1 influenza were notified on 26 April 2009. Starting with these first cases, an epidemic outbreak of a holomiantic nature was seen in Andalusia, with the primary cases in students who had travelled to Mexico and secondary cases in their families. There were 44 confirmed cases in this first epidemic wave until 5 May 2009. The average age of the cases was 24 years (range: 14-55 years). In 42 of them, the main symptoms were fever and cough, and 18 also had diarrhoea. All of them had mild clinical signs without complications [1].

During the first days of the outbreak, contingency plans were set up based on epidemiological surveillance, and outbreak control measures were adopted through early alert and rapid response systems. Protocols integrated the activities of the public health services, healthcare services, and the influenza reference laboratories [2]. The objective was initially to slow down the propagation of infection by identifying cases according to clinical and epidemiological criteria, reporting the first generation imported cases, their treatment, the measures adopted to prevent secondary cases and outbreaks, with an active search for any contacts. As a preventive measure, cases and contacts received treatment with oseltamivir with the recommendation to remain at home.

The declaration of pandemic phase 6 by the World Health Organization (WHO) on 11 June 2009 [3] indicated that it was no longer feasible to stop the spread of the new influenza virus. Since then, the epidemiological surveillance strategies have been aimed at defining scenarios that could aid healthcare services to respond to this emergency in order to reduce transmission and the number of affected people, and to identify and protect the most vulnerable population groups.

Surveillance of influenza in Andalusia

The epidemiological and virological surveillance of influenza in Andalusia is carried out through the Medical Sentinel Network of the Andalusian Epidemiological Surveillance System (SVEA), which consists of 128 sentinel physicians chosen according to population distribution, who are based in primary healthcare centres and cover 170,668 inhabitants (2.08% of the Andalusian population). The influenza reference laboratory, located at the 'Virgen de las Nieves' hospital in Granada, is part of this network.

The surveillance of severe cases is undertaken through the SVEA, by means of individualised notification of the cases admitted to the public hospitals of Andalusia. Information about the use of emergency services was also collected from the computerised emergency records of public hospitals.

A case of influenza was defined as established by the European Centre for Disease Prevention and Control (ECDC) [4]. The presence of influenza A(H1N1)v was confirmed by realtime PCR carried out with SW H1 forward and SW H1 reverse primers and Taqman SW H1 probe targeted at the H1 gene of this virus, as recommended [5].

Characteristics of pandemic H1N1 influenza cases in Andalusia

After the first pandemic wave in April and May 2009, the influenza activity in Andalusia decreased before the summer. New cases were seen in week 28 (beginning 6 July) and increased until week 39 (beginning 21 September), when the registered influenza incidence reached 147 cases per 100,000 inhabitants (Figure 1).

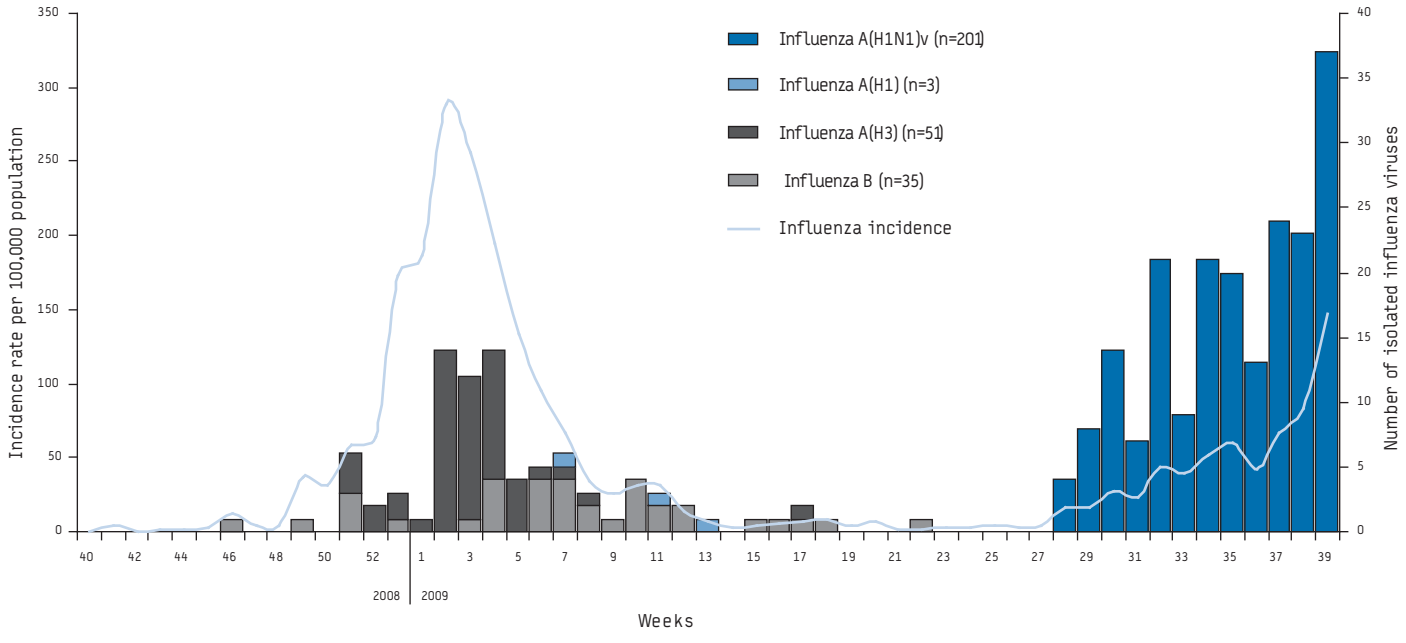
In weeks 38 and 39/2009, the incidence was higher than the epidemic threshold, established as 64.1 cases per 100,000. That implies a widespread dissemination of influenza within the population, two months ahead of the usual period for seasonal influenza. The increased influenza activity in this period is associated with a widespread escalation of the circulation of the

influenza A(H1N1)v virus. Since week 28, all circulating influenza viruses have been sub-typed and identified as influenza A(H1N1) v (see Figure 1).

The cumulative incidence rate for influenza until week 39 week was 643 cases per 100,000 inhabitants. In that week the highest rates were registered in the age group of 5-14 year-olds with 132 cases per 100,000, followed by the under five year-olds with 28 cases per 100,000. The incidence rate for those over 64 years of age was six cases per 100,000. Almost all cases showed mild symptoms lasting for a few days and responded to antipyretic

Figure 1

Weekly influenza incidence rate and isolated influenza viruses, Andalusia, 2008-9 and 2009-10 seasons



Table

Characteristics of severe cases of influenza A(H1N1)v, Andalusia, weeks 17-39/2009

	All hospitalised cases	Hospitalised cases <15 years old	Cases admitted to intensive care unit
Number of cases	**311**	**41**	**28**
Age (years)	Mean: 35.05 Median: 32 Range: 2-90	Mean: 8.56 Median: 9 Range: 2-15	Mean: 35.05 Median: 33.5 Range: 2-77
Sex	Male: 129 (41.5%) Female: 182 (58.5%)	Male: 26 (63.4%) Female: 15 (36.6%)	Male: 8 (28.6%) Female: 20 (71.4%)
Risk factors	**N**	**N**	**N**
Asthma	21	4	2
Cancer	10	1	1
Cardiopathy	24	2	0
Diabetes	18	0	4
Chronic hepatic disease	1	1	0
Active immunodeficiency	17	2	2
Obesity (body mass index ≥40)	8	1	1
Chronic respiratory disease	31	5	7
Convulsive disorders	4	1	0
Renal failure	2	1	2
Other metabolic diseases	2	2	1
Other risk factors	26	4	2
No risk factors	3	0	1
No information	**171 (55%)**	**22 (53.7%)**	**12 (43%)**

treatment. The most frequent symptoms were fever and cough in 94% and 88% of the cases, respectively.

In the study period, 311 of the confirmed cases notified in Andalusia were severe and required hospitalisation. Of those, 28 (9%) were admitted to intensive care units (ICUs). The hospitalisation rate for influenza was 3.7 per 100,000 inhabitants. Males represented 41% of the hospitalised cases, and 59% were female, a male/female ratio of 0.69.The age of the hospitalised cases ranged between 2 and 86 years, and 92% were under 65 years old. The average age of the cases admitted to ICUs was 38 years, with a median age of 35 years.

The most frequently registered complication during the course of the disease in severely ill patients was primary viral pneumonia, in 120 cases (39%). About 75% of them were 15 to 59 years of age.

For 137 of the 311 hospitalised cases (44%), information on risk factors was recorded (see Table). Main risk factors were: prior pulmonary pathology (especially asthma or chronic obstructive pulmonary disease) in 38% of them, a history of cardiovascular disease (18%), immunodeficiency (12%), diabetes (13%), cancer (7%), morbid obesity (6%), and convulsive disorders (3%).

Forty-one of the 311 hospitalised cases were under 15 years of age. Information on risk factors was recorded for 19 of them. Fifteen (80%) presented at least one risk factor (mainly asthma and other chronic pulmonary diseases).

Of the 28 cases admitted to ICUs (including adults and children), information on risk factors was obtained for 20 cases (Table 1). The most common factors were prior pulmonary pathology (chronic respiratory disease or asthma) in eight cases and diabetes in four cases. One case did not present any risk factor.

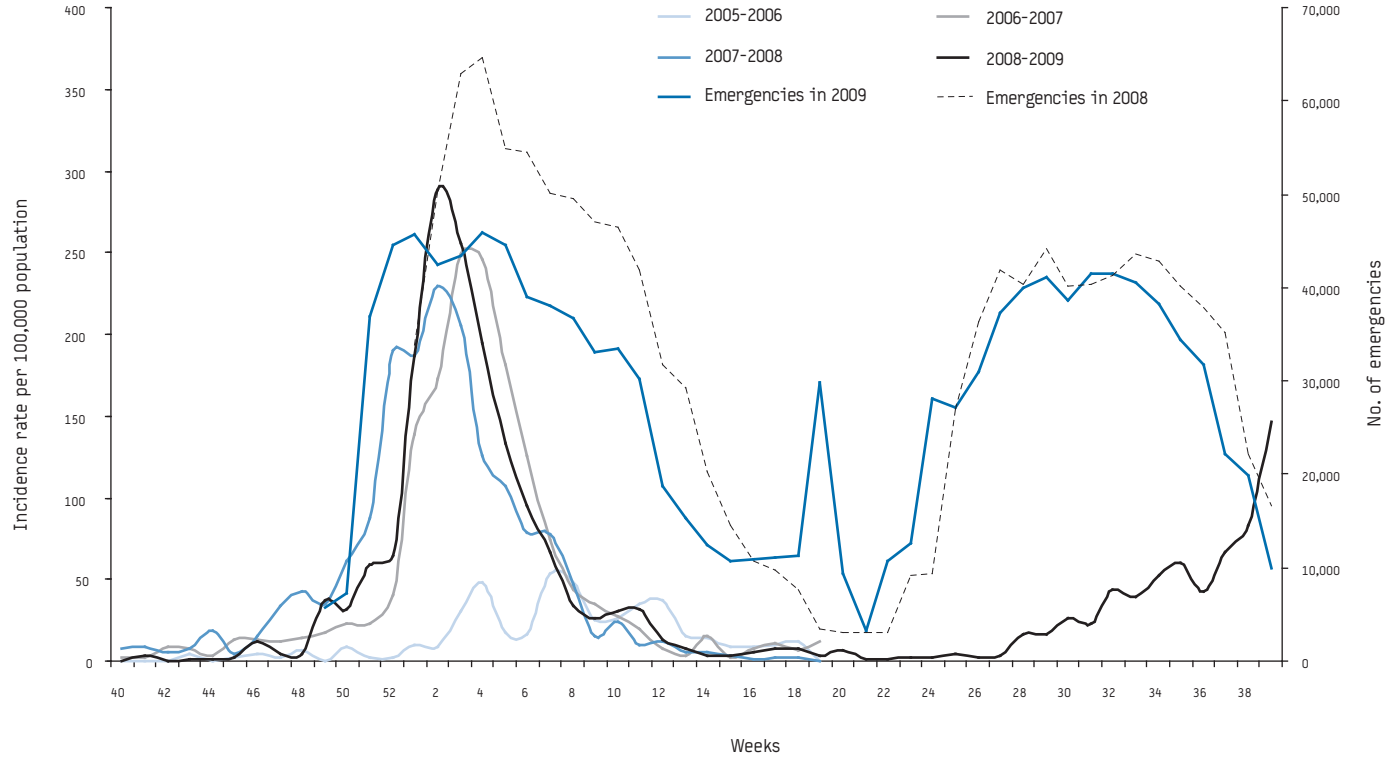
In the same period, 13 deaths due to influenza A(H1N1)v were registered in Andalusia. The estimated death rate was 0.02%. The average age of death was 44.3 years (range: 9 85 years). Information about risk factors was recorded in 10 of them. They were prior pulmonary pathology (especially chronic obstructive pulmonary disease), diabetes, morbid obesity (body mass index ≥40), renal failure, convulsive disorders and cardiopathy.

For 75 of all hospitalised cases, we had information on the time from beginning of symptoms to start of treatment. The median time was three days (range 0-24 days). This delay increased with the severity of cases: a median of three days for the 66 hospitalised, and of four days for the nine cases admitted to ICUs.

The impact of the H1N1 influenza pandemic on the health services in Andalusia was most obvious at the beginning, between weeks 17 (beginning 20 April) and 21/2009. Attendance of hospital emergency departments peaked during this period (Figure 2). This peak in emergencies represented the alarm the first cases of pandemic H1N1 influenza caused in the population and did not reflect the number of notified cases during this outbreak. The containment measures undertaken, together with environmental factors (increased temperatures), and a reduction in the flow of travellers returning from Mexico, contributed to the control of the first phase of the outbreak. From week 22 to week 39/2009, the frequency of emergencies was similar to that observed in the

Figure 2

Influenza incidence rate and hospital emergencies in Andalusia, 2005-2009



previous year, despite the increase in the incidence of influenza that took place after week 28.

Conclusions

Most cases of influenza caused by the pandemic influenza A(H1N1)v virus presented with a mild clinical picture similar to seasonal influenza. The majority of cases occurred in children of school age and in adults under 65 years of age, with the highest frequency of severe and fatal cases found in young adults. A significant proportion of those presented risk factors such as chronic pulmonary pathologies, cardiopathy, diabetes and morbid obesity. Similar results were observed in rest of Spain in the same period [1,2]. It was observed that a delay in the start of treatment increased the severity of the cases.

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Prolonged shedding of influenza A(H1N1)v virus: two case reports from France 2009

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We observed a prolonged shedding of virus 14 and 28 days after symptom onset in two patients with pandemic H1N1 influenza, who did not have immunodepression and were treated with neuraminidase inhibitor. This prolonged shedding was not associated with the emergence of resistance mutation H275Y in the viral neuraminidase gene.

From 1 May until the beginning of October 2009, the virology laboratory in Bordeaux received more than 1,200 nasopharyngal samples from the southwest of France for diagnosis of influenza A(H1N1)v virus by realtime RT-PCR, 186 of which were found positive. For five pandemic H1N1 influenza cases, we had the opportunity to monitor the duration of viral shedding and present here two cases of prolonged shedding.

Case report A

Case A was a man in his mid-fifties with body mass index (BMI) <30 and without relevant medical history. He developed fatigue and cough at the beginning of July 2009, shortly after his arrival to France from California, United States (US). On the day after symptom onset, he was admitted to the university hospital of Bordeaux with a fever of 39.4°C and breathing difficulties. Values of partial pressure of oxygen (PO2) and of carbon dioxide (PCo2) were at 7 kPa and 5.6 kPa, respectively, and the patient was transferred to an intensive care unit. A nasopharyngeal swab was taken and found positive on day 1 after symptom onset for influenza A(H1N1)v by realtime RT-PCR, and a treatment with oseltamivir was initiated at 150 mg/day. Since the patient's clinical condition improved rapidly, he was transferred to the infectious diseases department on 7 July. Oseltamivir treatment was continued and the presence of the virus was monitored via PCR from nasopharyngeal swabs. The signal remained positive during the following five days despite the patient's excellent clinical condition; oseltamivir was replaced by zanamivir on day 11. In five samples taken over the following seven days, influenza A(H1N1)v virus was still detected. The PCR was finally negative on day 15, and the patient was discharged. In order to exclude an immunodepression, we investigated biological parameters including IgG subclasses. Total IgG and subclass serum immunoglobulin levels were normal.

Case report B

Case B was a woman in her late twenties with a BMI >40 who had returned to France from holidays in Spain. On 25 July 2009, the day of the symptom onset, she consulted the outpatient clinic of her local hospital in France, where typical influenza symptoms were diagnosed. After staying at home for five days, she experienced severe breathing difficulties and was admitted to an intensive care unit. On 31 July, RT-PCR for influenza A(H1N1)v was positive and oseltamivir treatment was started at 150 mg/day. In the following days, she developed acute respiratory distress syndrome (ARDS) and required mechanical ventilation and subsequently extracorporeal membrane oxygenation (ECMO). The oseltamivir dose was increased to 300 mg/day from 2 August, and RT-PCR for influenza A(H1N1) v was positive in 13 samples (in deep respiratory secretions but interestingly not in nasopharyngeal swabs) for 19 days and negative on days 31 and 34 after symptom onset. In the meantime, the patient fully recovered and was discharged from the hospital at the beginning of September. No cellular or humoral immunodepression could be diagnosed by quantitation of IgG subclasses and B cell and T cell phenotyping.

Discussion

In two of our patients with confirmed pandemic H1N1 influenza who were treated with oseltamivir, the duration of viral shedding was prolonged. As confirmed by RT-PCR, starting from symptom onset, the shedding was 14 days in patient A and 28 days in patient B. For each patient, the neuraminidase N1 gene was amplified from a positive viral sample at the end of the shedding period and sequenced. No H275Y resistance mutation associated with oseltamivir-resistance was observed.

Viral shedding of seasonal influenza A viruses is estimated to occur over a period between five and seven days [1]. In humans experimentally infected with influenza A/Texas/36/91 (H1N1) virus, oseltamivir administration shortened the median duration of viral shedding from 107 to 58 hours [2]. Prolonged shedding of seasonal influenza viruses has been demonstrated in immunocompromised patients even when treated with antiviral drugs, potentially leading to the emergence of viral resistant mutations [3-5]. Similarly, most patients with pandemic H1N1 influenza infection may be shedding virus from one day before the onset of symptoms until five to seven days after the onset of symptoms [6]. For infections with the pandemic influenza A(H1N1)v virus, prolonged viral shedding has been reported in immunocompromised patients treated with oseltamivir, in association with emergence of viral resistance to the drug [7].

Our observations, although limited to PCR detection without an attempt to culture the virus, are noteworthy because long-term shedding of influenza A(H1N1)v occurred in two patients without immunodepression, who were treated with oseltamivir and in whom the virus did not develop resistance to the drug. However, it seems plausible that prolonged viral shedding in our patients was more likely to be associated with the rather severe clinical course in both cases. We cannot provide data on how frequently prolonged shedding for more than seven days occurred in our series because we only have the necessary data for few patients. However, in some non-severe clinical cases of pandemic H1N1 influenza where a longitudinal study was undertaken, the viral PCR was negative within five to seven days after symptom onset, which is clearly different from the observation presented here.

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Ongoing rubella outbreak in Bosnia and Herzegovina, March-July 2009 - preliminary report

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Between 24 March and 31 July 2009, 342 clinically diagnosed cases of rubella were notified in five municipalities in Republika Srpska, Bosnia and Herzegovina. Eight cases were laboratory-confirmed by positive IgM against rubella virus*. Four virus isolates were obtained and identified as genotype 2B strains, with one isolate differing by a single mutation in the region of the E1 gene. This ongoing outbreak revealed gaps in the immunisation programme during the war in BiH (1992-1995) and highlights the need to revise legislation to permit immunisation of children above 14 years of age with measles, mumps, rubella (MMR) vaccine and to introduce supplemental immunisation activities.

Introduction

Rubella is a notifiable disease in Bosnia and Herzegovina (BiH; estimated population 3,9 million) and is reported on the basis of clinical symptoms. Rubella immunisation was introduced in the 1980s. In 1999-2000 a two-dose schedule with the measles, mumps, rubella (MMR) vaccine was implemented, with the first dose given at the age of 12 months (since 2008 at 11 months) and the second dose at the age of seven years and no later than 14 years.

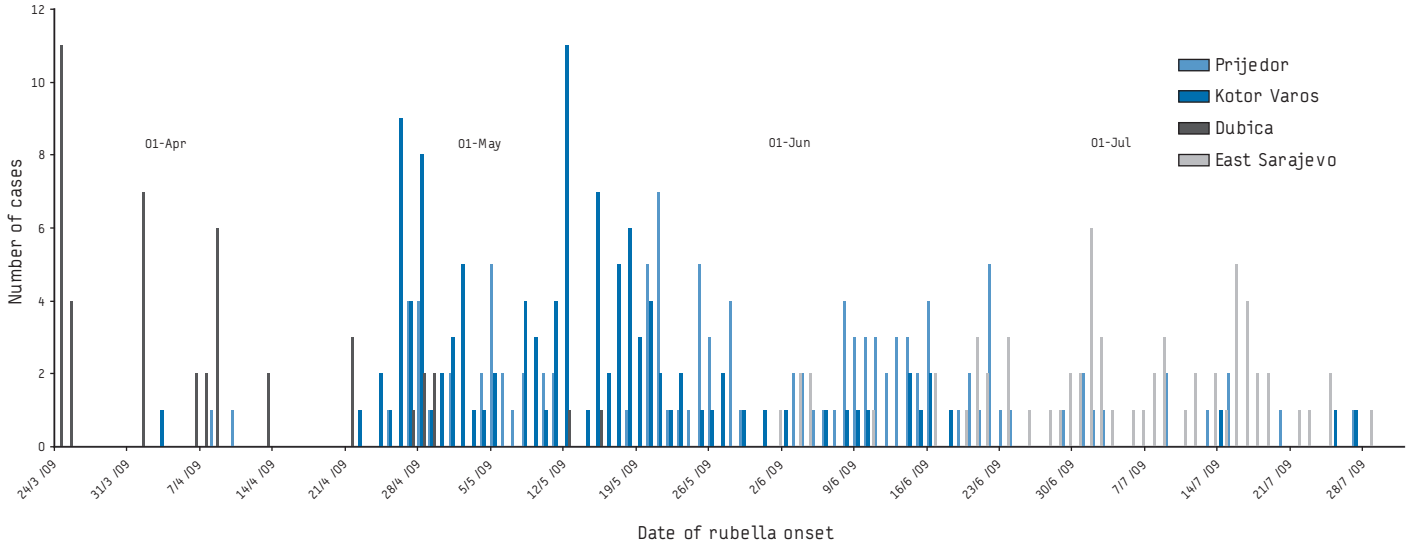
Between 24 March and 31 July 2009, 342 clinically diagnosed cases of rubella were notified in five municipalities in Republika Srpska (RS) which is one of two governing entities in BiH. At the time of publication of this report, the outbreak is ongoing, with ca. four cases per week. Epidemiological and laboratory investigation was started in early May 2009. Preliminary results are presented below.

Materials and methods

Serum samples were collected from 20 suspected rubella cases (six from Dubica, five from Kotor Varos, three from Prijedor, four from East Sarajevo-Pale and two from Trebinje). Throat swabs were obtained from the three patients from Prijedor and from two of the five patients from Kotor Varos. All sera were tested for IgM against measles and rubella and for rubella IgG (Dade Behring Enzygnost® immunoassays) at the Regional Reference Laboratory (RRL) of

Figure 1

Rubella cases, Bosnia and Herzegovina, 24 March - 31 July 2009 (n=342)



the World Health Organization Regional Office for Europe (WHO/Europe) in Luxembourg, and ten serum samples were also analysed for rubella IgM at the laboratory of the Public Health Institute of Republika Srpska (PHI RS). The throat swabs were used for PCR analysis as described previously [1] and for virus isolation [2]. Phylogenetic analysis based on the rubella virus E1 glycoprotein gene was done with MEGA [3] and sequences were compared to published sequences by BLAST.

Results

Outbreak profile

On 28 May 2009 the PHI RS declared a rubella outbreak in three municipalities in the Banja Luka Region: Prijedor, Dubica and Kotor Varos. Later, an outbreak occurred in the East Sarajevo region including the municipalities Pale and Sokolac. In addition, four suspected cases were reported in Banja Luka and eight in Doboj.

In Dubica, 44 rubella cases were reported between 24 March and 15 May 2009 (Figure 1) on the basis of a clinical case definition, i.e. acute onset of generalised maculopapular rash, body temperature higher than 37.2 °C and arthralgia/arthritis, lymphadenopathy, or conjunctivitis. The outbreak in this area appears to be over. The index case was not identified.

In Kotor Varos, Prijedor and East Sarajevo, where the outbreaks are still ongoing, 117, 116 and 65 rubella cases, respectively, were reported until the end of July. The last case to date was reported on 15 September 2009 in Sokolac, East Sarajevo.

Forty-five percent of the cases were male. The age ranged from those born in 1971 to those born in 2007. Most cases (82%, n=282) were observed among teenagers born between 1990 and 1994 still attending high school (Figure 2): 66% (29/44) in Dubica, 90% (105/117) in Kotor Varos, all of them attending the same school, 87% (101/116) in Prijedor and 72% (47/65) in East Sarajevo.

In Prijedor only five of the notified rubella cases had received one dose of MMR, while all the other patients were not immunised. The vaccination status of the cases in Dubica, Kotor Varos and East Sarajevo is still under investigation.

Laboratory findings

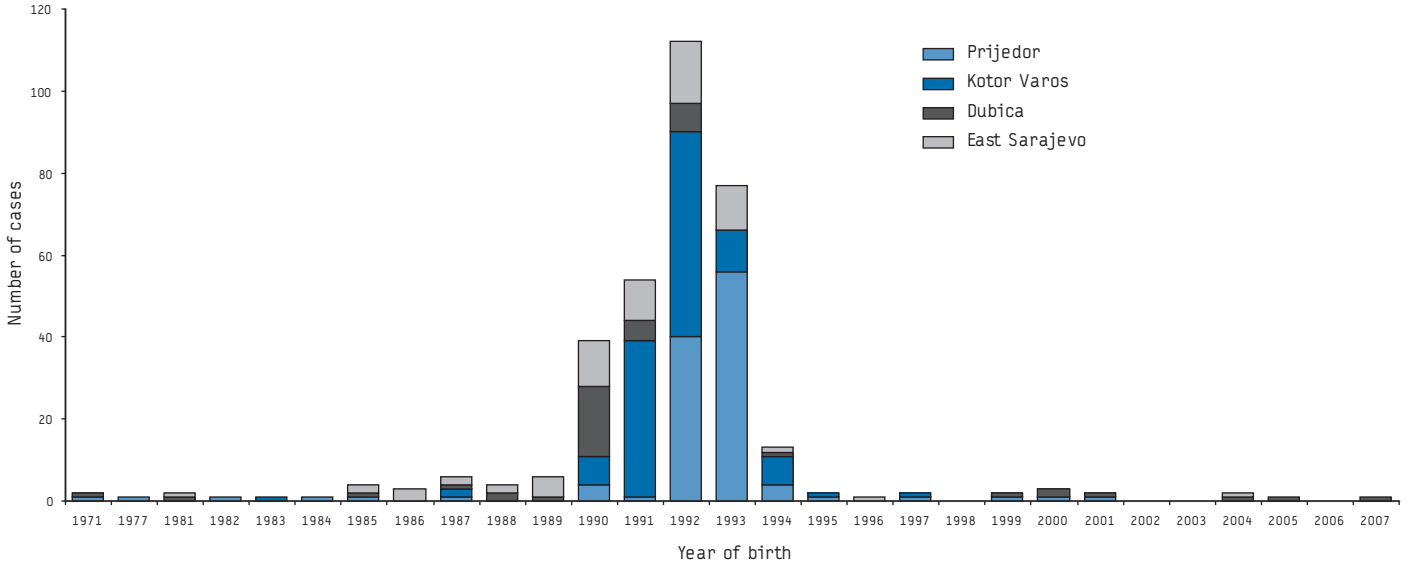
Eight samples were positive for rubella IgM, including three from Kotor Varos, one from Prijedor and four from East Sarajevo-Pale, confirming that the outbreaks in these regions were caused by rubella. Four sera were equivocal, and eight were IgM-negative for rubella (Table). There was a 100% concordance between the test results obtained at PHI RS and the Luxembourg RRL for the ten sera tested in both laboratories.

The rubella-positive samples were from seven 15-17 year-olds and from one 24 year-old. Five of them had received one dose of rubella vaccine, two were not vaccinated against rubella and for one patient no information on the vaccination status was available (Table). One rubella IgM-positive patient was negative for rubella IgG, while the other seven showed relatively low titres of rubella IgG (<77 IU/ml). In total 14 patients were positive for rubella IgG and six were negative. All 20 serum samples were negative for measles IgM.

Four of the five throat swabs were positive in the diagnostic PCR and for all four positives virus isolates were obtained. These samples were collected between one and four days after onset of rash from 16 or 17 year-olds of whom only one reported to have been vaccinated against rubella more than nine years ago. Of all four PCR-positive samples nearly complete E1 gene sequence data were obtained. Three of the sequences were identical and from Prijedor and the fourth showed one mutation at position 303 of the E1 gene and was from Kotor Varos. Phylogenetic analysis attributed the sequences to genotype 2B. According to a BLAST analysis, the most similar previously published sequence was an isolate obtained in the United States nine years ago (RVi/WA.USA/16.00, GenBank accession number AY968220) with a Kimura distance of more than 2%.

Figure 2

Rubella cases by year of birth, Bosnia and Herzegovina, 24 March - 31 July 2009 (n=342)



Discussion

This preliminary report describes a fairly large laboratory-confirmed outbreak of rubella affecting mainly unvaccinated or partially vaccinated 16-17 year-old school children in three contiguous municipalities and one distant region in RS. As the clinical diagnosis of rubella is unreliable, the real number of cases may be somewhat overestimated as for a few suspected cases there may have been different reasons for the symptoms observed. This may also explain why some of the sera tested negative for rubella IgM. On the other hand, several cases may have remained undetected due to a subclinical course of disease. No cases of rubella were diagnosed in BiH in 2008 nor in January and February 2009.

Due to lack of laboratory confirmation, the outbreak was recognised in the first community (Dubica)on 24 March 2009, The index case was not identified and therefore it is not clear when and from where the virus was introduced. As the most similar published sequence was found in the United States in 2000 and the genetic distance to that isolate was more than 2%, the origin of the virus remains obscure.

In early April 2009, the first cases were observed in two other municipalities, Kotor Varos and Prijedor, and in June in another two located 250-400 km away. In all of these areas, the epidemic is ongoing. Local epidemiologists speculate that the virus may have spread among teenagers during their stay in Mrakovica, Kozara mountain (56 km south from Dubica), which is a very popular place for regular school excursions in spring.

To date there is no information on occurrence of rubella in pregnant women or abortion in connection to the current rubella outbreak. Due to the risk of congenital rubella infection during the first trimester of pregnancy, which can lead to miscarriage, stillbirth, or infants with birth defects, rubella is of high public health importance.

Before the war in 1990, coverage with MMR vaccine was 93.6% in BiH. Vaccine procurement and implementation of the immunisation programme were difficult during the war, and in the last two years of war, MMR vaccine coverage was only 56.8%. The age groups primarily affected in the current outbreak were born during the war and most of them were not even vaccinated with the first dose of MMR. Surveys done in RS in 1999 and in 2006 showed MMR vaccination coverage rates of only 54% and 79%, respectively, among 12-23 months-old children [4]. Annual statistics from PHI RS show varying vaccination coverage rates in recent years (2006: first dose 83%, second dose 83%, 2007: 92% and 93%, 2008: 78% and 52%), indicating that other age groups may also contain people at risk for infection.

As a result of the outbreaks, the Minister of Health and Social Welfare and the PHI RS have initiated immediate actions to improve the coverage with the second dose of MMR vaccine in children under the age of 14 years, and have alerted the Regional Public Health Institutes and primary health care providers of the emerging outbreak. An action plan to initiate supplementary immunisation of children and young adults with measles and rubella vaccine or rubella vaccine is presently being developed with support from WHO/Europe. The ongoing rubella outbreak also highlights the need for a revised legislation that permits MMR vaccination in children older than 14 years as well as the need to improve the surveillance of congenital rubella syndrome.

Table

Laboratory results, rubella outbreak in Bosnia and Herzegovina, 24 March - 31 July 2009 (n=20)

Patient	Vaccination status	Rubella virus IgM	Rubella virus IgG	PCR
1	not vaccinated	negative	negative	positive
2	not vaccinated	positive	positive	positive
3	not vaccinated	equivocal	negative	positive
4	1 dose	positive	negative	positive
5	1 dose	equivocal	negative	negative
6	1 dose	negative	negative	not done
7	1 dose	positive	positive	not done
8	1 dose	positive	positive	not done
9	1 dose	negative	positive	not done
10	no information	negative	negative	not done
11	1 dose	negative	positive	not done
12	1 dose	negative	positive	not done
13	1 dose	equivocal	positive	not done
14	1 dose	equivocal	positive	not done
15	not vaccinated	negative	positive	not done
16	not vaccinated	negative	positive	not done
17	no information	positive	positive	not done
18	1 dose	positive	positive	not done
19	not vaccinated	positive	positive	not done
20	vaccinated	positive	positive	not done

* Author's correction: On request of the authors, this sentence was corrected on 2 October 2009

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Measles outbreak in Styria, Austria, March-May 2009

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In the last week of March 2009, five measles cases among students of an anthroposophic school were reported to the public health authorities in the Austrian province of Styria where only five cases had been reported in the whole of 2008. A descriptive epidemiological investigation of the measles outbreak was performed. Between 2 March and 10 May 2009, 37 cases of measles were identified in Styria: 33 confirmed outbreak cases and four probable outbreak cases. The measles outbreak spread from the general population (12 cases) to an anthroposophic community (25 cases). Cases outside of the anthroposophic community were mostly over 10 years of age (10/12). Thirty-five cases were unvaccinated, and two of the 37 had received one dose of measles, mumps, rubella vaccine. Following a measles outbreak in Salzburg in 2008 with 394 cases, this outbreak reemphasises the continued need for additional vaccination campaigns in population groups over the age of 10 years.

Introduction

In the last week of March 2009, five measles cases were reported to public health authorities in the Austrian province of Styria (total population: 1,2 million). All cases were pupils of an anthroposophic school (total school population: 305). No measles cases had been reported in the two previous months in Austria. In 2008, five cases had been reported in Styria during the whole year.

A bivalent measles, mumps (MM) vaccine was introduced in Austria in 1974 as part of the national childhood immunisation programme. This was replaced in 1994 by a trivalent measles, mumps, rubella (MMR) vaccine (two-dose regimen with the first dose at 15 months and the second dose at six years of age) [1]. The Ministry of Health estimates the average measles vaccine coverage with at least one dose for the birth cohorts 1997–2007 to be 84% [2]. Measles vaccination is not mandatory in Austria for enrolling a child in school.

The World Health Organization (WHO) set the year 2010 as the target for elimination of measles in the European Region [3]. Between 2004 and 2007, Austria was considered a low to moderate incidence country, according to the criteria of EUVAC.NET (< 1/100,000 population/year) [4]. In 2008, a measles outbreak with at least 394 cases in the Austrian province of Salzburg, linked to the anthroposophic community, changed Austria's status to a high incidence country [5].

The aim of the outbreak investigation was to describe the outbreak by person, place and time and to identify the proportion of cases who were vaccinated.

Methods

A descriptive epidemiological outbreak investigation was performed. Case data on demographics, date of rash onset, clinical symptoms, past history of contact with a known measles case, vaccination status, and disease outcome were assessed by telephone interviews.

A confirmed outbreak case was defined as a patient with a generalised macular-papular rash with fever accompanied by at least one of the following clinical signs: cough, coryza, or conjunctivitis, who fulfilled one of the criteria of a laboratory-confirmed measles infection as described elsewhere [6] or who was epidemiologically linked to a laboratory-confirmed measles infection within 7–21 days prior to rash onset, who fell sick after 1 March 2009, and was resident in the Austrian province of Styria. A probable outbreak case was defined as a patient who fulfilled the clinical criteria of measles, who fell sick after 1 March 2009, and was resident in the Austrian province of Styria.

Active case finding was conducted among contact persons of the measles cases who were notified to the district public health authorities. Infection with measles virus was defined as laboratory-confirmed if at least one of the following three laboratory criteria was fulfilled: detection of measles virus-specific IgM, detection of measles virus RNA, or isolation of measles virus from a clinical specimen [6]. The detection of measles virus RNA in clinical specimens as described by El Mubarak et al. [7] and genotyping as described by Santibanez *et al.* [8] were performed by the Austrian National Reference Centre for Measles.

Results

Thirty-seven cases fulfilled the outbreak case definition. Of these, 33 were confirmed and four were probable cases. Nine of the 11 laboratory-tested cases were confirmed for measles virus infection. The measles virus RNA from two outbreak case specimens was partially sequenced and was genotype H1. The outbreak affected four of the 17 public health districts of Styria between 2 March (week 10) and 10 May 2009 (week 19), and peaked with eight cases with onset of symptoms in week 17 (20-

26 April). Between March and May 2009, 11 unrelated measles cases were reported in the other eight Austrian provinces. The figure shows the outbreak cases by week of rash onset according to the outbreak case classification.

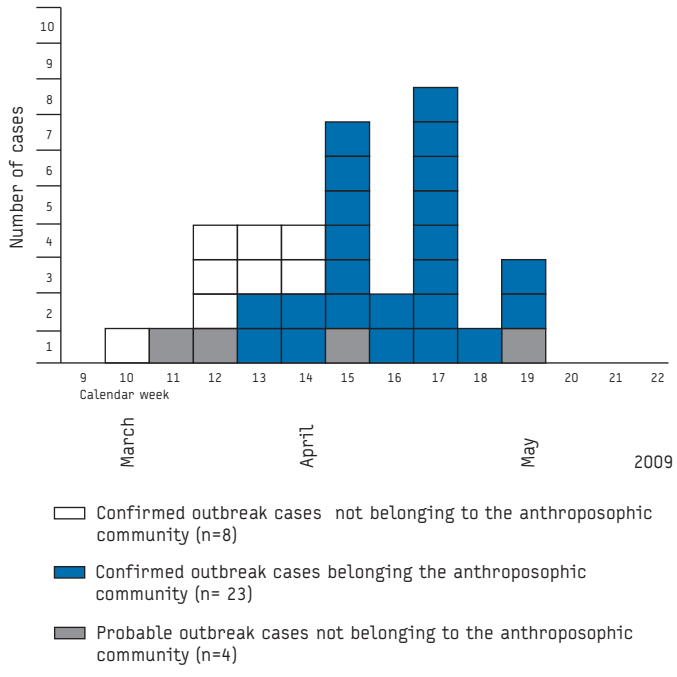
Of the thirty-five cases, 25 belonged to the anthroposophic community, including 12 pupils of the anthroposophic school - giving a school attack rate of 12/305 (3.9%) - four household members, and nine acquaintances. A likely source was identified as one of the first four anthroposophic community cases (including two cases in pupils), who fell sick at the same time. This anthroposophic case was a pupil who had visited a billiard pub within the three weeks prior to his rash onset. An earlier case from the general population had also reported having visited the same pub. This is one probable route which enabled the measles virus to spread from the general population to the susceptible anthroposophic community.

Among the cases belonging to the anthroposophic community, the age group of 5-9 year-olds was most affected with 14 of 25 cases. Among the cases in the general population, the age group of 10-14 year-olds was most affected, with five of 12 cases (Table). Most of the cases from the general population were over 10 years old (10/12).

The symptoms most commonly reported by all 35 cases were fever (n=35), cough (n=34), conjunctivitis (n=34) and cold-like symptoms (n=28). Two measles cases reported having otitis media.

FIGURE

Measles cases by week of rash onset, Styria, Austria, March-May 2009 (n=35*)



*Thirty-five of the 37 outbreak cases were accessible for telephone interviews.

Two cases were hospitalised during the course of the infection for five and eight days, respectively. All cases recovered.

None of the 37 outbreak cases had received both doses of MMR vaccine. Two cases had received one vaccine dose of MMR. Both belonged to the 12 cases in the general population. All cases in the anthroposophic community and ten cases in the general population were completely unvaccinated (Table).

The anthroposophic school was closed for two weeks and cases were asked to stay at home for the period of communicability (at least four days after the onset of the rash). An MMR post-exposure prophylaxis was offered free of charge to susceptible contacts of outbreak cases.

Discussion

We report a measles outbreak, which began in the general population in week 10 of 2009 and spread to an anthroposophic school in week 13. In a measles outbreak in 2008 involving 397 cases, the attack rate in the affected anthroposophic school was 44% (150/340 pupils), significantly higher than the 3.9%

TABLE

Outbreak measles cases by sex, age-group, clinical symptoms, laboratory testing and anthroposophic affiliation, Styria, Austria, March-May 2009 (n=37)

Case characteristics	N_{total}=37	
Sex ratio (m:f)	2.1:1	
Male	25	
Female	12	
Groups	Group A N= 12	Group B N= 25
Age distribution	Number of cases	Number of cases
0-4	0	1
5-9	2	14
10–14	5	8
15-19	1	1
20-24	0	0
25-29	4	0
30-34	0	0
35-39	0	1
Clinical symptoms		
Fever	35	
Cough	34	
Conjunctivitis	34	
Cold	28	
Otitis media	2	
Hospitalisation	2	
Laboratory-confirmed cases/tested	9/11	
Measles virus RNA positive/tested	2/9	
Measles virus-specific IgM positive/ tested	9/9	

Group A: not belonging to the anthroposophic community
Group B: belonging to the anthroposophic community

observed here. Assuming similar low vaccination coverage in the anthroposophic community as observed in the 2008 measles outbreak, the low attack rate in this outbreak was likely due to the prompt two-week closure of the anthroposophic school and the prompt isolation of cases at home for the period of communicability. The supplementary province-wide MMR vaccination campaign addressing the 15-25 years age group in the general population was implemented as a consequence of an outbreak affecting Austrian provinces other than Styria in 2008. In the first six months of 2008, 5,335 first doses (5.1% of those administered within the age group of 7–25 years) were administered, which is more than the number of first doses administered during the first half of 2009 (i.e. the period of the described measles outbreak) [unpublished data]. A concurrent rubella outbreak (ongoing since October 2008) may have also contributed to raise awareness for contagious rash diseases, which probably led to an early case presentation and case isolation [9].

Combating measles is still a high public health priority in Europe [10]. In Austria, a mumps outbreak in 2006, a measles outbreak in 2008, and a rubella outbreak in 2008-2009 have shown a clear shift of the age distribution of the cases to those older than ten years [1,5,10]. The age groups most affected were: 16-30 year-olds (mumps), 10-19 year-olds (measles), and 15-24 year-olds (rubella) [1,5,10]. The current outbreak of measles, in which the over 10 year-olds accounted for 10 of the 12 cases in the general population, justifies the introduction of supplementary MMR vaccination campaigns targeting the over 10 year-olds in Styria. Based on the vaccination register in Styria [unpublished data], an average vaccination coverage of 90% was reported for the birth cohorts 1999-2008.

Age group specific seroprevalence surveys could provide the required comprehensive information for designing supplementary age group-targeted vaccination campaigns Austria-wide. In neighbouring Germany, adolescents are often not fully vaccinated or unvaccinated [11]. Coverage is still insufficient to achieve wide enough herd immunity for measles elimination in central Europe. Continuing with suboptimal vaccination coverage in certain population groups such as the adolescents endangers the possibility of achieving the 2010 target for measles and rubella elimination in the WHO European Region.

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West Nile virus transmission with human cases in Italy, August - September 2009

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In 2009, to date 16 human cases of West Nile neuroinvasive disease (WNND) have been reported in Italy, in three regions: Veneto, Emilia-Romagna and Lombardia. The number of cases is higher compared with last year when nine cases were identified (eight cases of WNND and one case of West Nile fever) and the geographical distribution indicates spread from east to west.

Introduction

West Nile virus (WNV) infection is transmitted in natural cycles between birds and mosquitoes, particularly Culex spp. mosquitoes. Humans and horses are susceptible, dead-end hosts. Firstly identified in tropical Africa, WNV infection has been evidenced in northern Africa, Israel, India and Australia [1] and progressively spread in the Americas since 1999. WNV has been the cause of outbreaks and sporadic cases in central, eastern and Mediterranean Europe for more than 45 years.

In Italy, the first cases of equine WNV infection were detected in 1998, but no human cases were reported at that time [2]. The first human cases of WNV infection in Italy, including neuroinvasive forms, were identified in 2008 [3]. A total of nine human cases were reported by two regions: five confirmed cases of West Nile neuroinvasive disease (WNND) (four identified retrospectively) and one case of West Nile fever were recorded in Veneto, all in the province of Rovigo [4], and three confirmed WNND cases were detected in Emilia-Romagna [5,6].

Table 1

Case definition of West Nile neuroinvasive disease (WNND), surveillance programme in Veneto and Emilia-Romagna regions, Italy, 2008-2009

Subjects ≥ 15 yr with fever ≥ 38.5°C and neurological symptoms (e.g., encephalitis, meningitis, Guillain-Barré syndrome or acute flaccid paralysis).
Cases were classified as:
Possible: clinical symptoms and aseptic CSF.
Probable: clinical symptoms and at least one of the following laboratory criteria: - presence of IgM antibodies against WNV by ELISA; - seroconversion by ELISA; - fourfold increase of IgG antibodies against WNV in two consecutive samplings (>5 days, preferably 15-20 days between the two samples) by ELISA.
Confirmed: clinical symptoms and at least one of the following laboratory criteria: - isolation of WNV in blood or CSF; - presence of IgM antibodies in CSF (by ELISA); - detection of WNV-RNA by RT-PCR in blood or CSF; - detection of increased levels of WNV IgM and IgG by ELISA and confirmed by PRNT.

WNV: West Nile virus; CSF: cerebrospinal fluid; PRNT: plaque-reduction neutralisation test.

Veneto and Emilia-Romagna implemented an active surveillance of farm workers that yielded a seroprevalence of 1.5% and 3.1% respectively [3-6]. In the Emilia-Romagna region, a seroprevalence study of blood donors was also performed, showing a seroprevalence of 0.7-0.8% [6]. Apart from human cases, equine WNV infections have also been detected in the same regions [6]. No human cases were described in other Italian regions during the summer of 2008.

Human cases of WNND reoccurred in the summer 2009. Hereby we briefly describe these cases and discuss possible implications for public health.

WNND surveillance in Italy

Following the identification of the first human cases of WNV infection in Italy in 2008, specific WNND surveillance systems were set up in the affected regions of Emilia-Romagna and Veneto. The case definitions used are presented in Table 1 [3,5]. Both systems collect data on human cases of WNND every year between 15 June and 31 October. In both regions animal and vector surveillance for WNV is also in place.

In Lombardia region, a surveillance system for neuroinvasive diseases has been in place since 2008. Cases from all age-groups are tested for a large panel of viruses and bacteria, including WNV. No cases of neuroinvasive disease due to WNV were detected in Lombardia in 2008.

In addition to surveillance of human cases, a national veterinary plan for WNV surveillance has been implemented since 2008 [7].

Results

A total of 16 confirmed cases of WNND were reported to the regional surveillance systems in three Italian regions between August and September 2009. Detailed information is presented in Table 2.

The distribution of human cases of WNND by month of symptom onset and geographical location in the years 2008 and 2009 is shown in Figure 1 and Figure 2 (A and B).

A detailed description of the epidemiological situation in the affected regions is reported below.

Veneto

Since the end of August 2009, six human cases of WNND were reported to the regional surveillance system (Table 2). Five cases were observed in the area of Rovigo town and one case in the area between the provinces Rovigo and Venezia. The cases (four males and two females) were between 62 to 82 years old. Virus-specific IgM and IgG were detected in cerebrospinal fluid (CSF) and serum specimens by immunoglobulin M antibody (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA). The cerebrospinal fluid and serum specimens were obtained from the patients upon their first presentation to the clinic. Diagnosis was confirmed by the plaque-reduction neutralisation test (PRNT). All patients were hospitalised and they are still in critical condition. One patient from the province of Rovigo died.

Emilia-Romagna

Since the end of August 2009, eight human cases of WNND were reported to the regional surveillance system in the provinces of Modena (one case), Ferrara (five cases), Imola (one case) and Bologna (one case). Of these, seven are in critical condition and one died. Ages of cases ranged from 62 to 78 years (Table 2). Virus-specific IgM and IgG were detected in CSF and serum specimens by MAC-ELISA and immunofluorescence assays (IFA). Diagnoses were confirmed by PCR. To date, 57 possible cases of WNND have been referred to the Regional Reference Centre for Microbiological Emergencies (CRREM) laboratory in Bologna and excluded after negative results of laboratory test.

Lombardia

Since September 2009, two confirmed cases of WNND were hospitalised in Emilia-Romagna region (Modena) and they are still in critical condition. The two cases were resident in Lombardia, in the province of Mantua bordering Emilia-Romagna region (Table 2). Virus-specific IgM and IgG were detected in CSF and serum specimens by MAC-ELISA and IFA in the CRREM laboratory in Bologna. In all cases the diagnoses were confirmed by PCR.

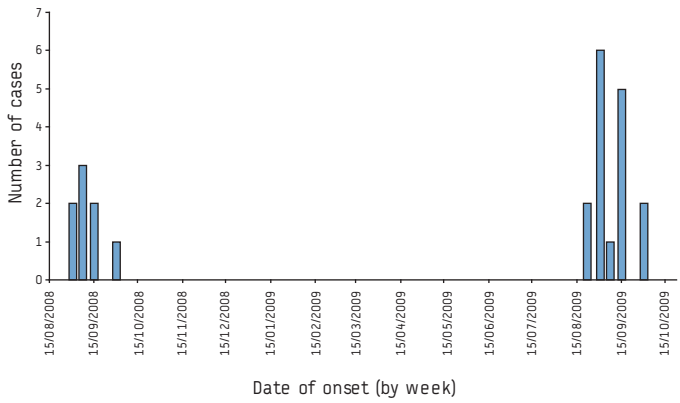
Table 2

Confirmed cases of West Nile neuroinvasive disease (WNND) in Italy, August - September 2009 (n=16)

Patient	Sex	Age	Province	Region
1	M	76	Rovigo	Veneto
2	F	78	Rovigo/Venezia	Veneto
3 (died)	M	82	Rovigo	Veneto
4	M	62	Rovigo	Veneto
5	M	78	Rovigo	Veneto
6	F	84	Rovigo	Veneto
7	F	73	Ferrara	Emilia Romagna
8	M	62	Ferrara	Emilia Romagna
9 (died)	M	72	Ferrara	Emilia Romagna
10	M	72	Ferrara	Emilia Romagna
11	M	68	Ferrara	Emilia Romagna
12	M	78	Bologna	Emilia Romagna
13	M	77	Imola	Emilia Romagna
14	M	64	Modena	Emilia Romagna
15	F	72	Mantova	Lombardia
16	F	72	Mantova	Lombardia

Figure 1

Human cases of West Nile neuroinvasive disease (WNND) in Veneto and Emilia-Romagna regions, Italy, 2008-2009 (n=24)



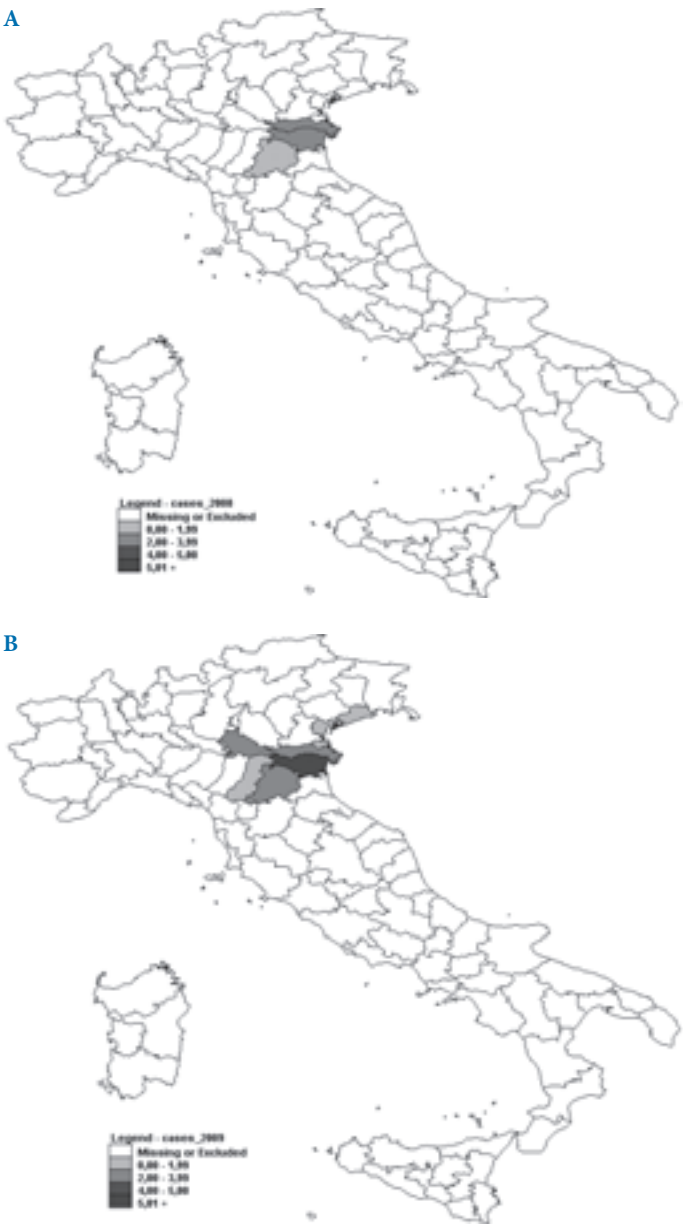
Control measures implemented

Vector control measures consisted in regular mosquito spraying activities (adulticide and larvicide) especially at public events, in the affected regions. In addition, Emilia-Romagna region implemented public education messages on self-protection from mosquito bites on the region's public health authority website.

Regarding blood, tissue and organ safety, between 1 August and 30 October 2009, Italy applies nucleic acid amplification technology (NAT) screening on all blood donations from residents in the provinces of Ferrara (Emilia-Romagna), Rovigo (Veneto) and Mantua (Lombardy). The objective of this screening is to quantify the viral circulation in these provinces among blood donors and to ensure the early implementation of appropriate blood safety measures. The first NAT-positive blood donation is considered as a trigger to defer further donations from the province of residence of the donor, independent of the identification of human cases of WNND. In case of positivity, blood donors who have spent at least one night in affected provinces are deferred for 28 days. This policy is implemented nationwide.

Figure 2

Geographical distribution of human cases of West Nile neuroinvasive disease (WNND), Italy, 2008 (A) and 2009 (B) (n=24)



Conclusions

The occurrence of human cases of WNND in Italy is indicative of the ongoing WNV activity. In Italy, the provinces of Ferrara (Emilia-Romagna), Rovigo (Veneto) and Mantua (Lombardy) are considered high risk areas of transmission of WNV, and equine cases of WNV infection were also confirmed there [8].

Compared to the summer of 2008, a larger geographical area was affected by WNV infection in 2009. In particular, the virus expanded its activity apparently moving from east to west. These changes were immediately detected by the public health authorities, which started the NAT screening of all blood donors in the newly affected provinces, in order not to defer donations from these areas. For this reason the exchange of data between human, animal and vector sector is crucial, as experienced in the Emilia-Romagna region where weekly reports with detailed description of WNV infections in humans, animals and vectors have been made since the beginning of 2009.

The national public health authorities are now considering the implementation of a nationwide enhanced human surveillance system in Italy, in order to include all those regions where the circulation of WNV has been reported (Emilia-Romagna, Lombardia, Veneto and Toscana) together with animal and vector surveillance [8].

Disseminating the information regarding the presence of WNV among clinicians could help public health authorities to rapidly identify new human cases of WNND, in order to implement control measures to reduce the transmission of the virus. This should be done in an integrated approach including veterinary and entomological surveillance in order to better monitor the situation in areas with favourable ecological conditions for WNV cycle.*

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*Authors' correction

On request of the authors, the last paragraph of the article was replaced and the acknowledgement was added on 9 October 2009.

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Genome sequence analysis of the first human West Nile virus isolated in Italy in 2009

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In 2009, six new human cases of West Nile neuroinvasive disease (WNND) were identified in Veneto region, following the six cases already reported in 2008. A human West Nile virus (WNV) isolate was obtained for the first time from an asymptomatic blood donor. Whole genome sequence of the human WNV isolate showed close phylogenetic relatedness to the Italy-1998-WNV strain and to other WNV strains recently isolated in Europe, with the new acquisition of the NS3-Thr249Pro mutation, a trait associated with avian virulence, increased virus transmission, and the occurrence of outbreaks in humans.

Figure 1

Sites where human cases of symptomatic West Nile neuroinvasive disease occurred in Veneto region, Italy, in 2008 and 2009

Dark grey dots: cases in 2008
Light grey dots: cases in 2009

Introduction

In Italy, the first outbreak of West Nile virus (WNV) infection was reported in 1998 among horses residing in Tuscany region [1]. The virus re-emerged in Italy in 2008, when equine and human cases of West Nile neuroinvasive disease (WNND) were notified in Veneto and Emilia Romagna regions [2,3]. In Veneto region, six clinical cases of WNV infection were identified with disease onset in August-September 2008 and all were from Rovigo province [4]. Three further human cases of WNND were notified in Emilia Romagna region in September-October 2008 [5]. Moreover, the veterinary and entomological surveillance documented that WNV infection was widespread in the same areas in north-eastern Italy [2]. In 2008, WNV strains were isolated from one horse in Rovigo province, Veneto region, and from one donkey, one pigeon and three magpies in Ferrara province, Emilia-Romagna region. Sequencing of 255 bp of the WNV *E* gene showed the virus had 100% amino acid identity with the equine strain isolated in Tuscany in 1998 [6]. The complete genome sequences of two WNV strains isolated from magpies in Italy in 2008 were also deposited in the Genbank database (Accession No. FJ483548 and FJ483549).

In 2009, further 16 human cases of WNND were notified in northern Italy, including six from Veneto region, eight from Emilia-Romagna region and two from Lombardia region, as recently reported in a detailed description of the epidemiological situation in Italy [7].

Here we report the results of genome sequencing of the first human WNV isolate reported in Italy, which provide evidence of the emergence of a strain more virulent than the WNV strain isolated in Italy in 1998. Moreover, we report further clinical and epidemiological details on human cases of symptomatic WNV infection detected in 2009 in Veneto region.

Samples and methods

Human cases of West Nile neuroinvasive disease in Veneto region, 2009

A surveillance programme for possible human cases of WNND has been implemented in Veneto region since September 2008, as reported previously [4]. According to this programme, all possible cases of WNV infection are referred to our Regional Reference Laboratory which performs the following diagnostic tests [4]: detection of WNV RNA in plasma and cerebrospinal fluid (CSF) samples by real-time RT-PCR and detection of IgM and IgG antibodies against WNV in serum and CSF samples by ELISA testing (Focus Diagnostics, Cypress, CA). ELISA-positive samples are further tested by plaque-reduction neutralisation test (PRNT) for confirming specificity of antibody response, while WNV RNA-positive samples are inoculated onto confluent monolayers of Vero E6 cells for virus isolation. Moreover, nucleic acid test (NAT) screening for WNV RNA has been applied to all blood, tissue and organ donations collected from 1 August to 30 October 2009 in the province of Rovigo.

In August-September 2009, six new cases of WNND were identified in Veneto region, following the six cases reported in 2008 [4]. Five of the patients in 2009 were resident in Rovigo province and one in a village in the south of Venice province, not far from Rovigo province (Figure 1).

To date, no cases of West Nile fever have been notified in 2009. Detailed clinical and laboratory data of cases are summarised in Table 1.

Genome sequence analysis of the first human West Nile virus isolate reported in Italy

At the end of August 2009, a WNV strain was isolated in Vero E6 cells from a NAT-positive blood donation of an asymptomatic individual resident in Rovigo province. At the time of blood donation, the donor was WNV IgM- and IgG-negative but after

Table 1

Clinical and laboratory data on cases of West Nile neuroinvasive disease notified in Veneto region, Italy, 2009

Referral date	Sex/age (years)	Symptoms	Laboratory data	Outcome*	Province
31 Aug	M/76	Fever, severe meningoencephalitis	IgM+/IgG+ in serum and CSF, PRNT confirmed, WNV RNA-negative.	Amelioration of symptoms, discharged from hospital on 13 October	Rovigo
8 Sep	F/78	Fever, severe meningoencephalitis	IgM+/IgG+ in serum and CSF, PRNT confirmed, WNV RNA-positive in plasma.	Still hospitalised, amelioration of symptoms	Venice
10 Sep	M/82	Fever, headache, severe meningoencephalitis	IgM+/IgG+ in serum and CSF, PRNT confirmed, WNV RNA-negative.	Death on 17 September	Rovigo
11 Sep	M/62	Fever, headache, severe meningoencephalitis	IgM+/IgG- in serum and CSF, PRNT confirmed, WNV RNA-negative.	Amelioration of symptoms, discharged from hospital on 25 September	Rovigo
24 Sep	M/78	Guillain-Barré syndrome	IgM+/IgG+ in serum and CSF, PRNT confirmed, WNV RNA-positive in plasma.	Still hospitalised with severe disease	Rovigo
28 Sept	F/84	Fever, arthritis, severe meningoencephalitis	IgM+/IgG- in serum and CSF, PRNT confirmed, WNV RNA-negative.	Still hospitalised with symptons	Rovigo

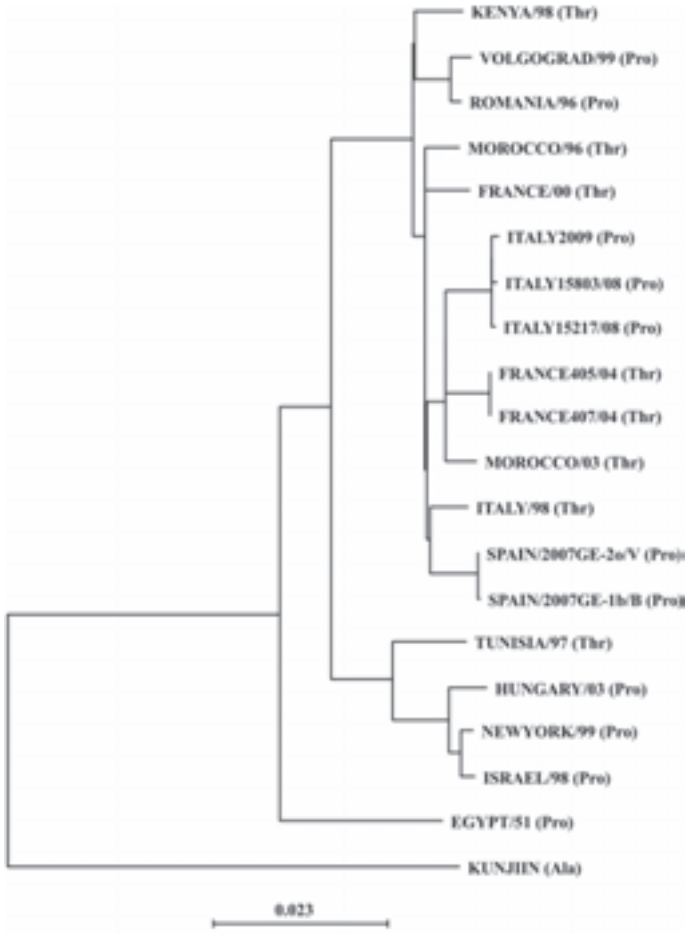
WNV: West Nile virus; CSF: cerebrospinal fluid; PRNT: plaque-reduction neutralisation test.
*As of 5 November 2009 (date of publication).

a few days showed seroconversion and remained asymptomatic. WNV growth in cell cultures was demonstrated by the presence of cytopathic effect in the monolayer and detection of WNV-RNA at real-time RT-PCR testing.

For WNV genome sequencing, the supernatant of infected Vero E6 cells at the first passage was collected for RNA and PCR amplification with a set of 21 primer pairs targeting overlapping sequences of ~600 nucleotides in WNV genome. Primer sequences are available upon request. Amplicons underwent bi-directional sequencing by using the BigDye® Terminator Sequencing Kit on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). After alignment and assembling with the SeqScape v2.5 software (Applied Biosystems), the consensus sequence (Genbank Accession No. GU011992) was aligned using ClustalW and Blastp with genome sequences of the following WNV strains: Kunjin 1973 (MRM61C; Westaway; Accession No. D00246), Egypt 1951 (Eg101; Accession No. AF260968), Romania 1996-mosquito (RO97-50, *Culex pipiens*, Bucharest, Romania; Accession No. AF260969), Italy 1998-equine (PaAn981, Tuscany, Italy; Accession No. AF404757), Volgograd 1999-human (Accession No. AF317203), NY 1999-human (Accession No. AF202541), Spain 2007 GE-1b/B and GE-2o/V (golden eagle *Aquila chrysaetos*; Accession No. FJ766331 and FJ766332, respectively), France 407/2004 and 405/2004 (house sparrow *Passer domesticus* and common magpie *Pica pica*; Accession No. DQ786573 and DQ786572, respectively), Morocco 2003-equine (Accession No. AY701413), France 2000-equine (PaAn001, Accession No. AY268132), Morocco 1996-equine (Accession No. AY701412), Kenya 1998-mosquito (KN3829; Accession No. AY262283), Tunisia 1997-human (PaH001; Accession No. AY268133), Hungary 2003-goose (*Anser anser domesticus*; Accession No. DQ118127), Israel 1998-goose (*Anser anser domesticus*; Accession No. AF481864). Two WNV strains, 15217 and 15803, isolated from magpies in Italy in 2008 (Genbank Accession No. FJ483548 and FJ483549, respectively) were also included in the analysis. The aligned nucleic acid sequences were used to construct a phylogenetic tree using the maximum likelihood algorithm within Phylo_Win v2.0 software with bootstrap resampling analysis (500 iterations) (Figure 2).

FIGURE 2

Maximum likelihood phylogenetic tree of aligned complete genome sequences of 20 West Nile virus strains, including the strain isolated from a blood donation in the province of Rovigo, Veneto region, in 2009, and two strains isolated from magpies in Italy, in 2008



ITALY2009: human, from blood donation in Rovigo province, Italy, 2009 (Genbank Accession No. GU011992)
ITALY15217/08 and ITALY15803/08: magpies, Italy, 2008
The amino acid at each NS3-249 site is indicated between brackets. Branch lengths are drawn to scale to indicate the number of nucleotide changes (genetic distances). Scale bar shows the number of base substitution per site. All WNV strains belong to lineage 1, clade 1a, with the exception of Kunjin virus (outgroup) which belongs to clade 1b.

Results

Phylogenetic tree analysis of the complete genome sequence of 20 WNV strains shows that the human WNV strain isolated in Italy in 2009 belongs to lineage 1, clade 1a, and is closely related to the two WNV strains isolated from magpies in Italy in 2008 (average nucleotide and amino acid divergence of 0.14% and 0.07%, respectively) (Figure 2). Both the human 2009 WNV isolate and the WNV strains isolated from magpies in Italy in 2008 were phylogenetically related to strains isolated since 1996 in the western Mediterranean area, including the Italy 1998-equine WNV strain (Figure 2). In particular, nucleotide and amino acid divergence of the 2009-human WNV isolate from the Italy 1998-equine WNV strain was 1.62% and 0.25%, respectively. All amino acid changes among Italian WNV isolates are detailed in Table 2.

The 2008-2009 Italian WNV isolates had a higher degree of divergence from the eastern European strains isolated in Romania in 1996 and in Russia in 1999 and from the American/Israeli cluster (Figure 2). Our findings obtained with WNV complete genome sequences, which confirm the results of a recently reported detailed genetic analysis of Mediterranean WNV strains [8], provide a more detailed picture of WNV evolution in Italy and in the Mediterranean area than the phylogenetic analysis performed on a partial sequence of the WNV *E* gene obtained from veterinary samples in Italy in 2008 [6].

Based on these results, we believe that the WNV strain responsible for the recent outbreaks might have originated from the Italy 1998-equine strain, since the virus seems to have had

a continuous low level, endemic circulation in Italy from 1998 to 2008. The virus might have also evolved somewhere else in western Mediterranean area and then it might have been reintroduced in Italy, for instance by migratory birds. The rapid spread in the last two years in Italy, with the occurrence of human cases of WNND, might be due to the positive selection of amino acid mutations in viral proteins conferring increased virulence and transmission capacity. In this regard, it is interesting to note that, in comparison with the Italy 1998-equine strain and with other western Mediterranean strains, the recent Italian WNV isolates have acquired the Thr249Pro mutation in the helicase domain of the NS3 protein, a trait associated with avian virulence [9]. In fact, this mutation is predicted to confer higher stability to the NS3 protein at high temperature conditions, such as in avian hosts, where the mutated virus can efficiently replicate leading to high levels of viraemia in birds that may facilitate the infection of new mosquito vectors. In support of this hypothesis, high mortality rates were reported among birds in the Unites States (US) and Israel, whereas seroprevalence studies in Romania indicated significant infection of resident birds [9,10]. It is important to note that the NS3 Thr249Pro mutation has emerged on at least three independent occasions (i.e., in the 1951 Egyptian isolate, in the 1996 Romanian isolate and within the Israeli/North American clade) and, in each case, viruses carrying this substitution have been associated with human disease outbreaks [9]. The WNV strains isolated from golden eagles in Spain in 2007 also carry the NS3 Thr249Pro change [8]. Studies in mice showed that the Spanish isolates do not have increased pathogenicity as compared with other strains, but virulence in birds has not been investigated [8].

Conclusions

Since 2008, an outbreak of WNV infection is ongoing in north-eastern Italy, in areas surrounding the Po river delta. The Italian outbreak is characterised by the occurrence of cases of severe meningoencephalitis [3-5,7], as also described in the recent outbreaks in the US [11], Romania [12], Israel [13], and Russia [14]. The number of human cases of WNND identified in the province of Rovigo represents about 1% of all cases of WNV infection occurring in 2009 in Rovigo province as estimated from the preliminary results of an ongoing seroepidemiological survey on blood donors.

Genome sequencing of WNV isolates is providing insight into the mechanism of re-emergence of this virus in Italy. In fact, the human WNV strain isolated this year and the strains isolated from magpies in 2008 are closely related to the Italy 1998-equine strain and to other western Mediterranean strains, with the acquisition of new amino acid mutations in non-structural proteins. These mutations include the Thr249Pro change in WNV-NS3 helicase, a trait associated with avian virulence and rapid geographic diffusion of WNV in North America [9]. In this regard, the veterinary and entomologic surveillance demonstrates that the virus is endemic in Italy and that it is rapidly spreading to other regions [15]. However, at variance with the WNV outbreaks in the US and Israel [16], the Italian outbreak does not seem to be associated with a particularly high mortality rate among birds [15]. The mechanisms of susceptibility of different bird species for WNV virulence is still unknown and might be related both to the genetic and immunological characteristics of the avian hosts and to the particular genetic backbone of each WNV strain [16].

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Table 2

Description of amino acid differences among Italian West Nile virus strains

AA position in WNV polyprotein	AA position in WNV proteins	Italy-98 AF404757 (equine)	Italy-08 FJ483548 (magpie)	Italy-08 FJ483549 (magpie)	Italy-09 GU011992 (human)
851	NS1-60	Val	Val	Val	Ala
1228	NS2A-85	Ile	Val	Val	Val
1248	NS2A-105	Ile	Ile	Thr	Ile
1494	NS2B-120	Ile	Ile	Val	Val
1754	NS3-249	Thr	Pro	Pro	Pro
2209	NS4A-85	Val	Ile	Ile	Ile
2224	NS4A-100	Pro	Ser	Ser	Ser
2581	NS5-53	His	His	Tyr	His
2786	NS5-258	Val	Ala	Ala	Ala
2950	NS5-422	Arg	Lys	Lys	Lys

AA: amino acid; NS: non-structural protein.

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First report of a North American invasive mosquito species Ochlerotatus atropalpus (Coquillett) in the Netherlands, 2009

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In late August and early September 2009, numerous larvae, pupae, and actively flying adult specimens of *Ochlerotatus atropalpus* were discovered in the Province of Brabant, southern Netherlands, during surveillance activities for *Aedes albopictus* at two trading companies that import used tires. No *Ae. albopictus* were found. Both companies mainly import used tires from countries in Europe, but also from North America. *Oc. atropalpus* is endemic to North America and has so far only been found outside of its endemic range in Europe, namely France and Italy, where it was subsequently eradicated. A preliminary modelling study shows that the weather conditions in the Netherlands are unlikely to prevent establishment of *Oc. atropalpus*. This species has so far only been shown to serve as a vector for virus transmission under laboratory conditions. Studies on potential human and veterinary health risks, as well as possible control strategies are currently ongoing.

Introduction

Following the discovery in 2005 of *Aedes albopictus* in the Netherlands at greenhouses of companies that import Lucky bamboo [1], surveillance activities to monitor this mosquito species were initiated. In 2006 a continuous surveillance programme was established and carried out by the Dutch Plant Protection Service (PPS) at these companies [2].

Gradually, other national surveillance activities for this mosquito species were established, including passive surveillance (since 2007) and active surveillance at parking lots along principle highways entering the country from the south and east (since 2008). The latter surveillance activity was initiated after reports of *Ae. albopictus* eggs found at parking lots in France, southern Germany, and Switzerland [3]. Since international trade of used tires is a well documented pathway dispersing *Ae. albopictus* around the world [4], surveillance at companies that import used tires was initiated in 2009. Except for the passive surveillance all *Ae. albopictus* surveillance activities are national surveys, carried out by the Plant Protection Service and funded by the Ministry of Public Health, Welfare, and Sports (Ministerie van Volksgezondheid, Welzijn en Sport, VWS).

During the surveillance at two companies that import used tires, the presence of *Ochlerotatus atropalpus* was observed at both companies. In Europe, the same species was found in Italy in 1996 [5] and in France in 2003 [6] and 2005 [7], but was eliminated in both countries by control measures directed against *Ae. albopictus* [8; F. Schaffner, pers. communication].

Methods

Two companies (subsequently called 'locations 1 and 2') were included in the survey. Both companies import used tires from airplanes, tractors, and large tires of rare sizes. One of the companies has two locations (location 2a and 2b). All three locations are in the south of the Netherlands, in the province of Brabant. All locations were inspected weekly.

Inspection of the sites consisted of checking tires for the presence of mosquito larvae and pupae, which were manually collected. Larvae collected during the first visit of location 1 were placed in alcohol and taken to the laboratory for molecular identification.

Larvae that were collected during the second and subsequent visits were either placed in alcohol and taken to the laboratory for morphological identification [9], or taken to the insectary to develop. A batch of eleven larvae was sent to an expert in mosquito taxonomy (F. Schaffner) at the University of Zurich, Switzerland, for morphological identification. Emerged adult mosquitoes were collected, identified morphologically, and stored in RNA-later tissue storage solution for future testing for viral RNA at the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM). Molecular identification of the larvae consisted of sequencing the cytochrome oxidase 1 (CO1) gene, a mitochondrial gene with a relatively high mutation rate which renders it suitable for molecular species differentiation tests.

In total, 23 visits were carried out (eleven at location 1, eight at location 2a, and four at location 2b). On seven visits after the first inspection (three at location 1 and four at location 2a), the inspector was accompanied with a colleague who manually collected actively flying mosquitoes using hand-held mouth aspirators (transparent tubes with mesh wire to prevent inhalation of mosquitoes, used to capture live mosquitoes). These were brought to the laboratory, stored at -20ºC for at least one hour, identified morphologically, pinned, and labelled to be kept as reference material.

Additionally, after the first visit, 20 oviposition traps and several adult traps (three CO_2 traps with octenol and, at locations 1 and 2a, two additional BG Sentinel traps) were placed in the immediate surroundings of all three locations, in zones of approximately 1 km^2 to determine possible spread of the species.

In order to predict whether *Oc. atropalpus* could become established in the Netherlands, a modelling study was carried out using 'Climex' [10], a software designed to match climates in ecology, which is used to carry out rapid, reliable assessment of the risks posed by the introduction of different organisms and to predict locations to which they could spread and become established. Parameters (temperature, moisture, heat stress, dry stress, wet stress, and degree-days) for suitable areas for *Oc. atropalpus* establishment were based on parameters of the known original distribution area [11] and determined by adjusting these parameters until they fitted the original distribution area.

Results

First visit

Initially, only location 1 was inspected. During that visit, seven larvae were retrieved. Sequence results for the seven larvae were negative for *Ae. albopictus*. However, the sequence of the CO1 gene from all seven specimens matched to 98.6-99.0% the CO1 sequence of *Oc. atropalpus* stored in GenBank.

Second and subsequent visits

Identification of *Oc. atropalpus* at location 1 prompted further inspection visits to this location as well as visits to locations 2a and b. During the second visit at location 1, 11 Culicidae were collected. A taxonomy expert confirmed five of them as *Oc. atropalpus* by morphological identification (the others were *Culex pipiens* (n=5) and *Culiseta annulata* (n=1)).

Surveillance activities that were carried out in all three locations after the first visit (at location 1), resulted in the finding of numerous larvae and pupae. Approximately 500, 250, and 100 larvae were collected from locations 1, 2a and 2b respectively. At locations 1 and 2a, larvae were found in almost every tire that contained water. At these two locations, also actively flying adult mosquitoes were collected.

Oc. atropalpus was present at two of the three locations (locations 1 and 2a). Not all larvae collected have developed into adults yet, but from the data that have been analysed so far, approximately half of the emerged adults were morphologically diagnosed as *Oc. atropalpus*. The other were *Culex pipiens/torrentium* and, occasionally, *C. annulata*. The same is true for the actively flying adults that were collected. To date, no *Oc. atropalpus* eggs have been collected in any of the oviposition traps placed in the surrounding areas. Virus detection tests have yet to be carried out on the emerged adults stored in RNA-later solution.

At none of the three locations that were visited in this survey, *Ae. albopictus* was detected.

Climex model study

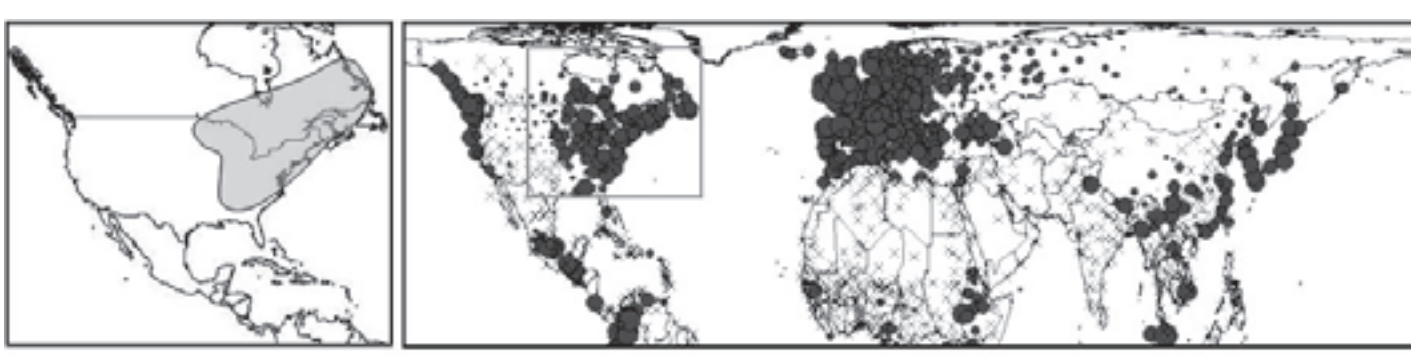
Using the 'match climates' module in 'Climex' software, parameters were set to fit the endemic geographic distribution of *Oc. atropalpus,* as described in [11] (southeastern Canada and mid-east-eastern United States). The model used this set of parameters to compare with meteorological data from locations in other areas of the world to predict likely areas where *Oc. atropalpus* could become established. This preliminary study shows that the climatological conditions in the Netherlands are not a limiting factor for establishment of *Oc. atropalpus* (see Figure).

Discussion and conclusion

The most likely introduction pathway of *Oc. atropalpus* from North America into the Netherlands is the passive transport of eggs through the import of used tires from airplanes, tractors, or soil-lifting vehicles. It is unlikely that the introduction has taken place this year, considering that tires containing *Oc. atropalpus* were found scattered over the premises of the two inspected companies, and only about 2% of the companies' tires are imported from overseas. Possibly, *Oc. atropalpus* was introduced several years ago. The fact that it was found in two different companies, separated by 100 km, could be explained by the fact that they occasionally exchange tires. Another possibility is that separate introductions

Figure

Climex model study for *Ochlerotatus atropalpus*



The grey area in Figure A shows the endemic geographic distribution of *Ochlerotatus atropalpus* [11; included here with permission from Journalof Genetics: Szymczak et al., 1986; J. Genet. 65(3):193-204, published by the Indian Academy of Sciences, Bangalore, India]. Areas with similar meteorological conditions as in the grey area (A) are depicted as dark grey dots in B (the larger the dot, the better the fit), thus predicting a relatively high likelihood that *Oc. atropalpus* could establish in that area. In contrast, crosses indicate areas with very low meteorological similarities to the area depicted in A, predicting that in such an area this mosquito species is unlikely to be able to establish.

of this species have occurred in the past. The species has been introduced into Europe on at least three separate occasions, once in Italy and twice in France, through import of used tires [5,6,7]. However, it is unlikely that the specimens found in the Netherlands were imported from other European companies since the three aforementioned known foci of *Oc. atropalpus* were successfully eradicated [8, F. Schaffner, pers. communication] and new introductions have not been reported since 2005.

The first results of the oviposition and adult traps in the surrounding areas of the two infested sites suggest that the species has not spread to the immediate surroundings. We are currently investigating whether the species (or other invasive mosquito species) are present at companies that import used truck and bus tires.

The results of the preliminary modelling study imply that *Oc. atropalpus* could become established in large areas of Europe.

In the field, *Oc. atropalpus* is not considered an important vector of infectious diseases. However, under laboratory conditions, the species is a competent vector for West Nile virus, Japanese encephalitis virus (JEV), Saint-Louis encephalitis virus (SLEV), La Crosse encephalitis virus (LACV), Murray valley encephalitis virus (MVEV), Western equine encephalitis virus (WEEV), and Eastern equine encephalitis virus (EEEV) [12,13]. SLEV and LACV can be transmitted transovarially by *Oc. atropalpus* [14,15], with laboratory studies that reported infection rates of up to 13.9% in adults that derived from eggs that were laid by LACV-infected females [15].

Oc. atropalpus was reported only once to be positive for virus infection: in one pool positive for WNV in the United States in the year 2000, out of 515 positive WNV pools consisting of 14 species [16]. It is possible that SLEV and LACV came into the Netherlands with the import of this mosquito species, but because of the limited role of *Oc. atropalpus* in the epidemiology of these viruses in its area of origin, this likelihood is considered very low. However, a role of the species in the spread of pathogens cannot be excluded.

The Dutch Ministry of Public Health, Welfare, and Sports considers this invasive mosquito species to be an 'unwanted organism' for the Netherlands, based on its putative role in the spread of infectious diseases important for public health. Control strategies are currently being investigated, including adequate treatment of used tires upon arrival and/or roofed storage of tires.

The aim of the surveillances at the tire import companies was initiated to monitor the presence of *Ae. albopictus*. The finding of *Oc. atropalpus* shows that other invasive mosquito species may be introduced as well and underlines the importance of mosquito surveillance systems.

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Trichinellosis acquired in Nunavut, Canada in September 2009: meat from grizzly bear suspected

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Five cases of trichinellosis with onset of symptoms in September 2009, were reported in France, and were probably linked to the consumption of meat from a grizzly bear in Cambridge Bay in Nunavut, Canada. Travellers should be aware of the risks of eating raw or rare meat products in arctic regions, particularly game meat such as bear or walrus meat.

Case detection and description

On 5 October 2009, the French National Reference Centre (NRC) for *Trichinella* was informed about a possible case of trichinellosis in an individual returning from Nunavut, Canada. This very asthenic patient had high eosinophil counts and elevated plasma levels for muscle enzymes. Specific antibodies were detected by ELISA and Western-blot (Diasorin & LDBio, France). The patient belonged to a group of five marine navigators who had travelled from the Aleutian Islands to Greenland and crossed the North-West Passage in northern Canada. The NRC started an investigation and identified four more cases among these travellers. Case 2 presented primary symptoms of shivers and fever without diarrhoea on 7 September. At the time she had been diagnosed with influenza but symptomatic treatment did not improve her condition. As high fever (40.4° C), intense muscular and joint pain, extreme asthenia and bilateral inferior limbs oedema persisted, the patient was hospitalised on 22 September. She also had elevated levels of eosinophils and muscle enzymes and was serologically positive on 30 September for trichinellosis (ELISA and Western-blot). The third and fourth crew members also had asthenia, high levels of eosinophils and muscle enzymes; one had a lasting diarrhoeal disease at the end of August; these two cases were tested positive by ELISA and Western-blot by the NRC and Biomnis lab in late October. The fifth traveller, living in Brussels, was also investigated and found to have been initially diagnosed with influenza but subsequently revised as trichinellosis (particularly when the link was made with the other cases) with manifestations of fever, myalgia, increased eosinophils and muscular enzymes levels and positive serology. Serological assays were not performed on one of the two patients with mild symptoms. No cardiac or neurological complications were observed. Only case 2 was hospitalised, discharge occurring 11 days later. All patients were treated with albendazole (7.5 mg/kg twice a day for 10 days) and corticosteroids were used in the first case and in the hospitalised patient (case 2).

Outbreak investigation

During the travel expedition many stopovers were made in Inuit's villages, and, on these occasions, the crew consumed meat of various wild animals: caribou, walrus, seal, polar bear and grizzly bear. Considering the occurrence, onset and duration of signs and symptoms, the source of infection were probably grizzly (*Ursus arctos*) steaks which were consumed in the Cambridge Bay area (Iqaluktuuttiaq), Victoria Island, Nunavut, Canada between the 19 and 22 August 2009. Information obtained from residents of Cambridge Bay indicated the grizzly bear was shot at Elu Inlet Lodge, at the beginning of August, transported fresh to Cambridge Bay where it was frozen for about a week. A leg was thawed, cut into pieces and given to the travellers. The pieces were frozen again for two days. After departure, the meat was stored for two additional days in the boat. All five members of the crew consumed this meat, barbecued or pan-fried, on several occurrences after the 19 August. All the remaining meat from the bear was consumed locally in Cambridge Bay, but well cooked and no suspected cases were reported. The Centre for Food-borne & Animal Parasitology, Canadian Food Inspection Agency, in Saskatoon, Canada was contacted on 6 October 2009 and informed of the outbreak. In the course of the investigations, it was established that, for some time, the boat of the five travellers sailed together with another one with four persons on board and members of both crews ate at the same places. The second boat was on the way for Halifax, Canada in mid-October when the crew was contacted by email and alerted of the possibility of trichinellosis infection and of specific preventive and treatment measures that might be necessary. According to their blog, one of the crew members had been affected by a persistent flu during the same period as the travellers on the first boat. But no additional information could be obtained from this second crew.

Discussion

This report illustrates well the fact that trichinellosis can be misdiagnosed for influenza, which is particularly important in the context of the pandemic H1N1 influenza outbreak when health professionals and the general public are more inclined to suspect influenza. Misdiagnosis of trichinellosis for influenza is not unusual because the initial clinical symptoms of these diseases occurring at the acute stage of infection are not pathognomonic. In another occurrence, Laurichesse *et al.* [1] emphasized that "general

practitioners could have misdiagnosed cases of trichinellosis because they did not routinely order serological tests". The presence of specific clinical and biological signs (facial oedema, elevated levels of eosinophils and muscle enzymes, and specific antibodies) can readily confirm the diagnosis of trichinellosis.

Trichinellosis is a widespread helminthic zoonosis endemic in northern Canada where the incidence rate among the indigenous population was estimated at 11 cases per 100,000 [2], which is 200 times the national Canadian rate [3]. Walrus (*Odobenus rosmarus*) meat is the most frequent source of trichinellosis infection in humans; polar bear (*Ursus maritimus*) seems to be less important. *Trichinella nativa* and the genotype T6 are widespread in northern Canada [4,5]. The precise genotype responsible for this small outbreak could not be determined, as the infected meat was not conserved and no muscular biopsies were performed. In an extensive survey recently performed on wildlife across northern Canada, Gajadhar and Forbes found that 29.4 % of grizzly bears examined harboured *Trichinella* larvae [5]. The prevalence was 65.9% among polar bears, 40.6% in walrus and 7.3 % in black bears (*Ursus americanus*). There are no other recent survey reports for *Trichinella* in wild fauna in Nunavut, except for a survey of wolverines (*Gulo gulo*) which found 87.8 % of these animals positive [6]. Outbreaks of trichinellosis among Inuit population have been described earlier in Nunavut on Baffin Island [7] and Repulse Bay [8]. They occurred in the local residents after consumption of walrus meat. Apparently, Inuit populations consume bear meat thoroughly cooked whereas walrus meat is eaten frozen, fermented or air-dried [9]. An earlier study has shown that traditional northern foods used by Inuit can harbour infective *Trichinella* larvae [10]. Other outbreaks, linked mainly to walrus meat consumption have been described in neighbouring Nunavik (from Inukjuak on south Hudson Bay and as far north as Salluit) leading to the development and implementation of a prevention program for trichinellosis in Inuit communities [8,9]. We also described, in 2005, an outbreak of trichinellosis among French hunters and their families in France after consumption of black-bear meat obtained from northern Quebec [11,12]. Apparently, French tourists, especially hunters, are particularly fond of bear meat. Including the present report, a total of 25 cases linked to bear meat consumption have been reported to the NRC since 1995 [12]. The present outbreak appears to be associated with the most northern geographic area described to date in Canada with grizzly bear meat as source. As shown in this report, the arctic species of *Trichinella* (*T. nativa* and T6) are resistant to freezing and are killed by sufficient cooking at 67°C. Travel in endemic regions is a classical driver for acquiring trichinellosis, and travellers should be aware of the risks of eating raw or rare meat products, particularly game meat such as bear or walrus meat [13].

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Botulism and hot-smoked whitefish: a family cluster of type E botulism in France, September 2009

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A family cluster of three cases of type E botulism were identified in south-east France in September 2009. The suspected food source of infection was a vacuum packed hot-smoked whitefish of Canadian origin purchased by the family during a visit to Finland and consumed several weeks later in France on the day prior to symptom onset. No leftover fish was available to confirm this hypothesis. Vacuum packed hot-smoked whitefish has previously been associated with cases of type E botulism in multiple countries, including Finland, Germany, the United States and Israel.

Case notification

A confirmed case of type E botulism in an individual residing in south-east France was reported to the French National Institute for Public Health Surveillance (Institut de Veille Sanitaire) by the National Reference Center (NRC) for Anaerobic Bacteria and Botulism at the Pasteur Institute in Paris on 10 September 2009. Two other members of the same family were reported as having clinical symptoms compatible with botulism. An investigation was undertaken to identify additional cases, the vehicle of transmission, and to put in place appropriate control measures.

Methods

Following notification of the cases, active case finding was carried out via contact with local health authorities, the NRC and the hospital services where cases were hospitalised. Hospital clinicians treating the patients, and thus likely to see other such cases, were reminded by telephone contact to immediately report all clinical suspicions of botulism to the local health authorities using the routine mandatory notification system for the disease.

Serum samples from the cases were analysed by the NRC. The presence of botulinum neurotoxin was confirmed by intraperitoneal administration of patient serum to mice, and the toxin type was ascertained by neutralisation with specific antibodies [1].

The food history of the cases in the three to four days before onset of symptoms was documented by the local health authorities, as were the details of purchase, transport and consumption of the suspected food product.

Based on patients' food history a fish product purchased during a family visit to Finland was suspected to have been the source of infection. A sample originating from the same batch of raw fish as the implicated product but processed one day later was collected from a local supermarket in Finland. The sample was sent to the Department of Food and Environmental Hygiene, University of Helsinki, Finland, for analysis of *Clostridium botulinum* by multiplex PCR targeted to the types A, B, E, and F neurotoxin genes [2]. Twelve 1gram samples from skin, gills or peritoneum were each inoculated into 10 ml of anaerobic tryptose-peptone-glucose-yeast extract (TPGY) medium and incubated at 30°C for three days. One ml of each culture was transferred to fresh TPGY medium and incubated overnight at 30°C. Lysed cells from 1 ml of each culture were used as template in PCR. PCR amplification products were visualised in 2% agarose gels against standard molecular weight markers.

Results

The three cases (two adults aged 52 and 46 years and one adolescent child aged 13 years) presented with classical clinical symptoms of botulism (gastrointestinal symptoms followed by

descending paralysis) on 7 September 2009 and were hospitalised the following day. One of the adult cases rapidly developed quadriplegia and required intubation and mechanical ventilation for 17 days. The other two patients presented with a milder form of the disease, did not develop paralysis of limbs or respiratory muscles and were released from hospital in mid-September. The severe case remained hospitalised as of 29 September (latest information available) but had regained motor function and begun to walk.

The NRC confirmed a diagnosis of type E botulism for the severe case. Botulinum toxin type E was identified in a serum sample (8 Mouse Lethal Dose/ml) and in two from three gastric juice samples (<20 MLD/ml). Serum samples from the two milder cases were negative for botulinum toxin. A faecal sample obtained from the child was negative for botulinum toxin and *C. botulinum*. No other botulism case associated with this episode was identified.

The food investigation carried out with the family identified the consumption of vacuum packed hot-smoked whitefish (*Coregonus lavaretus*) on 6 September 2009 (the day prior to symptom onset). All three sick members of the family reported having eaten the smoked fish and a fourth non-sick family member did not consume the product. There was no leftover fish to test for the presence of toxin. The family did not report consumption of any other foods usually associated with the risk for botulism (home-canned vegetables or home-prepared meat products such as ham, sausages and pâté) in the days preceding symptom onset.

The whitefish was purchased by the family in a supermarket in a village in east Finland on 22 August 2009. The fish was smoked in Finland but was originally from Canada. It was refrigerated after purchase. The family returned to France the following day. The fish was placed in a cooling bag with ice-packs for the duration of the 14-hour journey and then refrigerated upon arrival in the family home until the day of consumption on 6 September 2009, two days before the expiry date.

The fish was not heated prior to consumption. The entire product (800-1000 g) was eaten at the meal by the three patients. The adult with a severe form of the disease reportedly consumed a greater portion of the fish than the two milder cases.

An environmental investigation was carried out in the premises of the fishery production plant by the food control authority in Finland. The inspection focussed on the fish processing and storage temperatures, hygiene conditions and efficacy of in-house control of the producer. The storage temperature of the raw material, temperatures during the process and transport were found to be correct and in accordance with the in-house control plan and legislation. The raw fish was imported from Canada two months earlier and stored frozen at the premises' freezer (-18°C). The processing of the batch was started on 16 August 2009 with thawing and salting of the fish (temperature below 3°C). After hot smoking (two hours; maximum temperature 68°C) the fish was rapidly chilled (until 0.5° C), vacuum packed and stored below 3°C. The batch (about 600 kg) was transported at 0°C to the retail on 18 August 2009. The fish sample representing the same batch of raw material but processed one day later than the implicated fish product was negative for *C. botulinum* in the PCR analysis. Temperature controls carried out at the supermarket of purchase by the local food control authority showed storage temperatures for fishery products of 0.8-2.8°C.

Public health measures

European countries were informed of the event via the 'Early Warning and Response System' (EWRS) and an alert in the 'Rapid Alert System for Food and Feed' (RASFF), both issued on 11 September 2009. The information in the RASFF was subsequently transmitted to the Canadian food safety authorities. No other cases of botulism associated with this product were identified in Finland, France or other European Member States, as of 9 November 2009.

Discussion and conclusion

C. botulinum type E is an aquatic bacterium endemic in areas such as Canada and Alaska [3-5]. Type E botulism is characteristically associated with the consumption of improperly prepared foods of aquatic origin, either fresh water or marine [6]. Cases of type E botulism are very rare in France with the last episode declared in 2003 [7]. Foods associated with the occurrence of this form of botulism in France include salted herring, grey mullet, canned carp and canned sardines [8].

The negative mouse bioassay results of the serum samples of the two patients with a milder form of the disease could be explained by a lack of circulating toxin in the patients' blood. It is known that botulinum toxin cannot be detected in serum once it becomes irreversibly bound to its cell receptors and thus the detection of toxin in serum samples is believed to depend on the timeliness of sample collection and on the ingested dose of toxin, among other factors [6,9].

The epidemiological investigations support the hypothesis of the vacuum packed hot-smoked whitefish as the source of contamination of the three cases. No leftover fish was available for testing to confirm this hypothesis. An association between hot-smoked whitefish and type E botulism has been previously documented in Finland, Germany, the United States and Israel [10-13]. On two previous occasions, cases of type E botulism have been associated with whitefish imported from Canada and processed in Finland, as was the situation with the whitefish consumed by the three French cases [10,11].

Vacuum packed hot-smoked fish is a known risk food for type E botulism [14]. It is believed that the hot-smoking processes carried out on this type of fish, which typically reach temperatures of 60-80°C, are often insufficient to eliminate *C. botulinum* spores [15]. Among factors believed necessary for controlling growth and toxin production in this fish is the continuous storage of the fishery products below 3°C [10,11], information which is clearly labelled on this food product. According to the national legislation, modified atmosphere package (MAP) and vacuum packed fishery products must be stored below 3°C in production and at retail in Finland. Temperature controls carried out at the fishery production plant and the supermarket of purchase showed that storage temperatures were in accordance with the legislation. It is probable that the whitefish consumed by the three French cases was not stored below 3°C for the duration of the 14-hour return journey to France. Also, French domestic fridges are estimated to have an average temperature of 6.6°C [16] and thus well above 3°C. Assuming that the temperature of the family's fridge corresponds approximately to the estimated national average (the actual fridge temperature was not measured) the two weeks of refrigerated storage could have allowed ample time for growth and toxin production in the anaerobic environment created by vacuum packaging.

The absence of additional cases in Finland could be explained by a limited contamination of the whitefish by *C. botulinum*. The fish sample representing the implicated batch of raw material was negative for *C. botulinum* spores. In a previous case of human infection reported in Finland, 10 fish samples from an implicated batch were also negative for *C. botulinum* [11]. This is consistent with a previous prevalence study showing that 18% of raw and 5% of processed and packaged whitefish carry type E spores [14]. The absence of further cases may also be explained by a difference in storage habits of hot-smoked whitefish between the Finnish population and foreign tourists.

This family cluster provides further evidence of the risk of type E botulism associated with consumption of vacuum-packed hot-smoked whitefish. This episode also highlights the potential public health threat of *C. botulinum* spores in incorrectly stored processed food products and underlines the importance of clear labelling of storage conditions for products purchased in the refrigerated sections of supermarkets.

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Detection of human norovirus from frozen raspberries in a cluster of gastroenteritis outbreaks

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We describe a cluster of norovirus outbreaks affecting about 200 people in Southern Finland in September and October 2009. All outbreaks occurred after consumption of imported raspberries from the same batch intended for the catering sector. Human norovirus genotype GI.4 was found in frozen raspberries. The berries were served in toppings of cakes in separate catering settings or mixed in curd cheese as a snack for children in a daycare center. The relative risk for consumption of the berry dish was 3.0 ($p \leq 0.05$) at the daycare centre. Human norovirus GI.4 was also detected in samples from two patients, and in berries. Both shared identical partial capsid sequences. Based on the results of epidemiological, trace-back and laboratory investigations it was concluded that one particular batch of frozen raspberries was the source of all outbreaks.

Introduction

Human norovirus is a common cause of outbreaks of acute gastroenteritis worldwide [1]. Food-borne outbreaks caused by contaminated shellfish occur commonly, but fresh products, especially raspberries have also been found to be the vehicle [2, 3, 4]. In Finland, from 1997 to 1999, several norovirus outbreaks were linked to imported frozen raspberries [2,5]. It still remains unclear how the berries were contaminated, but it seemed to have occurred already in the countries of origin. Pre-harvest irrigation or hygiene failures during harvest/freezing have been suggested as possible sources of contamination [6]. A proper heating of frozen raspberries prior to consumption has been recommended by the Finnish Food Safety Authority since 2000 but it is occasionally neglected.

Here we describe a trace-back investigation in a cluster of three food-borne norovirus outbreaks linked to consumption of imported raspberries affecting about 200 people in Southern Finland in September and October 2009. The epidemiological investigation was performed of one of the outbreaks, at a daycare centre.

Outbreak in the daycare centre

A curd cheese dish mixed with raspberries (originally frozen) was served without heating the berries and eaten by about 90 persons (majority children, less than 7 years old) at a daycare centre on 2 October 2009 at 2-2.30 pm. On Saturday evening, 3 October, more than 20 of the 90 persons started symptoms of vomiting and diarrhoea (Table 1). The food inspection authorities were informed about the outbreak on 6 October and started an epidemiological investigation. No samples of the dish were available for investigation but the remaining frozen raspberries were sent for bacteriological and virological examination on 7 October. Also samples from patients were collected, and questionnaires were distributed to the children's parents and the personnel on 7 October to investigate the possible source of the outbreak. The outbreak occurred at a daycare centre in a city of 100,700 inhabitants in Southern Finland.

Epidemiological analysis

Questionnaires were obtained from 69 people at the daycare centre. A case was defined as a person who was working, or at daycare at the daycare centre, and fell ill with vomiting and diarrhoea between 2 and 5 October 2009. A two-by-two table for consumption of berries was performed (a cohort study). A chi-squared test was used to calculate the statistical significance.

Most cases (45/46, 98%) had eaten berries. The epidemic curve shows a point-source pattern with some secondary cases (Figure). The incubation period was determined at 32.5 h (range 14-76) and the mean duration of symptoms was 22.4 h (range 1-72). Based on

Table 1

Symptoms among cases in a daycare centre, norovirus outbreak, Finland, October 2009 (n=46)

Symptoms	N (%)
Vomiting	42 (91)
Nausea	33 (72)
Stomach pain	30 (65)
Diarrhoea	17 (37)
Fever	12 (26)
Headache	10 (22)

a cohort study, those who ate berries were 3.0 times (relative risk) more likely to develop disease than those who did not ($p \leq 0,05$).

Food and patient samples from three outbreak settings

Three samples of frozen raspberries obtained from three outbreak settings (Table 2) and two samples from the wholesaler's stock (total batch size 20,000 kg) were analysed for norovirus at the laboratory of the Department of Virology, Helsinki University Central Hospital (HUSLAB), Helsinki, Finland. The raspberries, packed in bags of 2.5 kg, originated from Poland. Patient samples from two outbreaks were sent for norovirus analysis to HUSLAB and sequencing and genotyping was performed at the laboratory of the Finnish Institute of Health and Welfare. A norovirus real-time RT-PCR was performed targeting the polymerase-capsid gene junction [7]. The sequence analysis was performed on the polymerase and capsid region with primer-pairs MJV12-RegA and SKF-1-SRI-3, respectively [8-10]. The expected lengths of amplicons were 320 and 240bp, respectively. The accession numbers for the norovirus sequences are GU188278 (capsid; berries) and GU 188279 and GU188280 (capsid and polymerase; patient).

Figure

Epidemic curve, norovirus outbreak, day-care center, Finland, October 2009 (n=46)

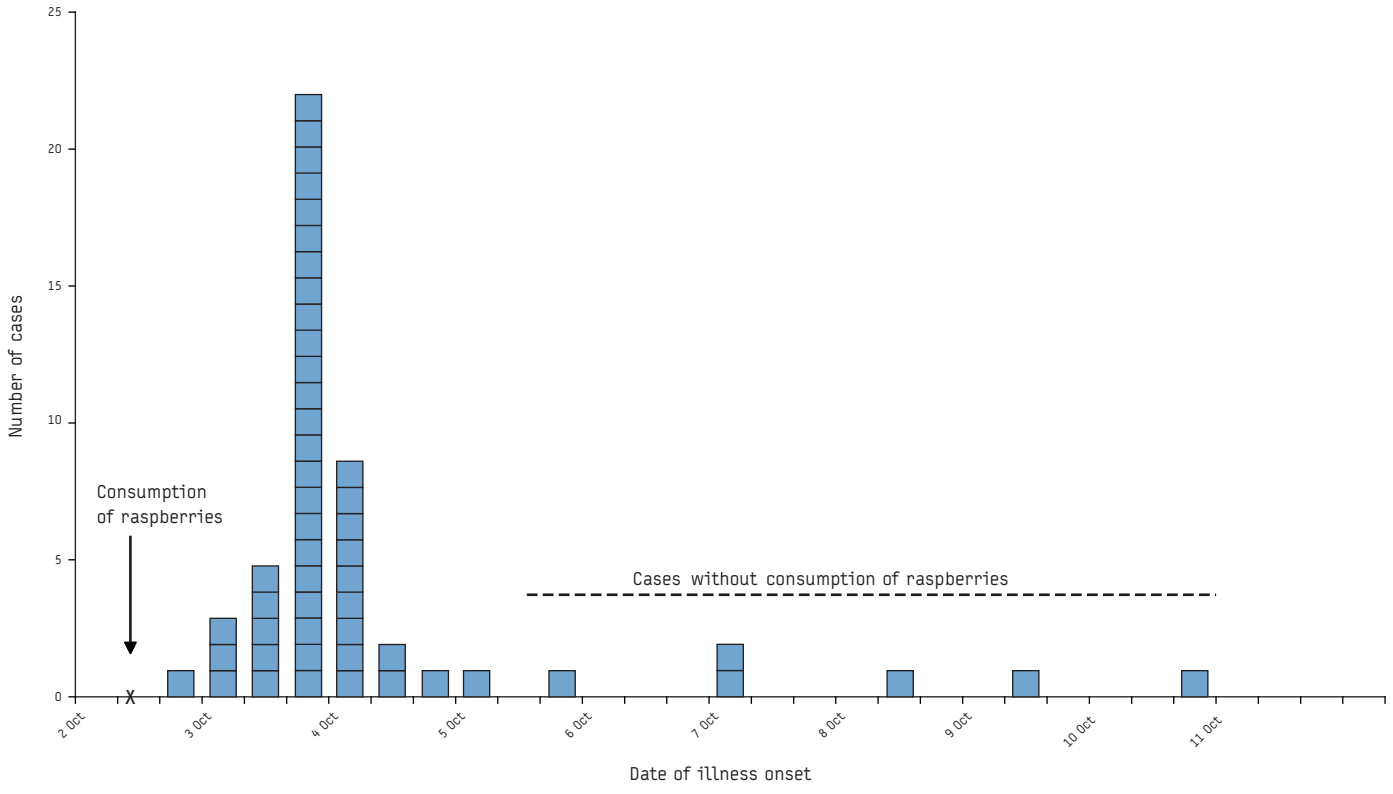

Table 2

Onset of outbreak, number of cases among exposed and detected norovirus, by place, Finland, September-October 2009

Place (provider of food)	Start date	Cases/exposed (estimation)	Virus in raspberries	Virus in patients
Restaurant (catering)	26 September	(15/30)	**1/1 NoV GI.4 (Cp 34,8)**	0/0
Daycare centre (prepared on site)	**2 October**	**46/90**	**1/1 NoV GI (Cp 40,1 1:10 37,0)**	**2/3 NoV GI.4**
Cafeteria (catering)	3 October	(15/30)	1/1 NoV GI (Cp 37,8)	1/2 NoV
Raspberries from wholesaler's stock	NA	NA	0/2	NA

Cp = crossing point -value in norovirus real-time RT-PCR (LightCycler, Roche); NoV: norovirus; NA: not applicable.

Microbiological findings

In total, norovirus GI was detected in three of five raspberry samples analysed by norovirus real-time RT-PCR. In one case the concentration of virus was high enough to allow exact genotyping and the virus could be identified GI.4 by conventional RT-PCR and sequence determination. In the two patient samples available for genotyping, norovirus GI.4 was detected. The viruses in berries and patients showed identical nucleotide sequences in the short 181 bp-capsid gene region. A positive polymerase RT-PCR result could only be amplified from patient samples.

In addition to the three outbreaks described, several smaller outbreaks involving only few cases (e.g. bakery, bank) that were most likely berry-related, were reported to the local food health authorities between 26 September and 9 October, but no samples were obtained for virological investigation. Taken together, about 200 people were affected in all these outbreaks.

Discussion and conclusions

Strong laboratory evidence supported the epidemiological findings that imported raspberries were the source of the norovirus outbreaks, since the identical genotype was detected in samples from berries and patients. The outbreaks occurred outside of the norovirus outbreak season that usually occurs from December to May in Finland. The detection of GI.4 virus is in line with a large study of norovirus outbreaks in which the proportion of non-GII.4 outbreaks was found to be higher in food-borne outbreaks, whereas GII.4 outbreaks were mostly linked to person-to-person transmission [11].

The berries that caused the outbreaks were likely to contain a considerable number of viruses, since they were detected without prior concentration of the samples. While the present real-time RT-PCR method is quite sensitive, the positivity in foods is mostly weak, partly due to PCR inhibitors. To determine the viral genotype with the less sensitive conventional RT-PCR is therefore challenging. In this study, a short sequence in a capsid gene region, not normally used for genotyping could be determined in berries, independently from the patient sample analysis.

Our findings highlight the importance of routine investigations of food samples for viral pathogens in addition to bacterial analyses. So far, all our virus findings in foods have been directly linked to outbreaks. In spite of analysing several samples of the same batch of raspberries epidemiologically linked to human cases, norovirus could not be detected in all samples. This could be due to an uneven distribution of viruses in the berries.

The norovirus gastroenteritis outbreaks rapidly died out, after the contaminated batch was withdrawn from the market. Furthermore, the Finnish authorities issued an alert through the Rapid Alert System for Food and Feed (RASFF) on 20 October to inform other European Union countries of the outbreaks caused by norovirus-contaminated raspberries. It is noteworthy that a month earlier, in August, another food-borne outbreak in east Finland was epidemiologically linked to crushed frozen raspberries also imported from Poland. No viruses were found in the berries, but genotype GI.4 norovirus was found in the patients.

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Quantifying the risk of pandemic influenza in pregnancy and Indigenous people in Australia in 2009

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An increased relative risk of infection with the 2009 pandemic H1N1 influenza virus associated with pregnancy and Indigenous status has been a common finding in many countries. Using publicly available data from May to October 2009 in Australia, we estimated the relative risk of hospitalisation, admission to intensive care unit and death as 5.2, 6.5 and 1.4 respectively for pregnant women, and as 6.6, 6.2 and 5.2, respectively for Indigenous Australians. Pregnancy and Indigenous status were associated with severe influenza. More complete analyses of risks in these groups are required to understand and prevent influenza morbidity and mortality.

Introduction

The 2009 H1N1 influenza pandemic in Australia corresponded with the expected influenza season, although pandemic virus circulation began relatively early. In the populous states of New South Wales and Victoria, pandemic influenza virus circulated for about 10-13 weeks [1,2]. The death rate due to pandemic H1N1 influenza was reported as approximately 9 per million for Australia, in the middle of the range of 5-15 per million that was reported for other populous countries in the southern hemisphere [3]. Groups most at risk in the pandemic were recognised to be Indigenous people, pregnant women, the morbidly obese and people with recognised comorbidities [4]. Before the end of the 2009 pandemic in Australia, we used publicly available data to estimate the increased risk of hospitalisation for pregnant women as 3.2 (95% confidence interval (CI): 2.6 to 4.1) [5]. We now use the same data sources to provide estimates of the relative risk of hospitalisation, intensive care unit (ICU) admission and death for pregnant and Indigenous Australians throughout the entire pandemic period.

Methods

We obtained population data from the Australian Bureau of Statistics [6]. Data extracted included estimated total population in 2009, population by sex and age group, estimated number of live births and proportion of the Australian population identifying themselves as Aboriginal or Torres Strait Islanders (Indigenous Australians). We obtained data on the hospitalisations, ICU admissions and deaths in pregnant women and Indigenous Australians due to pandemic H1N1 influenza from reports published by the Australian Department of Health and Ageing [7].

We estimated the cumulative incidence of all outcomes for the entire pandemic period, from May to October 2009. To estimate the relative risk (RR) for the two nominated risk groups, we compared the cumulative incidence of each outcome in the risk group with the same outcome in the entire population minus the estimated population in the risk group. Confidence intervals for RR were calculated using the method outlined in Bland and Altman [8]. We estimated the number of at-risk pregnant women as previously described by using the fertility and abortion rates in women aged 15-44 years [5] and compared this number with the estimated number of live births in 2009. We used the estimate of the proportion of Indigenous Australians in 2009 from the projected Australian census data.

Results

Our previous estimate of at-risk pregnant women in Australia was 237,215 and equivalent to about 1.1% of the Australian population [5]. The minimum prevalence of pregnancy should be 40 weeks divided by 52 weeks multiplied by 296,600, which is the estimated number of live births for 2008 [9] and the estimate we used for the number of live births in 2009. The fraction of live births represents the expected duration of pregnancy and leads to a minimum estimate of the number of pregnant women in Australia which was 228,154. The proportion of the Australian population who identify themselves as Aboriginal or Torres Strait islanders is estimated as 2.5%, i.e. 534,350 Indigenous Australians [10]. This estimate attempts to correct for under counting in census data and we could find no more exact estimate of the number of Indigenous Australians.

More than 4,800 hospitalisations, 650 admissions to ICU and almost 200 deaths due to pandemic H1N1 influenza were reported in Australia between May and October 2009. Estimations of the RR of hospitalisation, ICU admission and death for pregnant and Indigenous Australians ranged between 5.2 and 6.6, with the exception of the RR for death in pregnant women, which was only 1.4 (95% CI: 0.3 to 4.3). This imprecise estimate was based on only three deaths (see Table). We also calculated the RR of

hospitalisation in pregnant women compared with not pregnant women of reproductive age (15-44 years). Of an estimated 4,492,701 women of reproductive age, 1,030 were hospitalised. This gave an RR of 5.1 (95% CI: 4.5 to 5.8), similar to the comparison with the general population.

Our estimate of pregnant women at risk was 3.8% higher than the minimum number of pregnant women estimated from the number of live births. Using the minimum estimate of pregnancy did not change RR estimates for pregnancy to any appreciable degree (data not shown).

Discussion

Before the end of the 2009 pandemic in Australia, we had estimated the RR for hospitalisation of pregnant women due to pandemic H1N1 influenza as approximately 3.2 [5], comparable to an early estimate from the United States of 4.3 [11]. At the end of the 2009 pandemic in Australia, this risk appeared to be higher, of the order of 5.2. We had not previously estimated the increased risks associated with Indigenous status. These risks appear to be at least as high as the risk associated with pregnancy, with a much higher risk for death in Indigenous Australians (RR=5.2) compared with pregnant women (RR=1.4).

Limitations of these results include the potential under-ascertainment of cases, but this is more likely for those perceived not at increased risk (the denominator) than those at increased risk, pregnant and Indigenous Australians (the numerator). For the entire pandemic period, efforts were concentrated in identifying pandemic H1N1 influenza in vulnerable population groups, and testing was also prioritised for hospitalised patients. Increased ascertainment of the group perceived not to be at risk would result in lower estimates of RR than we have reported. We therefore think it is unlikely that our estimates of RR for any of the outcomes are spuriously low. A further limitation of the reported RR estimates results from necessarily imprecise estimates of the at-risk populations. Moreover, with access only to data in the public domain, we could not report age-stratified or age-adjusted rates or adjust for the presence of co-morbidities. A more thorough analysis of risk is warranted, with risk during pregnancy stratified by gestational age.

In a 2008 review of influenza vaccination in pregnancy, Mak and colleagues concluded that during severe influenza seasons and the pandemics of 1918-19 and 1957-58, pregnant women were at increased risk of influenza-related hospital admission compared with not pregnant women or women post-partum [12]. They also noted that the risk rose with increasing gestation and the presence of co-morbidities. A study from Tennessee between 1974 and 1993 found the excess rates of hospitalisation of pregnant women for an acute cardio-respiratory illness in the second trimester to be 6.3 and in the third trimester 10.8 per 10,000 healthy woman-months. Much lower estimates of excess hospitalisation rates, in the range of 0.4-2.0 per 10,000 healthy woman-months, were reported for influenza-attributable hospital admissions 1990-2002 in Nova Scotia [12]. Reflecting the non-systematic approach to risk quantification in the influenza literature, none of the reported risks were due to laboratory-confirmed disease. In a more recent systematic review of influenza immunisation in pregnancy, Skowronski and De Serres confirmed that studies using laboratory-confirmed outcomes are scarce [13]. This lack of quality data continues to frustrate our understanding of the burden of influenza and prevents direct comparison with the data presented here [5].

Point estimates for RR, defined as the incidence rate ratio, of up to 3.8 for hospital admission coded as influenza in Aboriginal children in Western Australia between 1996-2005 have recently been made (personal communication, Hannah Moore, Telethon Institute for Child Health Research, Perth, Western Australia). This outcome is more specific than the outcomes studied in pregnant women but again is not strictly comparable to the data presented here.

While it is generally accepted that both pregnancy and Indigenous status increase the risk of adverse outcomes due to

Table

Estimated relative risk of the cumulative incidence of hospitalisation, admission to an intensive care unit or death from pandemic H1N1 influenza in pregnant and Indigenous Australians, May-October 2009

Outcome	Number	Population at risk	Rate/100,000	Relative risk	95% confidence interval	Comparator
Hospitalisation, all	4,833	21,373,998	22.6	n.a.	n.a.	Comparison of at-risk population derived from total population
ICU admission, all	650	21,373,998	3.0			
Death, all	186	21,373,998	0.9			
Hospitalisation, pregnant women	278	237,215	117.2	5.2	4.6 to 5.8	Pregnant women versus all non-pregnant
ICU admission, pregnant women	47	237,215	19.8	6.5	4.8 to 8.8	
Death, pregnant women	3	237,215	1.3	1.4	0.4 to 4.5	
Hospitalisation, Indigenous status	803	534,350	150.3	6.6	6.2 to 7.2	Indigenous versus non-Indigenous
ICU admission, Indigenous status	100	534,350	18.7	6.2	5.0 to 7.6	
Death, Indigenous status	24	534,350	4.5	5.2	3.4 to 7.9	

ICU: intensive care unit; n.a.: not applicable

laboratory-confirmed influenza, quantification of these risks is surprisingly scarce. We have provided estimates of RR from data available in the public domain from the Australian pandemic of 2009, but acknowledge the need for more complete analyses.

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An update on an ongoing measles outbreak in Bulgaria, April-November 2009

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Earlier this year, an outbreak of measles was detected in Bulgaria, following an eight–year period without indigenous measles transmission, and continues to spread in the country. By the end of 48 week of 2009 (first week of November), 957 measles cases had been recorded. Most cases are identified among the Roma community living in the north-eastern part of the country. Measles has affected infants, children and young adults. The vaccination campaign that started earlier in the year in the affected administrative regions continues, targeting all individuals from 13 months to 30 years of age who have not received the complete two-dose regimen of the combined measles-mumps-rubella (MMR) vaccination.

Introduction

This is an update of an article published in July 2009 that reported an outbreak of measles in Bulgaria. The outbreak was first clearly noticeable in April 2009 and had involved 79 cases by mid-June [1]. Since then, the outbreak has intensified and continues to spread throughout the country. It occurred eight years after the last indigenous cases of measles in Bulgaria were reported in 2001 [2].

Measles has been a statutorily notifiable disease in Bulgaria since 1921, obliging medical practitioners and microbiologists to immediately report suspected measles cases to the Regional Inspectorate for Protection and Control of Public Health (RIPCPH). Notifications of measles cases are collected and analysed centrally at the National Centre of Infectious and Parasitic Diseases in Sofia. In 2005, the Council of Ministries of the Republic of Bulgaria approved the Bulgarian national programme for the elimination of measles and congenital rubella infection (2005-2010) [3]. National case-based notification was initiated in 2004 and the European Union (EU) case definition and case classification have been adopted since 2005 [4,5].

In Bulgaria, the measles vaccine is given as the combined measles-mumps-rubella (MMR) vaccine. Since 1993 the first dose has been recommended at the age of 13 months and the second dose at the age of 12 years, but at least one month after the first dose. For 2005-08, the national vaccine coverage was estimated at 95.9-96.2% for the first MMR dose in two year-old children and at 92.4-94.3% [6,7] for the second dose in 12 year-old children.

We aim to report an update on the ongoing measles outbreak in Bulgaria by analysing measles data provided for the first 48 weeks of 2009.

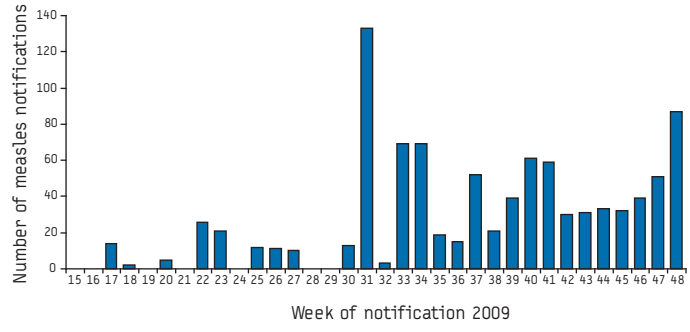
Outbreak description

The outbreak has spread to five more administrative regions since the last report [1], now affecting nine regions (Figure 1). By week 48 of 2009 (week beginning 23 November), there have been 957 notifications of measles, giving a crude incidence of 12.5 per 100,000 inhabitants, with large regional variations. Most cases (97%) were reported from the north-eastern part of the country, i.e. the regions of Dobrich, Silistra, Burgas, Varna, Shumen and Razgrad (Figure 2). Although no data by ethnicity are available, it was clear to the outbreak investigators that at least 90% of cases occurred in the Roma ethnic community. Members of this community usually belong to large families and frequently travel within and across borders. So far, during the current outbreak, several family clusters have been recorded among this group.

Of the total, 429 cases (45%) were laboratory-confirmed by detection of measles IgM antibodies in serum. An epidemiological link to laboratory-confirmed cases was identified in 337 (35%) cases. The remaining 191 cases (20%) were classified as clinical cases only. The World Health Organization (WHO) Regional Reference Laboratory (RRL) for Measles and Rubella in Berlin identified the virus as measles genotype D4. The nucleotide sequence was identical to that detected between January and June 2009 in northern Germany, confirming the epidemiologically link with the index case who had stayed in Hamburg during that period. Apart from the index case all cases acquired measles in the country and are therefore indigenous cases.

Figure 1

Notified measles cases by week of notification, Bulgaria, April-November 2009 (n=957)



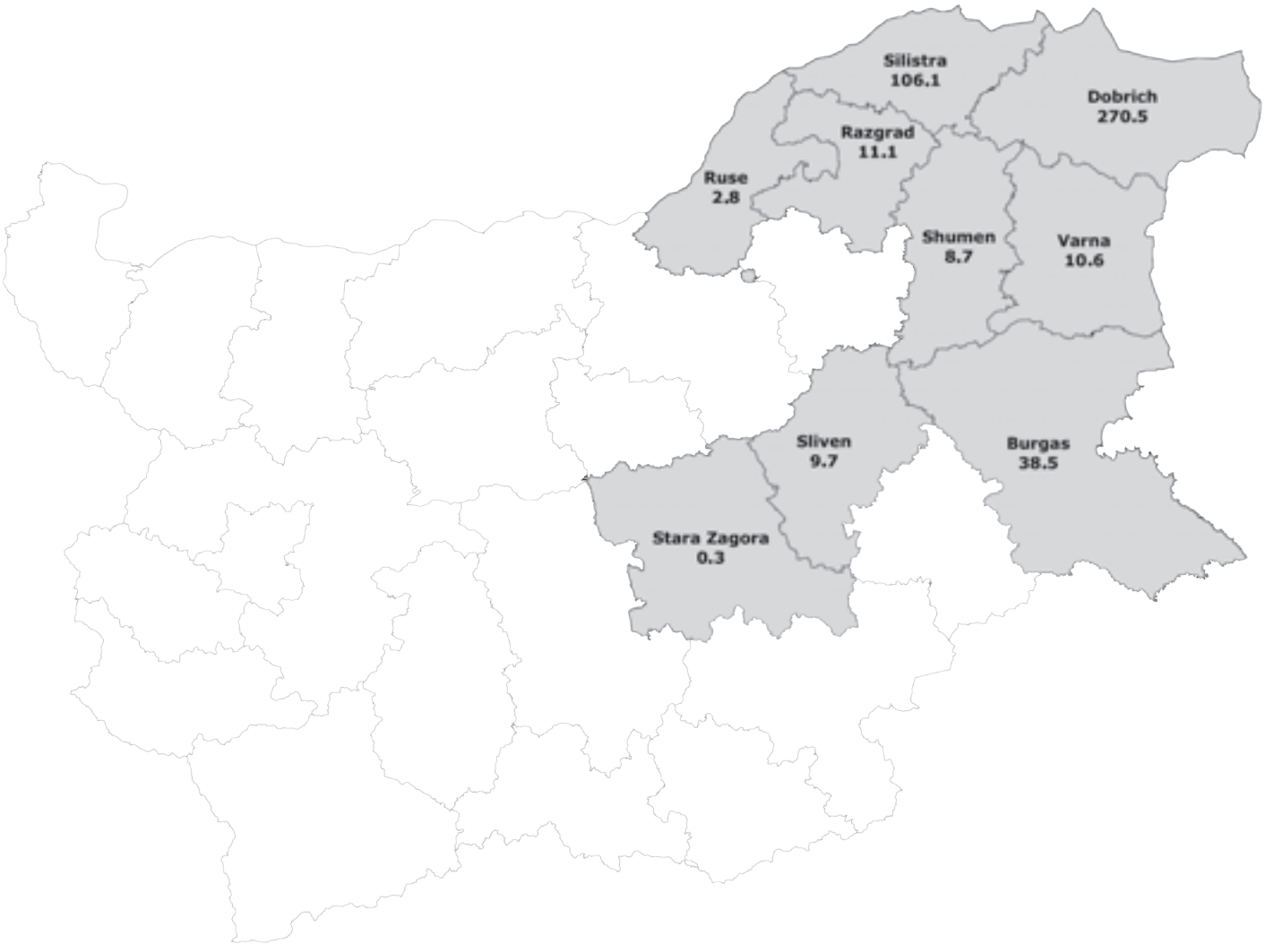
Our analysis on age, vaccination, hospitalisation and complications variables was based on the 748 case-based reports received by week 44 as data on the remaining 209 cases reported in weeks 45-49 are still being processed. The age was known for 730 cases (98%). The median age was 10 years (range: four days to 38 years). The cases were distributed between age-groups with 96 (13%) aged under one year, 149 (20%) aged 1-4 years, 123 (17%) aged 5-9 years, 131 (18%) aged 10-14 years, 137 (19%) aged 15-19 years, 73 (10%) aged 20-29 and 21 (3%) older than 30 years. The status of measles vaccination was known in 482 cases (64%). Overall, 142 were unvaccinated (29%), 248 (52%) had received one dose of measles-containing vaccine and 91 (19%) had received two doses (Figure 3). A total of 522 cases (69.7%) were hospitalised, and 303 cases (40.5%) were reported with measles-related complications including pneumonia (n=95; 31.3%) and abdominal symptoms and diarrhoea (n=35; 11.5%). No cases of acute encephalitis or measles-related deaths were reported.

Control measures

Several control measures continue to be implemented by local health authorities, according to the Bulgarian national programme for the elimination of measles and congenital rubella infection. Activities have been undertaken to increase awareness of the ongoing outbreak among the public in general and healthcare professionals in particular. General practitioners and other medical staff were requested to pay special attention to rash/fever symptoms and to strengthen routine immunisation of children aged 13 months (first dose) and 12 years (second dose) by directly reaching out to the parents and explaining the benefits of vaccination. In addition, a supplementary MMR vaccination campaign that had started earlier in the year in the affected administrative regions continues targeting all individuals from 13 months to 30 years of age who had not received the complete two-dose vaccination regimen. The MMR vaccine is supplied by the Ministry of Health and is offered free of charge through the routine immunisation services (family doctors). Special outreach teams consisting of regional epidemiologists, health inspectors and local Roma community leaders have been deployed in the campaign to immunise the Roma community.

Figure 2

Measles incidence per 100,000 population by region, Bulgaria, April-November 2009 (n=957)



Discussion

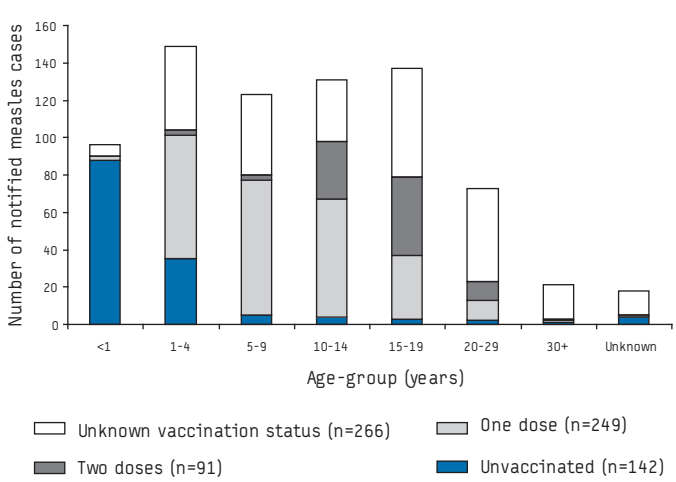
Despite the high national immunisation coverage with MMR vaccine, this outbreak highlights the presence of pockets of vulnerable individuals, particularly those members of the Roma community that are still susceptible to measles infection. They are only brought to light when the measles virus is imported from abroad. A similar experience was made in Croatia in 2008 [8]. It is generally believed that the vaccination coverage among members of the Roma community in Bulgaria does not differ from that of the rest of the population, since all citizens are well integrated into the primary healthcare system that provides easily accessible and free immunisation services. However, travelling members of the Roma community may be overlooked, if they delay or even fail to use the immunisation services. There is therefore a need for innovative ways to improve vaccination coverage in such groups that are hard to reach by standard immunisation programmes. In doing so, the herd immunity would be maintained at a high level conducive to measles elimination in Bulgaria.

The age distribution changed towards increasing numbers of older children, adolescents and young adults compared with what we noticed during first 10 weeks of the outbreak [1]. This provides more accurate insight into the susceptible age groups. Obtaining an accurate vaccination history presents challenges, but the large proportion (50%) of cases who reported having received one measles vaccine dose is indicative of vaccine failure and raises concerns about the maintenance of the cold-chain. However, a proportion of these cases may have received a vaccine dose offered as part of the outbreak control measures, when they were already infected with the measles virus and in the incubation period. Further data including the date of vaccination of such cases would need to be collected for more in-depth analysis of this hypothesis. The high hospitalisation rate noted is explained by the large number of patients from crowded households and poor living conditions of affected Roma families.

The current measles situation in Bulgaria underlines the need for more urgent preventive and control measures to be taken. To achieve the goal of measles elimination, awareness of the disease as well as a commitment by the public health authorities in Bulgaria are essential to strengthen vaccination programmes. The WHO's strategic plan for the elimination of measles from the European region stipulates that vaccination programmes should achieve and sustain a minimum of 95% coverage with two doses of vaccine and better target susceptible individuals in the general population and high-risk groups [9].

FIGURE 3

Notified measles cases by vaccination status, Bulgaria, April-October 2009 (n=748)



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Mumps outbreak in Jerusalem affecting mainly male adolescents

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From mid-September 2009 to 7 December 2009, 173 cases of mumps have been reported in the Jerusalem District. Most cases (82.1%) were male adolescents (median age 14.5 years) who are students in religious boarding schools. The majority of them (74%) are appropriately vaccinated for their age; 67% had received two doses of mumps-containing vaccine. An epidemiologic connection has been reported with visitors from New York, some of whom had recently had mumps.

Mumps is a notifiable disease in Israel by law. From mid-September 2009 to 7 December 2009, 173 cases of mumps have been reported to the Jerusalem District Health Office. The patients were mainly (147/173; 85%) students in yeshivas (religious academies operated as boarding schools) in several Jerusalem neighbourhoods and two neighbouring cities, and 142 of 173 (82%) were males. The epidemic curve is presented in Figure 1 and shows a pattern compatible with person-to-person transmission. The median age of the patients was 14.5 years and the mean was 14.8±7.3 years. Their age and sex distribution are presented in Figure 2. Altogether, 60 schools have been affected (see Table). The outbreak spread to other regions of the country, and up to 7 December over 250 cases have been reported with similar demographic and epidemiologic characteristics.

The clinical picture included unilateral and bilateral parotitis. One patient (a 19 year-old) was hospitalised in a urology department with orchitis and another three were admitted to ear, nose and throat departments. A further six patients were observed for varying periods in hospital emergency departments and discharged.

Case ascertainment included: positive mumps IgM antibody (in 20 patients) and positive real-time RT-PCR in urine (in four patients). The virus was classified by the central virology laboratory of the Israel ministry of health as genotype G5. The remaining 149 cases were diagnosed on the basis of clinical features together with an epidemiologic association.

Of the 173 patients, 116 (67%) had received two doses of measles-mumps-rubella (MMR) vaccine (Priorix GlaxoSmithKline Biologicals – Jeryl Lynn strain), 29 (16.8%) had received one dose (age-appropriate in 12 of them), 20 (11.6%) were not immunised, and in another eight patients (4.6%) the immunisation status was unknown (see Figure.3).

A number of patients reported contact with yeshiva students from the United States (New York¬) who visited Israel during the High Holidays in mid-September 2009 and some of whom were reported to have recently had mumps.

Outbreak control measures included investigations in the relevant schools to determine the students' vaccination status and referral for completion of MMR vaccination where necessary. Information on the outbreak was circulated to all health maintenance organisations in the District and to the public via the mass media.

Discussion

Mumps is an acute viral infection; a third of infections are subclinical, another 30-40% are expressed clinically as unilateral or bilateral parotitis. Complications occur more frequently in adults than in children; 10-15% of mumps patients develop meningo-encephalitis. Orchitis occurs in 20-50% of post-pubertal men, but sterility is rare. Other complications include pancreatitis, oophoritis, deafness, arthritis, thyroiditis, and myocarditis. Transmission is through droplet infection. Confirmation of mumps infection includes serological testing (for IgM antibodies by vari¬ous methods), identification of mumps RNA by RT-PCR and viral isolation in cell culture [1].

Mumps vaccination was included in the routine childhood immunisation schedule in Israel in 1984, and since 1994 has been administered in a two-dose schedule at ages 12 months and six years (first grade in school) in the form of the MMR vaccine, and since 2008 as measles-mumps-rubella-varicella (MMRV) vaccine. The average overall immunisation coverage for the first dose of mumps vaccine (MMR/MMRV) in the Jerusalem District has been maintained between 93 and 96.7% over the past decade [Jerusalem District Health Office, unpublished data]. It is to be noted that in 1992, the coverage for the first dose of MMR among the Jewish population of Jerusalem was a mere 82.3%.

Mumps control in Israel improved significantly during the 1990's [2], although periodic outbreaks still occurred due to under-vaccination, primary vaccine failure and waning immunity. In 1998 and 2005, two outbreaks (each of the order of 100 cases) occurred in Israel. In 2006, 12 cases were reported; six were reported in 2007 and 13 in 2008. Serological studies performed in the late 1990s revealed relatively low mumps antibody levels among adolescents and army recruits in Israel, ranging from 59 to 83.3% positivity; such levels do not guarantee adequate herd immunity [3,4].

Mumps outbreaks, mainly involving adolescents and young adults, have emerged recently in several countries. A nationwide

mumps outbreak occurred in the United Kingdom in 2004-2005, with 56,390 reported cases. The majority (79%) were aged 15-24 years; two thirds were unvaccinated. Non-availability of MMR vaccine probably contributed to susceptibility of the birth cohorts 1983-1986 [5].

Figure 1

Mumps outbreak in Jerusalem September-December 2009, epidemic curve (n=173)

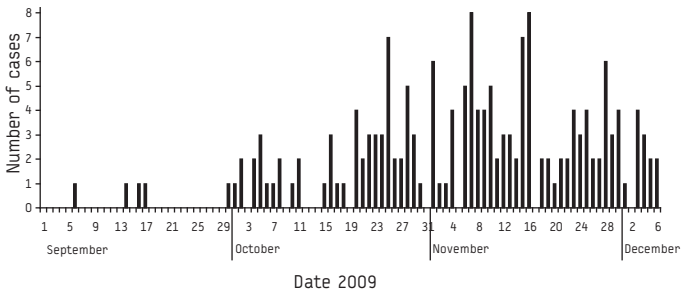
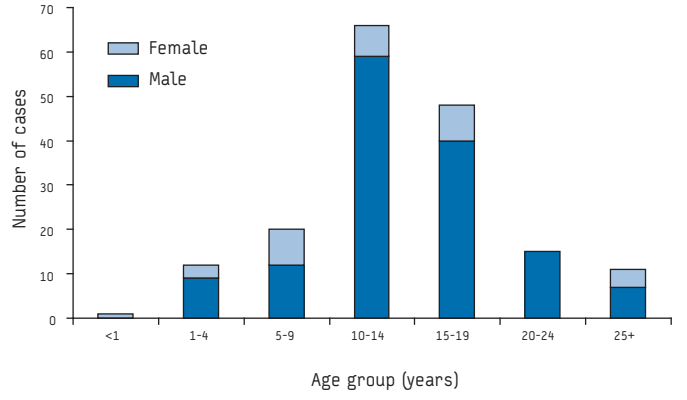


Figure 2

Mumps outbreak in Jerusalem September-December 2009, cases by age and sex (n=173)



Table

Mumps outbreak in Jerusalem, September-December 2009, distribution of cases in the affected schools (n=147)

Number of Mumps cases per school	Total number of cases	Number of schools
1	37	37
2	18	9
3	9	3
4	12	3
5	10	2
6	6	1
9	9	1
10	10	1
11	22	2
14	14	1
Total	**147**	**60**

In the United States, the largest outbreak in 20 years occurred in 2006-2007, encompassing more than 6,000 cases centred in college campuses. Of the students aged 18-24 years, 84% had been vaccinated with two doses of mumps vaccine [6]. The epidemic occurred despite high vaccination rates and low mumps activity in the community [7].

England and Wales are currently in the throes of an outbreak of mumps centred in college campuses, with 998 cases reported in January-February 2009, and further cases still being reported, mainly among college students. The circulating genotype is G5 [8].

Other European outbreaks have been reported in recent years. In an Austrian outbreak involving over 200 cases [9], 49% of the patients were unvaccinated – a very different situation from the outbreak we report. In the Republic of Moldova, an extremely large outbreak of nearly 20,000 cases was reported in 2007-2008 [10]. Most of the patients (96%) had received only one dose of MMR. A two-dose schedule was introduced in that country in 2002, for birth cohorts from 1995 onwards.

In an ongoing mumps outbreak in the United States (New York, New Jersey), and Canada (Quebec), 179 and 15 cases, respectively, were reported in August-October 2009. The affected individuals are mainly members of a Jewish religious community (83% males; median age 14 years). Of those for whom vaccination status is known 72% were vaccinated with two doses. The virus was of genotype G [11].

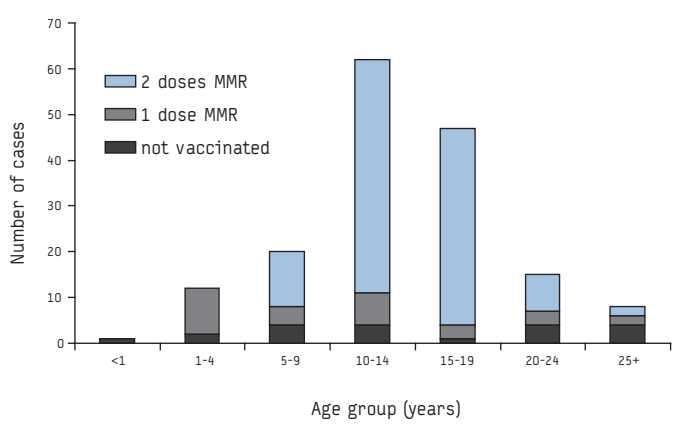
Conclusions

The two main characteristics of the current outbreak in Jerusalem are the predominance of male adolescents in religious boarding schools and the fact that most cases (74%) are appropriately vaccinated for their age. The male predominance is striking, and requires further study.

It had been observed that the mumps component of the MMR vaccine provides inferior protection compared to the measles and rubella components. Unlike the levels of 95% and 98% provided by the latter two, the mumps protection levels are approximately 62 85% and 85 88% for the first and second doses, respectively. Recently, the effectiveness in the United Kingdom was determined as 88% and 95%, respectively. However, the effectiveness of one

Figure 3

Mumps outbreak in Jerusalem September-December 2009, cases by age and vaccination status (n=165)



dose waned from 96% in two year-olds to 66% in 11-12 year-olds, and the effectiveness of two doses from 99% in 5-6 year-olds to 86% in 11-12 year-olds [12].

The reasons for the particular characteristics of these mumps outbreaks are unclear. Possible explanations include a combination of primary and secondary vaccine failure, waning immunity, inadequate vaccine effectiveness and previous low immunisation coverage. Contributory factors include living conditions in specific population groups such as college freshmen, army recruits and adolescent students in boarding schools.

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First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009

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We report the first worldwide case of Usutu virus (USUV) neuroinvasive infection in a patient with diffuse large B cell lymphoma who presented with fever and neurological symptoms and was diagnosed with meningoencephalitits. The cerebrospinal fluid was positive for USUV, and USUV was also demonstrated in serum and plasma samples by RT-PCR and sequencing. Partial sequences of the premembrane and NS5 regions of the viral genome were similar to the USUV Vienna and Budapest isolates.

Introduction

Usutu virus (USUV) is an arthropod-borne virus of the family *Flaviviridae*, genus *Flavivirus*. It is included in the Japanese encephalitis virus (JEV) group [1] being closely related to human pathogens such as JEV and West Nile virus (WNV). In the last decade, USUV was detected in a variety of central European birds with encephalitis, myocardial degeneration, and necrosis in liver and spleen [2-5]. As far as we know, the virus had never been associated with severe or fatal disease in humans [6]; it was isolated once in the Central African Republic in a man with fever and rash [7]. Here we report evidence of a neuroinvasive infection clinically related to USUV in Italy.

Case report

In May 2009, a woman in her 60s from Emilia Romagna region, Italy, underwent hemicolectomy because of a diffuse large B cell lymphoma. Six courses of chemotherapy were administered (including rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), with last administration on 21 August 2009. Some days later, there was a reactivation of genital herpes treated with valacyclovir. On 1 September, a fever of 39.5°C with resting tremor appeared and antibiotic (moxifloxacine and amoxicilline clavulanate) therapy started however the temperature persisted. On 5 September, the patient was admitted to hospital for hyperpyrexia resistant to antipyretic and intravenous antibiotic treatment (meropenem and teicoplanine). Once admitted, the patient received blood transfusion because of a critical anaemia.

Examination of blood, urine and stool cultures and virological assessment for herpes virus simplex (HSV1/2) and cytomegalovirus (CMV) antigen were negative. A total body computerised tomography was performed without evidence of lymphoma. Suspicion of meningoencephalitis was addressed by neurological examination which showed distal resting tremor, positivity to the Romberg test, dysmetry and weakness at four limbs without cranial nerve affection. Magnetic resonance imaging (MRI) of the brain showed a signal alteration of the substantia nigra of the parietal and frontal subcortical areas that did not change after injection of contrast medium. On 11 September, the cerebrospinal fuid (CSF) was therefore collected and examined. The CSF was limpid without any alteration detected in the clinical-chemical analysis, activated lymphocytes were evident in the sediment. As further analysis of the same CSF specimen revealed the presence of flaviviruses (see below), steroid treatment was started. This therapy resolved the fever but did not lead to any improvement of the neurological symptoms. The electroencephalogram still registered diffuse slow theta waves and slow spike prevalent in left frontal parietal areas. The neurological functions, mainly the resting tremor, improved following the administration of levodopa and carbidopa.

Virological analysis

When tested for the presence of viral agents, the CSF collected on 11 September was negative in molecular tests for CMV, HSV1/2, Epstein-Barr virus, adenoviruses, parvovirus B19, polyomavirus JC and BK, enteroviruses, mumps virus and WNV and positive to a heminested RT-PCR specific for the NS5 region of the *Flavivirus* genus [8]. The amplicon was directly sequenced and analysed by BLAST (http://www.ncbi.nlm.nih.gov/blast), revealing a 98% identity with both the USUV Budapest (gb|EF206350.1) and Vienna (gb|AY453411.1) isolate.

To confirm the identification of the species Usutu virus, we performed two USUV-specific RT-PCRs targeting the NS5 [2] and premembrane (preM) regions (primer sequences available on request) of the USUV genome on two plasma specimens collected on 8 and 11 September 2009 and one serum specimen collected on 14 September. The amplified products were sequenced (583 bp of NS5 and 602 bp of preM) and aligned with the corresponding sequences deposited in Genbank (gb|AY453411.1;

gb|EF206350.1) using ClustalW. The alignment of the preM gene shared 99% nucleotide identity with the USUV Budapest and Vienna sequences, whereas the NS5 gene sequences shared 100% nucleotide identity with USUV Vienna and 99% with USUV Budapest.

Further specimens of serum (26 May and 13 October) and plasma (19 October) before and after the acute phase of meningoencephalitis were analysed to demonstrate the absence of the virus. The two USUV-specific RT-PCRs performed on these three samples did not detect any USUV RNA. These samples were also analysed for WNV because a WNV outbreak was ongoing in the area at the time [9], and were negative.

Discussion

To our best knowledge this the first human disease with neurological involvement caused by USUV. The detection of USUV only in those samples collected during the acute phase of clinical manifestation is clear evidence that the virus caused the meningoencephalitis in the patient. Its capability of causing neurological lesions and death has already been reported in birds of central Europe [10]. The presence of USUV in Emilia Romagna has also been reported [4] and, in the past few months, the virus was isolated from black birds found dead in Northern Italy [G. Savini, personal communication 22 October 2009]. A surveillance programme in sentinel chicken flocks to monitor the possible appearance and/or circulation of WNV and other flaviviruses has been in place for several years. In the clinical case reported here, the immunosuppressed status of the patient due to both the underlying disease and the treatment, particularly with rituximab, may have played an important role in USUV infection and in its pathogenicity. It is known that rituximab can reactivate hepatitis B virus in patients with lethal fulminant hepatitis.

However, a possible unusual neuroinvasiveness and neurovirulence of this particular USUV strain cannot be excluded. The fact that neurological symptoms occurred prior to hospital admission excludes the transfusion as a possible source of infection. Conversely, since USUV as well as competent viral vectors are circulating in the patient's area of residence [4], it is likely that the infection was transmitted to the patient through mosquito bites.

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Usutu virus infection in a patient who underwent orthotropic liver transplantation, Italy, August-September 2009

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We report a case of Usutu virus (USUV)-related illness in a patient that underwent an orthotropic liver transplant (OLT). Post transplant, the patient developed clinical signs of a possible neuroinvasive disease with a significant loss of cerebral functions. USUV was isolated in Vero E6 cells from a plasma sample obtained immediately before the surgery, and USUV RNA was demonstrated by RT-PCR and sequencing. This report enlarges the panel of emerging mosquito-borne flavivirus-related disease in humans.

Introduction

In recent years, several mosquito-borne flaviviruses were identified as new emerging pathogens in animals and humans worldwide. The widespread occurrence of flaviviruses, such as West Nile virus (WNV), Dengue virus (DENV), Japanese encephalitis virus (JEV), yellow fever virus (YFV) and tick-borne encephalitis virus (TBEV) represents an important global health problem [1]. In the past ten years, infections with Usutu virus (USUV), a mosquito-borne flavivirus of the JEV serogroup and related to WNV, has been detected in a variety of birds in central European areas such as Austria, Hungary and Italy [2,3,4]. To date, USUV did not show considerable pathogenicity for humans [5]. In particular, no clinically evident USUV-related infections have so far been documented in humans.

Here we report a case of USUV-related disease in a female patient who, during a viraemic episode caused by USUV, received an orthotropic liver transplant (OLT) as a final consequence of a thrombotic thrombocytopenic purpura (TTP). This patient developed a neurological disease with severe impairment of the cerebral functions within the first days after OLT.

Case report

On 10 August 2009, a few days after returning to Italy from a holiday in Egypt, a woman in her 40s developed a TTP and received 18 plasma exchanges until 4 September 2009. Two weeks later, on 14 September, the patient presented with fever of 39.5°C, headache, skin rash, mild increment of cytolitic liver enzyme, without signs of TTP relapse, and was treated with antibiotics (moxifloxacin and amoxicillin clavulanate) without any response. On 18 September, the patient was admitted to hospital for persisting fever and headache. Any sign of TTP was excluded by total body computed tomography (CT) scan, and a peripheral blood smear did not show schistocytes or other fragmented red blood cells. Within a few days, a fulminant hepatitis and impairment of neurological functions were observed and rapidly developed into a coma. The molecular and serological laboratory diagnosis for the most common viruses associated with hepatitis (hepatitis A, B and C virus, cytomegalovirus and Epstein-Barr virus) gave negative results.

Two weeks after the OLT the patient slowly regained a low level of consciousness as well as some motor function of cranial nerves and limbs, and an intensive rehabilitative programme was started.

Virological analysis

Since 3 September 2009, systematic screening has been performed on blood, tissue, stem cell and organ donations from idividuals living in the Emilia Romagna region in Italy, where WNV transmission was observed in summer 2009 [6]. This screening activity was undertaken following the data about WNV circulation in wildlife, horses and mosquitoes obtained from the regional integrated surveillance system that was in place from 15 June to 31 October. Screening for WNV was done using a nucleic acid amplification test (NAAT-Transcription-Mediated Amplification (TMA): PROCLEIX WNV, Novartis Diagnostics).

On 24 September, a plasma specimen obtained from the above patient immediately before surgery, was positive in the WNV NAAT assay. The test was repeated twice and the results were confirmed. A second sample was obtained from the patient one day after the OLT and the WNV NAAT was again positive.The level of positivity obtained with the two specimens was quite low, suggesting either an extremely low concentration of WNV RNA in the blood or a false

positive reaction. Additional blood samples obtained during the following 15 days gave negative results.

The liver's donor was also investigated. The donor had been living in the area of Parma and her plasma, obtained before liver donation, was NAAT-negative for WNV.

The NAAT result was further investigated by real-ime RT-PCR targeting the WNV envelope (env) gene [7]. Surprisingly, the result was negative. Consequently we extended the investigation to additional members of the Flaviviridae family, including at first TBEV, because this agent was already reported in Italy and because the illness caused by this virus can involve the central nervous system with a possible association with liver injury [8]. The plasma specimens were analysed by real-time RT-PCR specific for the 3' non-coding region of the TBEV genome [9], and resulted negative.

A further step in the aetiological investigation was the use of a heminested RT-PCR with primer pairs which amplify the NS5 region of the Flavivirus genus. This method was developed for the detection by PCR of the principal pathogenic flaviviruses (including DENV, JEV, USUV, WNV YFV, and Zika virus) and subsequent identification by sequencing [3]. We performed the heminested RT-PCR as reported by Scaramozzino *et al*. [10] with minor modifications (details available on request) and obtained a single amplicon of the expected size (220 bp). Both strands of the amplicon were sequenced using the PCR primers and analysed by BLAST (http://www.ncbi.nlm.nih.gov/blast). This analysis revealed 98% sequence identity (over 203 nt) to the USUV genome sequences available in GenBank (please give the accession numbers), and no higher homology with any other published DNA sequence. Low homologies were observed to the WNV genome sequence (80 % identity) and to the JEV genome sequence (79% identity); this partial homology is very likely due to the fact that these flaviviruses are closely related.

In conclusion, the sequencing results demonstrated the presence of USUV in the clinical samples of our patient. Additional confirmation of USUV viraemia was obtained by a PCR assay specific for USUV, performed as reported by Weissenbock *et al*. [11]. USUV was subsequently isolated in Vero E6 cells, and the identity of this isolate was confirmed by the heminested RT-PCR test reported above. As expected, the sequence obtained from the cultured virus isolate was identical to the one obtained from the amplified plasma sample. Complete sequencing of this human pathogenic USUV isolate is in progress.

Discussion

The results presented in this report, demonstrate USUV viraemia in an immunocompromised OLT recipient suffering from severe neurological impairment caused by an encephalitis. It is noteworthy that the NAAT test PROCLEIX WNV was capable of detecting a WNV-related virus, which indicates a potential problem with the specificity of this method.

The clinical findings observed closely resemble those reported in an animal model of USUV-related neurological disease [10]. To our knowledge, this report is the second description of the involvement of USUV in a human disease. Before, USUV-related infections had been reported as a cause of disease in animals, mainly birds, with no demonstrated pathogenicity for humans. Recently, it has been observed that USUV is circulating in owls and blackbirds in the North Eastern part of Italy, suggesting the possibility of USUV transmissions to humans in that area [12].

We are currently involved in an extensive serological investigation for USUV antibodies in the blood donors that were used for the plasma exchanges for our patient in order to define whether this therapy could have been the source of the infection or whether it was acquired naturally through a mosquito bite. In addition, a study is in progress to identify the presence of USUV in additional plasma and tissue specimens obtained from the same patient in order to quantify the viral load and the persistence of the USUV viraemic stage and to assess the possible involvement of USUV in the original liver disease. This case of USUV-related illness in humans has added this virus to the list of those that can be transmitted to humans by local mosquitoes and can cause severe diseases in immunocompromised individuals.

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