Eurosurveillance

Volume 14, Issue 23 - 11 June 2009

Rapid communications

Research articles

News

ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

Preliminary analysis of influenza A(H1N1)v individual and aggregated case reports from EU and EFTA countries

ECDC working group on influenza A(H1N1)v (PHE.H1N1v@ecdc.europa.eu)[1,2]
1. European Centre for Disease Prevention and Control, Stockholm, Sweden
2. The members of this group are listed at the end of this article

Since the first importation of influenza A(H1N1)v virus to Europe in late April of this year, surveillance data have been collected in the Member States of the European Union and European Free Trade Association. This is the first preliminary analysis of aggregated and individual data available as of 8 June 2009 at European level.

Introduction

On 21 April 2009, the United States Centers for Disease Control and Prevention (US CDC) reported two cases of influenza due to a new virus strain of mixed swine, avian and human origin, the so-called new influenza A(H1N1) virus (hereafter named A(H1N1)v virus) [1]. On 25 April, the European Centre for Disease Prevention and Control (ECDC) published a risk assessment, started developing tools to monitor the situation and support the countries of the European Union (EU) and European Free Trade Association (EFTA), and initiated its first situation report distributed daily to more than 700 stakeholders since then. After the World Health Organisation (WHO) raised its pandemic alert level to phase 4 on 27 April and up-scaled again to phase 5 on 29 April, ECDC was monitoring the situation around the clock and provided epidemiological updates on global case numbers three times a day. Subsequently, the European Commission published a case definition for surveillance of the new disease [2], ECDC published information for travellers, updated its risk assessment on 8 May, published several documents on case and contact management, and coordinated the surveillance of influenza A(H1N1)v at EU level.

The objective of this paper is to present the epidemiological situation in the 27 EU and the three countries in the European Economic Area (EEA) and EFTA, Iceland, Liechtenstein and Norway, hereafter called the EU+3 countries, on the basis of the surveillance data provided by the EU+3 countries through individual and aggregated case reports.

Methods

Data used in this analysis of the epidemiological situation in the EU+3 countries, as of Monday 8 June 2009, 08:00 CEST, include individual case reports posted by countries in the Early Warning and Response System (EWRS) and aggregated case reports provided daily through the EWRS or through other official communication channels.

Confirmed cases are defined as persons in whom the infection has been confirmed by RT-PCR, or by viral culture or by a four-fold rise in influenza A(H1N1)v-specific neutralising antibodies. The latter implies, according to the EU case definition, the need for paired sera from the acute phase of illness and from the convalescent stage 10-14 days later [2].

While countries with fewer cases are uploading data on their cases directly into the surveillance database at ECDC, Spain and the United Kingdom (UK), who both have high number of cases, and Belgium are providing extracts from their own national databases, which are then entered into the ECDC database. Re-coding of some of the variables was necessary for Spain and the UK, and data were subsequently validated by the countries. The data from Belgium were imported manually after re-coding the variables.

Cases which are not explicitly reported as having been exposed during travel in an affected country (imported cases) are considered to have been infected in their own country.

Results

As of 8 June, 1,128 laboratory-confirmed cases of influenza A(H1N1)v have been reported from 25 of the EU+3 countries through aggregated case reports. Spain (26%) and the UK (49%) together account for 75% of confirmed cases. Of those 1,128 cases, 498 (44%) were also reported through individual case reports (Table 1). Latvia, Liechtenstein, Lithuania , Malta and Slovenia have not reported confirmed cases so far.

Epidemic curves

The first confirmed case in EU+3 countries was a traveller returning from Mexico to the UK. He was identified on 27 April 2009 and reported onset of symptoms on 16 April. Figure 1 compares the distribution of cases by date of onset from the individual case reports (n=498) with the distribution of cases by reporting date from the aggregated case reports (n=1,024). It shows a delay of one week between date of onset and date of reporting in the first weeks of the outbreak, up to 20 May, followed by an increasing discrepancy in the number of cases reported by the two systems.

Figure 2 shows the distribution of imported and domestic cases in EU+3 countries by date of onset. The first case reported as in-

Table 1

Distribution of confirmed cases of influenza A(H1N1)v reported until 8 June 2009 by source of information, EU+3 countries (n=1,128)

Member State	Aggregated case reports	Individual case reports	Percentage
Austria	6	6	100
Belgium	14	14	100
Bulgaria	2	0	0
Cyprus	1	1	100
Czech Republic	2	2	100
Denmark	5	4	80
Estonia	3	3	100
Finland	4	4	100
France	57	18	32
Germany	63	63	100
Greece	5	0	0
Hungary	3	3	100
Iceland	1	0	0
Ireland	11	11	100
Italy	50	39	78
Luxembourg	1	1	100
Netherlands	10	6	60
Norway	9	9	100
Poland	5	5	100
Portugal	2	2	100
Romania	9	9	100
Slovakia	3	3	100
Spain	291	113	39
Sweden	14	13	93
United Kingdom	557	169	30
Total	1128	498	44

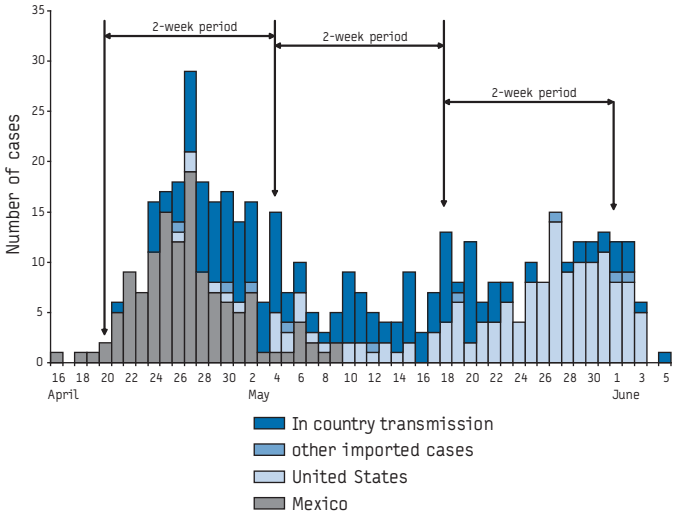
country transmission had onset of symptoms five days after the first imported case. During the first two-week period, 65% of cases were reported to have been imported, compared to 40% during the second and 73% during the third two-week period. The majority of imported cases in the first two-week period were imported from Mexico and in the third two-week period from the United States (US).

Demographic characteristics of cases

The male to female ratio was 1.1. The median age was 23 years (range: eight months to 73 years). Seven cases were younger than

Figure 2

Distribution of confirmed cases of influenza A(H1N1)v infections by date of onset and type of transmission, as of 31 May 2009*, EU+3 countries (n=457)



* Individual case reports from Spain were last updated on 14 May, from the UK and France on 29 May, from Italy on 4 June and from Germany on 6 June

Figure 1

Distribution of confirmed cases of A(H1N1)v infections by date of onset (n=498) and date of reporting(n=1,024), as of 5 June 2009, EU+3 countries

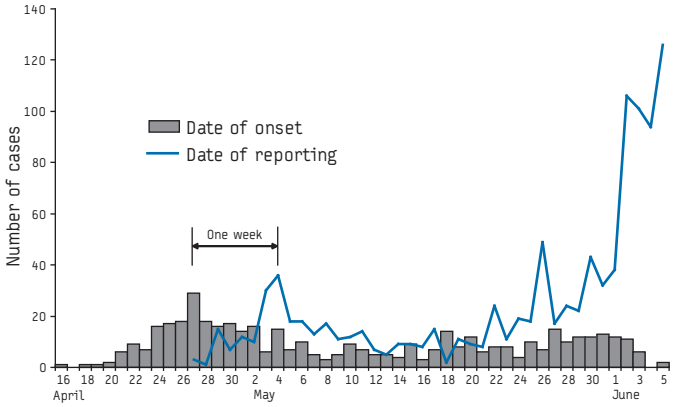
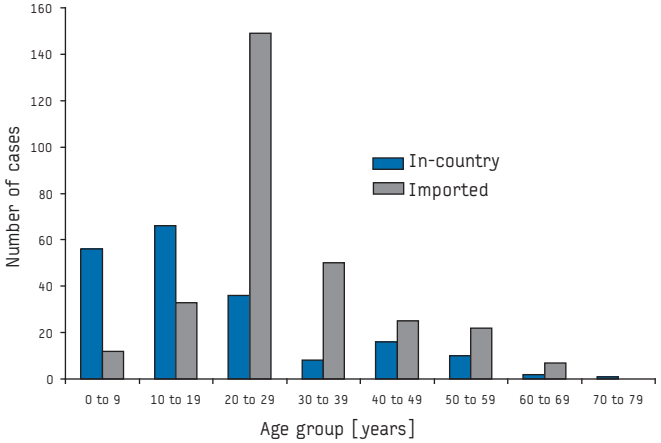


Figure 3

Distribution of cases of influenza A(H1N1)v infection by age group and type of transmission, as of 8 June 2009, EU+3 countries (n=493)



two years. Of 494 cases with known age, 168 (34%) were undee the age of 20 years. The most affected age group was the group of 20-29 year-olds and accounted for 37% of cases.

The proportion of imported cases older than 20 years (78%) was significantly higher than the proportion of over 20 year-old cases who were infected in their own country (27%, p<0.0001). The median age of imported cases was 25 years compared to 13 years for non-imported cases (Figure 3).

Symptoms

In the analysis of symptoms, the data from Spain and Belgium were excluded due to recoding issues, leaving 371 cases for analysis. Asymptomatic cases constituted 8% of reported cases (28/371), and were more common among cases under the age of 20 years (11%) when compared with older cases (5%, p=0.02).

The most commonly reported symptoms were respiratory symptoms (79%), followed by fever or history of fever (78%). Gastro-intestinal symptoms were reported from 86 cases (23%). Presence of gastro-intestinal symptoms was not significantly associated with travel exposure but was significantly more common among cases under the age of 20 years (32%) than among older cases (18%, p=0.001). Table 2 shows the distribution of symptoms by category of symptom.

Pre-existing conditions

Underlying disease was reported for 24 cases: lung disease for 12, heart disease for four, renal disease from three, human immunodeficiency virus (HIV) infection from three, and seizures from two cases (one of these two also had a not further specified cancers). One 14 months-old child was reported with combined heart, lung and renal disease. None of the cases was reported to be pregnant. Several cases with other underlying conditions such as hypertension, iodine sensitivity, allergic rhinitis or facial paralysis were reported, which are not considered classical risk groups for seasonal influenza [3].

Table 2

Distribution of symptoms among cases of influenza A(H1N1)v infection, as of 8 June 2009, EU+3 countries (n=371)

	Number	Percentage
At least one symptom	344	93
GENERAL	317	85
Fever or history of fever	290	78
Headache	160	43
Muscle pain	145	39
Joint pain	79	21
RESPIRATORY	295	80
Dry cough	188	51
Productive cough	60	16
Sore throat	172	46
Runny nose	120	32
Sneezing	72	19
Shortness of breath	34	9
GASTRO INTESTINAL	34	24
Diarrhoea	45	12
Vomiting	49	13
Nausea	57	15
OTHERS	146	39
Conjunctivitis	21	6
Nose bleeding	9	2
Altered consciousness	2	1
others (various)	117	32

Treatment and prophylaxis

Of 292 cases for whom information is available, 258 (88%) received antiviral treatment. Oseltamivir was the most commonly used drug (255), zanamivir was reported to have been used for treatment of three cases. Post-exposure prophylaxis was reported to have been administered to 13 (7%) of 198 cases for whom information was available. Twelve received oseltamivir and one received zanamivir as prophylaxis. Six of the cases who received prophylaxis were imported cases.

Complications

Seven (2%) of the 286 cases for whom information is available were classified as having complications. Four patients were reported with pneumonia, one with otitis, one with elevated liver enzymes and one with the need for steroid treatment. Fifty-three cases reported shortness of breath, one of whom had underlying heart disease.

Previous influenza vaccination

Twenty (8%) of the 260 cases for whom information is available were reported to have received seasonal influenza vaccination in the past season. Vaccinated persons were aged between 8 months and 76 years. Eighty percent of vaccinated persons were returning travellers. Two were reported to have asthma, one with underlying heart disease, one with chronic disease not further specified and one with myalgic encephalopathy.

Hospitalisation

Among 291 cases, 36% (105) were reported to have been hospitalised. The rate of hospitalisation varies by country. In several countries, e.g. France, Austria, Belgium and Romania, cases were hospitalised for isolation purposes.

Discussion

On the basis of the aggregated case reporting, two EU Member States account for 75% of the cases reported in the EU+3 countries. It is unlikely that a difference in the sensitivity of surveillance systems alone could explain such a difference. The one-week delay between date of onset (individual case reports) and reporting date (aggregated case-reports) observed in the first weeks of the epidemic probably reflects the delay in seeking medical care after onset and getting laboratory confirmation (see Figure 1). The discrepancy observed since the third week of May in the numbers reported through aggregated case reports versus individual case reports highlights the increasing difficulties of the Member States in investigating and reporting individual cases as the number of case increases.

This preliminary analysis does not allow an accurate description of the level of in-country transmission, as the data are still incomplete. However, a recent Eurosurveillance article suggests that in the UK, most of the recent cases are due to in-country transmission, although sustained community transmission still has to be confirmed [4].

The age distribution of cases is significantly different among imported and domestic cases. Imported cases tend to be young adults, exposed while travelling abroad, and their demographic characteristics are more representative of travellers than of the population susceptible to A(H1N1)v infection. Domestic cases tend to be younger (median age 13 years) and reflect school children and teenagers among whom transmission is amplified. Therefore, the demographic characteristics of cases documented in the EU so far do not reflect the overall population at risk of infection, but rather the population contributing to seeding events (travellers) and amplification of transmission (school children and teenagers) in the early stage of the spread of a new influenza virus strain.

The relatively high proportion of asymptomatic cases, especially among under 20 year-olds, is probably due to intensive contact tracing during school outbreaks. The difference in the number of cases with gastro-intestinal symptoms observed in under 20 year-olds compared to older cases has been previously described for seasonal influenza and is not significantly associated with an exposure abroad [3]. The hospitalisation rate cannot be considered as a factor of severity because many of the cases were reported to be admitted to hospital for isolation. There was great variation among countries in this respect.

Information on the interval between exposure and the start of prophylaxis is not available and therefore no conclusions can be drawn regarding the effectiveness of antiviral prophylaxis.

Individual case reports for less than half of the cases (498/1,128) were available for this analysis, which may bias the results. The bias will particularly affect conclusions drawn on cases from the last three weeks of the dataset, for which information from the most affected Member States were not available. Bias may have been introduced in the age distributions and the frequencies of symptoms and underlying conditions, since the missing data particularly concern in-country transmission. Therefore, the comparisons between cases affected in their won country and travel-associated cases should still be considered preliminary and a change in disease patterns during the period for which data are missing cannot be ruled out. Due to delay in reporting from the Member States to ECDC, the Europe-wide picture presented here may not fully represent the reality of what was known at country level on 8 June.

With the currently available information, conclusions about the severity of the infection are limited. In addition, if cases deteriorate while they are ill, this information would probably not be reported to the ECDC.

Conclusions

The preliminary analysis of the initial few hundred cases reported at European level shows that the epidemiological pattern in the EU+3countries does not differ from what was documented in the Americas. Currently, the disease seems to be relatively mild and comparable with seasonal influenza. However, it is still too early to define, on the basis of this analysis, the age groups most at risk of infection.

These data are important to guide appropriate policy decisions. In 2008, a working group on surveillance in a pandemic, including ECDC, WHO and experts from the Member States, identified nine strategic parameters which would need to be assessed early in an influenza pandemic [5]. Out of these, six parameters (including disease severity, incidence by age-group and known risk-factors, confirmation/modification of case definition and modes of transmission) can only be properly evaluated using individual case reports.

As the number of cases grows, it will become increasingly difficult for the Member States to investigate and report individual cases. The surveillance currently in place may soon reach its limits. It may well be that targeted outbreak studies will provide better information on risk factors for more severe disease. A switch to sentinel surveillance and/or surveillance of severe cases, as implemented by countries outside the EU, has to be considered. However, the case-based reporting should be continued at least until countries experience community spread or large-scale epidemics. ECDC is currently working with the Member States to automate the upload of data in their own national formats.

In the meantime, aggregated case reporting complementing individual case reports has proven very useful in describing recent trends and anticipating future developments. As recent trends suggest that Europe may be entering the acceleration phase [6], it is important to continue collecting aggregated case reports.

Acknowledgements

These data were provided by the national focal points for the Early Warning and Response System and the contact points for influenza surveillance of the EU and EFTA countries. ECDC wishes to acknowledge the serious commitment and effort of all these individuals and their teams in ensuring the timely reporting of case-based data from their respective countries. The full list of names is indicated below.

The final preparation of the report was made by ECDC working group on influenza A(H1N1)v, see below.

List of collaborators from Member States

Gabriela El Belazi, Hubert Hrabcik, Peter Lachner, Reinhild Strauss, Robert Muchl, Theresia Popow – Kraupp, Françoise Wuillaume, Leen Meulenbergs, Sophie Quoilin, Mira Kojouharova, Rositsa Kotseva, Teodora Georgieva, Avraam Elia, Chryso Gregoriadou, Chrystalla Hadjianastassiou, Despo Pieridou Bagatzouni, Olga Kalakouta, Jan Kyncl, Jitka Castkova, Martina Havlickova, Andreas Gilsdorf, Brunhilde Schweiger, Gabriele Poggensee, Gerard Krause, Silke Buda, Tim Eckmanns, Anne Mazick, Annette Hartvig Christiansen, Kåre Mølbak, Lars Nielsen, Steffen Glismann, Inna Sarv, Irina Dontsenko, Jelena Hololejenko, Natalia Kerbo, Olga Sadikova, Tiiu Aro, Amparo Larrauri, Gloria Hernandez–Pezzi, Pilar Perez–Brena, Rosa Cano–Portero, Markku Kuusi, Petri Ruutu, Thedi Ziegler, Sophie Vaux, Isabelle Bonmarin, Daniel Lévy-Bruhl, Bruno Lina, Martine Valette, Sylvie Van Der Werf, Vincent Enouf, Ian Fisher, John Watson, Joy Kean, Maria Zambon, Mike Catchpole, Peter Coyle, William F Carman, Stefanos Bonovas, Takis Panagiotopoulos, Sotirios Tsiodras Ágnes Csohán, Istvan Jankovics, Katalin Kaszas, Márta Melles, Monika Rozsa, Zsuzsanna Molnár, Darina O'Flanagan, Derval Igoe, Joan O'Donnell, John Brazil, Margaret Fitzgerald, Peter Hanrahan, Sarah Jackson, Suzie Coughlan, Arthur Löve, Gudrun Sigmundsdottir, Isabella Donatelli, Maria Grazia Pompa, Stefania D'Amato, Stefania Iannazzo, Sabine Erne, Algirdas Griskevicius, Nerija Kupreviciene, Rasa Liausediene, Danielle Hansen – Koenig, Joel Mossong, Mathias Opp, Patrick Hau, Pierre Weicherding, Antra Bormane, Irina Lucenko, Natalija Zamjatina, Raina Nikiforova, Charmaine Gauci, Christopher Barbara, Gianfranco Spiteri, Tanya Melillo, Adam Meijer, Frederika Dijkstra, Ge Donker, Guus Rimmelzwaan, Paul Bijkerk, Simone Van Der Plas,Wim Van Der Hoek, Katerine Borgen, Susanne Dudman, Siri Helene Hauge, Olav Hungnes, Anette Kilander, Preben Aavitsland, Andrzej Zielinski, Lidia Brydak, Magdalena Romanowska, Malgorzata Sadkowska

– Todys, Maria Sulik, Carlos Manuel Orta Gomes, Jose Marinho Falcao, Raquel Guiomar, Teresa Maria Alves Fernandes, Adriana Pistol, Emilia Lupulescu, Florin Popovici, Viorel Alexandrescu, Annika Linde, Asa Wiman, Helena Dahl, Malin Arneborn, Mia Brytting, Eva Grilc, Irena Klavs, Katarina Prosenc, Maja Socan, Hana Blaskovicova, Margareta Slacikova, Mária Avdicová, Martina Molcanová, Šárka Kovácsová, the HPSC Influenza A(H1N1)V Epidemiology team and the CIDR team and Surveillance Group for New Influenza A(H1N1) Virus Investigation and Control in Spain.

The ECDC working group on influenza
A Ammon, B Ciancio, D Coulombier, I Devaux, P Kreidl, F Plata, M Salminen, P Zucs

References

1. Centers for Disease Control and Prevention (CDC). Swine influenza A (H1N1) infection in two children – Southern California, March-April 2009. MMWR Morb Mortal Wkly Rep. 2009;58(15):400-2.
2. Commission Decision of 30 April 2009 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision n° 21/19/98/EC. 2009/363/EC. Official Journal L 110/58. 01.05.2009. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:110:0058:0059:EN:PDF
3. Chin J. Control of Communicable Diseases Manual. 17th ed. Washington, DC: American Public Health Association; 2000.
4. Health Protection Agency, Health Protection Scotland, National Public Health Service for Wales, HPA Northern Ireland Swine influenza investigation teams. Epidemiology of new influenza A (H1N1) virus infection, United Kingdom, April – June 2009. Euro Surveill. 2009;14(22):pii=19232. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19232
5. World Health Organization (WHO). Global Surveillance during an Influenza Pandemic. Version 1. Updated draft April 2009. Available from: http://www.who.int/csr/disease/swineflu/global_pandemic_influenza_surveilance_apr09.pdf
6. European Centre for Disease Prevention and Control. Likely Evolution of the Pandemic of the new Influneza Virus A(H1N1) 2 June 2009. Available from: http://ecdc.europa.eu/en/Health_Topics/Pandemic_Influenza/Likely_evolution_of_epidemics_pandemic_new_A-H1N1_influenza_090602.ppt#294,11,Age-specific clinical attack rate in previous pandemics

This article was published on 11 June 2009.

Citation style for this article: ECDC working group on influenza A(H1N1)v. Preliminary analysis of influenza A(H1N1)v individual and aggregated case reports from EU and EFTA countries. Euro Surveill. 2009;14(23):pii=19238. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19238

Enhanced influenza surveillance on Réunion Island (southern hemisphere) in the context of the emergence of influenza A(H1N1)v

E D'Ortenzio (Eric.Dortenzio@sante.gouv.fr)[1], C Do[1], P Renault[1], F Weber[2], L Filleul[1]
1. Cellule interrégionale d'épidémiologie (Cire) Réunion-Mayotte, Institut de Veille Sanitaire, Réunion Island, France
2. Institut National de Veille Sanitaire (Institute for Public Health Surveillance, InVS), Saint-Maurice, France

With the winter season on the southern hemisphere that starts in Réunion Island in June seasonal influenza activity usually increases shortly afterwards. The new influenza A(H1N1)v virus is rapidly spreading worldwide and may reach the island during the coming winter season. We have therefore enhanced influenza surveillance to detect the introduction of influenza A(H1N1)v, monitor its spread and impact on public health and characterise potential viral changes, particularly if seasonal influenza A(H1N1), resistant to oseltamivir, co-circulates with A(H1N1)v.

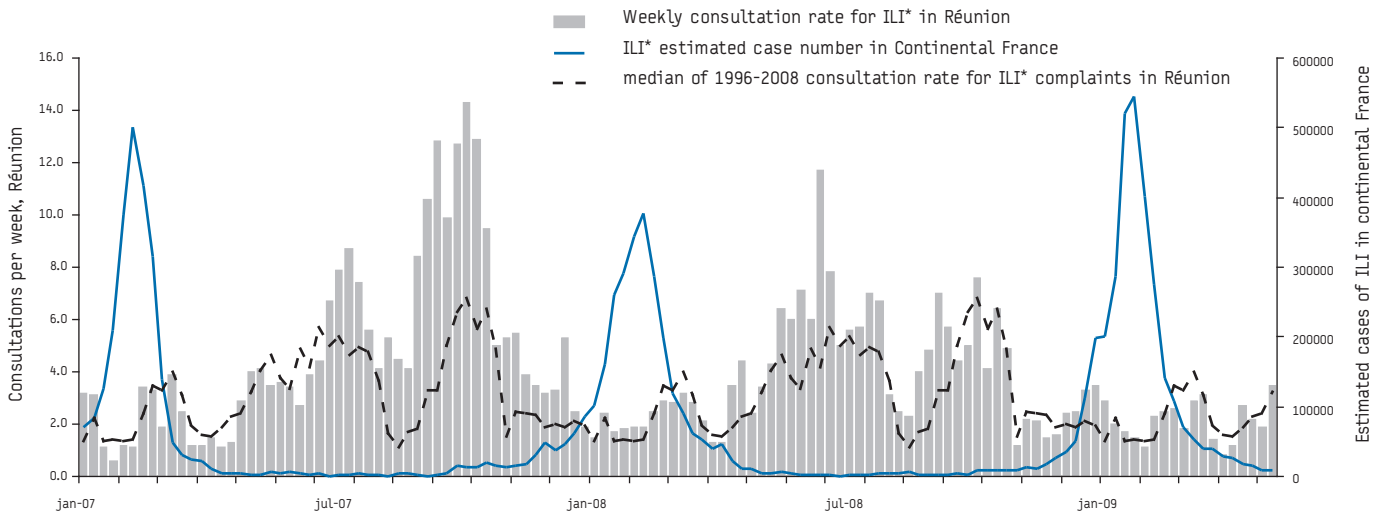
Background

Influenza virus type A is associated with annual epidemics and occasional large-scale global pandemics. Both are characterised by increased morbidity and mortality [1]. In temperate regions, a clear seasonality exists in the influenza activity with a marked peak in cold winter months. In tropical regions however, where there is less fluctuation in seasonal temperature this is not noticeable to the same extent [2].

Réunion Island, a French overseas administrated territory with 800,000 inhabitants, is located in the southern hemisphere in the south-western Indian Ocean, 700 km east of Madagascar and 200 km south-west of Mauritius, at a longitude of 55°3 east and latitude of 21°5 south, above the Tropic of Capricorn. In Réunion Island, influenza activity has been monitored since 1996 [3], but influenza virus circulation remains poorly documented. Results of past monitoring suggest that annual influenza activity increases in June-July [4] and the last reported seasonal influenza epidemic occurred in August-October 2007 [5]. The island is presumed to have a double exposure to seasonal influenza, one from the southern hemisphere and the other one from the intense link with metropolitan France [4,6] (Figure 1).

Figure 1

Seasonal influenza activity on Réunion Island and in continental France, 2007-2009

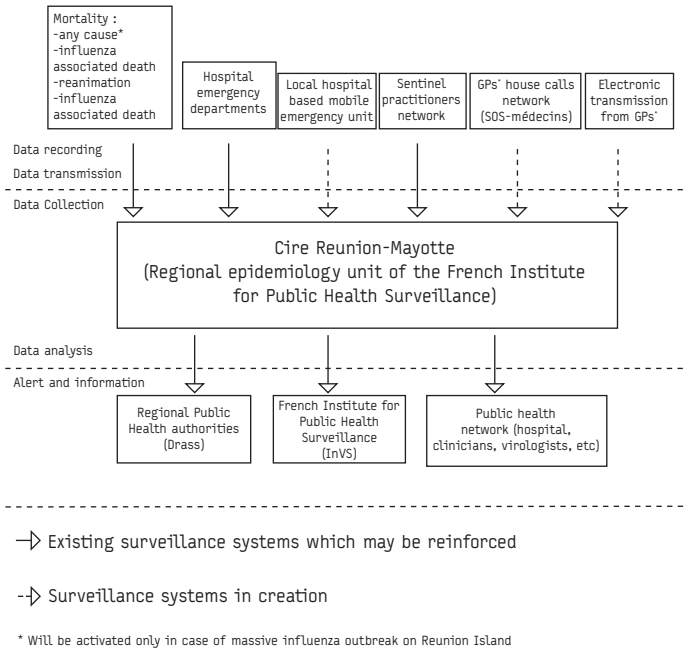


*Influenza like illnesses
Source for continental France data: Réseau Sentinelle, France ; Source for Réunion Island data: Observatoire Régional de la Santé and réseau sentinelle, Réunion

In April 2009, a new strain of human influenza A(H1N1) virus, the influenza A(H1N1)v virus, was identified in USA and Mexico [7]. As of 10 June 2009, a total of 74 countries reported 27,737 cases and 141 associated deaths to the World Health Organization (WHO) demonstrating the pandemic potential of the virus [8]. Anticipating the start of the influenza season in Réunion Island sometime in June (Figure 1), the Regional epidemiology unit of Réunion-Mayotte (Cellule interrégionale d'épidémiologie, Cire) of the French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS) is implementing an enhanced surveillance system to face the likely introduction and spread of influenza A(H1N1)v during the coming winter months in Réunion. The aim of this system is to detect the introduction of influenza A(H1N1)v timely on the island, monitor its spread and impact on public health and characterise potential viral changes, particularly if seasonal A(H1N1) resistant to oseltamivir co-circulates with A(H1N1)v. Furthermore, the surveillance we describe here is an attempt to include the specific surveillance of influenza A(H1N1)v virus into the global influenza surveillance system. It could be an example for other countries in the tropics and results will provide useful data about the effectiveness and limits of such system. Our experience might guide northern hemisphere countries in how to adapt their surveillance system before the upcoming influenza season in the winter.

Figure 2

Organisation of the influenza surveillance on Réunion Island, 2009



Organisation of the influenza surveillance on Réunion Island, 2009

Figure 2 shows the organisation of the enhanced surveillance for imported cases of influenza A(H1N1)v. Timely detection of the introduction of cases by travellers coming or returning from affected areas is crucial to implement control measures around each case and limit the indigenous spread of the virus. Our enhanced surveillance is based on the national protocol set up by InVS [9] and the management of patients follows recommendations of the French pandemic plan [10]. Case definitions of possible, probable, confirmed, excluded and close contacts of cases are shown in the Table.

Community surveillance

Sentinel practitioners network

A sentinel network, consisting of 40 general practitioners (GP) and two paediatricians, scattered across the island conducts prospective influenza surveillance on Réunion Island [3,4]. On a weekly basis, they report the percentage of consultations for influenza-like illness (ILI) using the following case definition: sudden onset of fever > 38°C AND cough OR breathing difficulty. Every physician is expected to perform a nasal swab for each first patient of the week presenting with ILI symptoms that started within less than 48 hours.

Table

Case definition and classification, influenza A(H1N1)v infection, France, 10 June, 2009

Clinical criteria	Any person with an acute respiratory illness: -Fever (>38°C) OR myalgia OR asthenia -AND respiratory symptoms: cough OR dyspnoea
Epidemiological criteria	At least ONE of the following in the seven days prior to disease onset: -travel to an area where sustained human-to-human transmission of influenza A(H1N1)v is documented (as of 10 June 2009: Argentina, Australia, Canada, Chile, Dominican Republic, Japan, Mexico, Panama, United Kingdom, United States). -close contact to a possible, probable or confirmed case of influenza A(H1N1)v infection while the case was contagious (24h prior to symptom onset until seven days after).
Close contact definition	At least one of the following: -a person living with a case: family, roommate etc. -a person who had direct contact with a case, within 1 m while the case was coughing, sneezing or talking ; flirt ; close friends ; classmate, working neighbour; plane or train neighbour
Case classification	1- Possible case: Any person meeting the clinical and epidemiological criteria. 2- Probable case: At least one of the following: -Any possible case with a positive RT-PCR for influenza A virus -Any possible case with a severe symptomatolgy (acute respiratory distress syndrome or death with an acute respiratory infection) -Any possible case which was a close contact to a probable or confirmed case while the case was contagious. 3-Confirmed case: Any possible case with a positive RT-PCR for influenza A(H1N1)v virus. 4-excluded case: At least one of the following: -Any person who does not meet possible case criteria. -Any possible case with a negative influenza A virus RT-PCR

Hospital emergency departments

Data are collected daily directly from patients' computerised medical files that are filled in during medical consultations regardless of the diagnosis. All data are extracted automatically and transmitted electronically in real-time to the InVS. Items collected include diagnosis, coded according to ICD-10, with a severity score ranked from 1 to 5 after medical examination, date of admission to emergency department, orientation (hospitalisation or discharge), age, sex, postal code, and main symptoms. Each patient corresponds to a single record, including all variables [11]. Moreover, data concerning ILI patients will be extracted using influenza associated ICD-10 diagnosis codes (codes for influenza and more acute respiratory tract infections). On Réunion Island three out of the four existing hospitals participate in the network. The forth one is being integrated and should participate starting in July 2009. Nasal swabs will be performed daily for every first adult and paediatric patient seen in emergency departments.

General practitioner house calls network (SOS Médecins)

In the western coast of Réunion Island, SOS Médecins is composed of eight GPs that are involved in more than 100 interventions per day for a population of about 100,000 inhabitants (one eighth of the population). Telephone calls are handled by a call center and logged in a local database. This database is linked via internet to electronic notebooks held by GPs who can update the database with additional information following the visit of a patient. The data collected include: date of the visit, postal code, age, sex, symptoms of the patient and the medical diagnosis. Each morning, data for all visits logged during the previous 24-hour period (midnight to midnight) are downloaded [12].

Local hospital based Mobile Emergency Unit (Samu Centre 15)

On Réunion Island, a single 'Samu Centre 15' operates for the entire island. This mobile unit receives emergency calls and provides emergency healthcare and medical transport of patients. Total phone calls (regardless of diagnosis), phone calls for ILI and for advice on influenza will be analysed weekly.

Hospital surveillance

To monitor and describe severity, cases hospitalised for ILI will have a nasal swab for viral testing. Clinical and epidemiological information will be collected by Cire in collaboration with a clinical research project for hospitalised cases currently under preparation.

Mortality surveillance

The National Institute for Statistics (Institut National de la Statistique et des Etudes Économiques, Insee) conducts the administrative recording of deaths from all causes in France. For several years, Insee has been monitoring and centralising daily mortality in France including Réunion Island. In case of an influenza epidemic on the island, we will analyse this total number and excess of deaths from all causes. This system will be completed by analysis of all death certificates received by the regional public health authority that mention 'influenza'. These certificates will be recorded as influenza-associated deaths. Electronic death certification which is being implemented in France will be used by the Intensive Care Department of Saint-Denis Hospital, and be analysed in real-time by the Cire.

Cluster identification

Despite a well functioning surveillance system, imported cases of influenza A(H1N1)v might be missed and result in outbreaks of ILI in closed communities (schools, children, workers, elderly). In order to prevent this from happening, reporting of outbreaks in such communities, particularly in the early phase of the influenza season, has been fostered and will lead to prompt investigation including virological testing. Furthermore, to improve self-notification of clusters, heathcare professionals have been informed on the relevance of such measures.

Virological surveillance

An enhanced virological surveillance will be implemented in order to identify and characterise circulating influenza viruses during the coming winter season in Réunion Island. Specimens will be collected by members of the sentinel network and hospitalised patients with ILI symptoms will also be tested. We estimate an average of 80 specimens to be tested weekly at the Laboratory of Virology of Saint-Denis Hospital, one of the 24 laboratories approved by the French Ministry of Health. Specimens will be tested for influenza A and B virus by RT-PCR. For positive influenza A specimens, specific RT-PCR for influenza A(H1N1)v will be performed. All positive influenza specimens (A(H1N1)v and others) will be sent for further viral isolation and complementary analysis, including oseltamivir resistance monitoring, to one of the two French National Reference Centres (NRC) for influenza.

Discussion

The beginning of the winter season in Réunion Island in June is usually followed by an increase of seasonal influenza activity shortly afterwards. As influenza A(H1N1)v is rapidly spreading worldwide, it can be expected that it emerges very soon in the upcoming winter season in the southern hemisphere (as it already has for example in Australia), including Réunion Island. Therefore, the surveillance of influenza on the island has been enhanced to be able to detect the introduction of influenza at an early stage and to monitor the spread and impact of the infections in order to guide the implementation of control measures foreseen in the French national pandemic plan. The usefulness of our enhanced surveillance will be guaranteed by a good collaboration between clinicians, virologists, epidemiologists and public health authorities. Close viral monitoring is of paramount importance since the circulation of seasonal influenza A(H1N1) resistant to oseltamivir with the A(H1N1)v virus is possible during the winter in the southern hemisphere. Such virological approach combined with epidemiologic description of a potential outbreak will assist local public health authorities to adapt control measures to limit the spread of the infection and mitigate the epidemic including use of information on the effectiveness of antivirals. Results of our enhanced surveillance, if an influenza epidemic occurs in Réunion Island, could provide relevant information for continental France or other European countries in preparation for the coming influenza season in the northern hemisphere.

Acknowledgements

We are very grateful to Jean Claude Desenclos (InVS) for scrutinising the manuscript.

We are thankful to all the sentinel network practitioners, the practitioners of the adult and paediatric emergency departments, Dr Emmanuelle Rachou (Observatoire Régional de la Santé de la Réunion), Dr Marie-Christine Jaffar (Laboratoire de Biologie, Centre Hospitalier Régional, Saint Denis, Réunion), Dr Arnaud Bourdé (Samu Centre 15, Centre Hospitalier Régional Saint Denis, Réunion), SOS Médecins-Ouest Réunion, Drass de la Réunion and the two National Reference Centres for Virus Influenza, Lyon and Paris for their participation in collecting and kindly providing data for this surveillance system.

We thank all the clinicians providing their assistance to patients and for their participation in providing clinical data.

This enhanced surveillance project has received funding from the Agence Régionale d'Hospitalisation de la Réunion.

* Erratum: On 12 June 2009 Figure 2 was replaced and the titles in the References were translated into English.

References*

1. Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC. The genomic and epidemiological dynamics of human influenza A virus. Nature. 2008;453(7195):615-9.
2. Viboud C, Alonso WJ, Simonsen L. Influenza in tropical regions. PLoS Med. 2006;3(4):e89.
3. Lassalle C, Grizeau P, Isautier H, Bagnis O, Michault A, Zeller H. Surveillance épidémiologique de la grippe et de la dengue. La Réunion, 1996. [Epidemiological surveillance of grippe and dengue. Reunion, 1996]. [Article in French]. Bull Soc Pathol Exot. 1998;91(1):61-3.
4. Brottet E, Renault P, Pierre V, Lassalle C, Jaffar MC, Rachou E. Surveillance épidémiologique et virologique de la grippe à La Réunion: juillet 2006-mai 2007. [Epidemiological and virological influenza surveillance in Reunion Island: July 2006-May 2007] [Article in French]. Bull Epidemiol Hebd. 2007;39-40:337-8.
5. D'Ortenzio E, Renault P, Brottet E, Balleydier E, Jaffar MC, Valette M, et al. Epidémie de grippe à la Réunion (France), hiver austral 2007. [Outbreak of influenza on Réunion Island (France), Southern winter 2007]. [Article in French]. Bull Epidemiol Hebd. 2008(34):306-7.
6. Vaux S. La surveillance de la grippe en France. [Influenza surveillance in France] [Article in French]. Rev Prat. 2008;58(15):1655-9.
7. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans. N Engl J Med. 2009 Jun 3. [Epub ahead of print].
8. World Health Organization. Influenza A(H1N1) - update 46. June 10, 2009. Available from: http://www.who.int/csr/don/2009_06_10a/en/index.html.
9. New influenza A(H1N1) investigation teams. New influenza A(H1N1) virus infections in France, April - May 2009. Euro Surveill 2009;14(21). pii:19221 Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19221
10. Secrétariat General de la Défense Nationale, France, 2009. Plan national de prévention et de lutte "Pandémie grippale". [National plan for the prevention and control «Influenza Pandemic»] [In French]. Available from: http://www.sante-sports.gouv.fr/IMG//pdf/planpandemiegrippale_200209.pdf.
11. Josseran L, Nicolau J, Caillere N, Astagneau P, Brucker G. Syndromic surveillance based on emergency department activity and crude mortality: two examples. Euro Surveill 2006; 11(12):pii=668. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=668
12. Flamand C, Larrieu S, Couvy F, Jouves B, Josseran L, Filleul L. Validation of a syndromic surveillance system using a general practitioner house calls network, Bordeaux, France. Euro Surveill 2008; 13(25).pii:18905 Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18905

This article was published on 11 June 2009.

Citation style for this article: D'Ortenzio E, Do C, Renault P, Weber F, Filleul L. Enhanced influenza surveillance on Réunion Island (southern hemisphere) in the context of the emergence of influenza A(H1N1)v. Euro Surveill. 2009;14(23):pii=19239. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19239

A methodological approach to investigating a nationwide clinical specimen contamination problem in England

L Pezzoli (lorenzo.pezz@gmail.com)[1,2], C Campbell[1], T L Lamagni[1], E Johnson[3], A Saei[1], G Duckworth[1]
1. Health Protection Agency, (HPA) Centre for Infections, London, United Kingdom
2. European Programme for Intervention Epidemiology Training (EPIET)
3. Mycology Reference Laboratory Regional HPA Laboratory, Bristol, United Kingdom

Outbreaks of pseudo-infection due to contamination of specimens have been described, often as localised incidents. From August 2006, several English hospital laboratories began to refer an unusually high number of isolates of the fungus *Paecilomyces variotii* from clinical specimens to the national mycology reference laboratory for microbiological testing. We describe the methods used during the outbreak investigation in order to provide infection control specialists with an overview of how such national incidents may be investigated. We surveyed the hospitals reporting the contamination problem and conducted microbiological and environmental sampling. We applied analytical epidemiology to supply chain data, comparing the supply lines of key equipment to affected and unaffected hospitals in England. The survey was useful to describe procedures and equipment in use in the hospitals reporting the problem. The microbiological aspects of the investigation helped us understand how the fungal spores were distributed in the hospital environment. In the supply chain investigation we used data that was previously only used for logistical purposes. Overall the investigation were methodologically challenging, with no existing protocol to guide the investigators. To our knowledge, this is a novel approach to the investigation of such a widespread contamination problem, affecting geographically disparate hospitals at the same time.

Introduction

Hospital equipment contamination can lead to a so called pseudo-infection: the isolation of a pathogen in clinical specimens without clinical relevance [1]. Outbreaks of pseudo-infection are referred to as pseudo-outbreaks. Clinical specimen contamination in multiple hospitals occurs, they are however more commonly seen in the form of localised problems due to inadequate sampling techniques, presence of the contaminants (e.g. fungal spores) in the hospital or laboratory environment, or decontamination failures [2-6]. Simultaneous pseudo-outbreaks in multiple hospitals are rare, being more likely the result of contamination in single hospitals or laboratories [7-9]. A pseudo-outbreak involving *Ochrobactrum anthropii* contamination of blood culture bottles occurred in the United Kingdom in 2001 [10]. It is important to investigate such incidents even in the absence of clinical infections as the laboratory results may lead to patients being treated with drugs which may be toxic or cause side effects and which are often expensive.

Between August and September 2006, 34 laboratories across England and one from Northern Ireland reported identification of 77 isolates of the fungus *Paecilomyces variotii* from clinical specimens, primarily blood cultures, to the Health Protection Agency (HPA) Mycology Reference Laboratory (MRL) for species confirmation [11]. Given the unusually high number of isolates (the MRL would usually receive only five or six *P. variotii* isolates per year) [11] the MRL subsequently notified the healthcare-associated infection and antimicrobial resistance department of the HPA Centre for Infections of this increase. Initial communication with referring laboratories indicated that the fungus had been isolated directly from blood culture bottles, that different blood culture systems were used in the hospitals and that, in most instances, the isolates were considered not to be clinically significant. Contamination of blood sampling equipment was therefore hypothesized and a national Incident Management Team (IMT) established. The team included experts in epidemiology of hospital acquired infections, mycology, and laboratory standards [11-13].

We describe the methods used to investigate the outbreak in order to provide infection control and public health specialists with an overview of how such national incidents may be investigated and to provide recommendations for future investigations.

Investigation

To our best knowledge, no standardised or field-tested methods existed to guide the investigation for this multisite outbreak of pseudo-fungaemia. We devised and pursued four investigative strands following active case finding which included:

- constructing and performing a descriptive survey;
- characterising samples microbiologically;
- performing environmental investigations; and
- investigating the supply chain.

Active case finding

The IMT notified the Medicines and Healthcare products Regulatory Agency (MHRA) of the fact that an unusually high amount of clinical samples from across the country were found

positive with *Paecilomyces variotii* and of the planned investigation. Relevant experts in microbiology, infection control and public health were alerted about the event and the forthcoming investigation through an article in the Communicable Disease Report (CDR) Weekly public health bulletin [12] and an email alert cascaded to all consultant microbiologists in England via the HPA Regional Microbiology Network. All stakeholders were asked to notify the investigation team of any isolates of *Paecilomyces variotii* after 1 July 2006. Alerts were also transmitted via the relevant public health bodies of Northern Ireland (Department of Health, Social Services and Public Safety), Wales (National Public Health Service for Wales) and Scotland (Health Protection Scotland). Furthermore, an article was published in Eurosurveillance to generate information about whether a rise in *Paecilomyces variotii* isolates had been noticed elsewhere in Europe [11].

Descriptive survey

Preliminary information indicated that the fungus was being identified directly in blood culture bottles from two different brands of blood culture systems. We conducted a questionnaire survey in order to understand how samples were taken in the affected hospitals and to generate hypotheses on the source of the contamination.

Methods

We sent a questionnaire to staff of every hospital laboratory that reported an isolate of *Paecilomyces* to collect descriptive data on the contaminated specimens and asked about all species isolated, including non-*Paecilomyces* contaminants, the specialty from which the contaminated samples were referred and the procedures and equipment used for collection of the samples. We also asked if the laboratory had made any changes in the supplies of equipment or in the standard procedures used for blood sampling and processing the samples. Furthermore, we asked about the assumed clinical relevance of the findings and if antifungal therapy had been initiated for patients that were associated with the positive *Paecilomyces* samples.

The questionnaire was sent via email to the reporting laboratories, which then had the option to send it back via email or post. Data from the questionnaire were entered into a customized MS Access database. Analysis was conducted with MS Excel and STATA version 8 (Stata, College Station, TX).

Lessons learned

With the survey we were able to describe how the contaminated samples were collected in the hospitals and how they were processed in the laboratories, although we were not able to formulate hypotheses to test. To collect timely, accurate and comprehensive information to identify the source of a pseudo-infection with a questionnaire is difficult. We speculate that the investigation of pseudo-outbreaks due to contamination of equipment is of little priority for physicians and hospital microbiologists and this leads to a low response rate and delay in responding. In our case the questionnaires provided insufficient answers and further investigation was required. In order obtain results and a high response rate, a web based survey may be more suitable for such incidents instead of sending a questionnaire via email as we did for this investigation.

Microbiological characterisation

Since different species of *Paecilomyces* present differences in response to treatment (antifungal sensitivity), the differentiation between members of the *Paecilomyces* complex is clinically relevant [14].

Methods

Isolates received by the MRL were initially characterised using phenotypic identification methods in which the macroscopic and microscopic morphology was examined. Strains were then subjected to broth microdilution susceptibility testing with a range of antifungal agents with systemic activity by means of the National Committee on Clinical Laboratory Standards (NCCLS now Clinical and Laboratory Standards Institute - CLSI) method for filamentous fungi M38-A [15]. *Paecilomyces* environmental isolates and isolates from clinical specimens were sent by the MRL to a laboratory in the Netherlands (the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre), which specialises in typing mould isolates, for sequencing of part of the beta-tubulin gene in order to compare the profiles of the isolates.

Lessons learned

Molecular typing of these organisms requires highly specialised laboratories. This may present difficulties in logistics, turnaround time and cost. In order to overcome this current limitation we recommend typing a representative sample of isolates received in any similar occurrences.

Environmental investigation

Environmental contamination, such as through *Aspergillus* spores released during construction work, is known to play a part in fungal infections [7,16]. Information on the ecology of *Paecilomyces* indicates that it is commonly associated with soil and decaying vegetable matter and has been proven to colonise also plastic surfaces, saline solutions and water damaged organic material like wood, cardboard or fiberboard [17-19]. Consequently our investigation included environmental investigations to assess whether:

- any equipment implicated could be identified;
- evidence could be provided to prove that contaminated equipment had been in, and contaminated the patient care areas sampled, and
- specimens for typing could be provided [20].

Methods

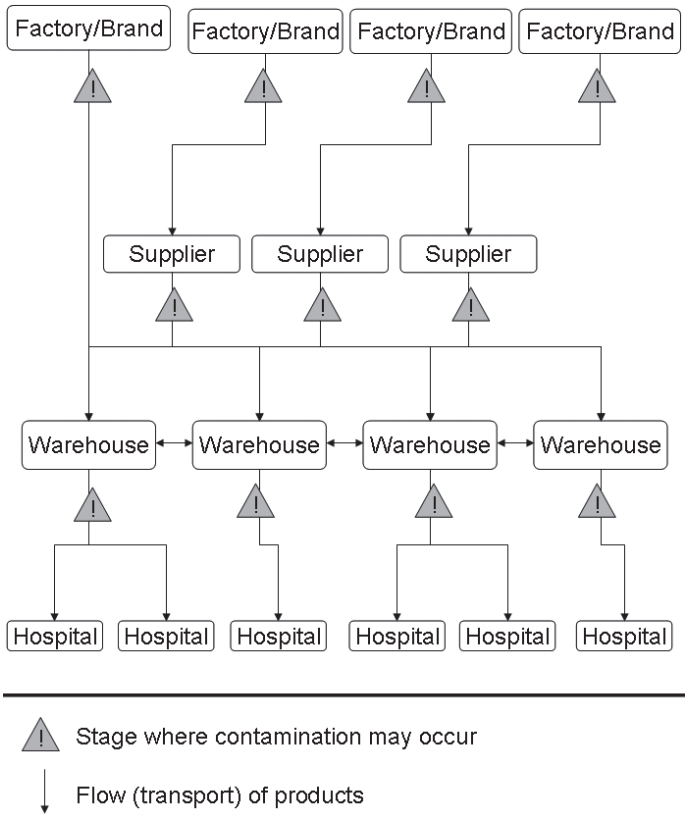
The hospitals that reported isolates (specimens taken less than four weeks before notification to the IMT) were targeted to increase the chance of any contaminated equipment still being present on the premises. We asked laboratories reporting Paecilomyces to undertake microbiological sampling of premises and equipment used when, or associated with, sampling eventually found to be positive. To increase the chance of any contaminated equipment still being present on the premises we asked only the hospitals that had found isolates within four weeks before notification to the IMT to perform microbiological investigations.

Samples of consumable equipment (i.e. syringes, needles, skin swabs, adaptor caps and butterfly needles) obtained from the wards where the blood samples were taken were either sent to the HPA Mycology Reference Laboratory for testing or tested at site of collection. Where possible, equipment belonging to the same batch as that used during the contamination episode was requested, as well as sampling of the outside packaging of these items for testing of fungal contamination (e.g. empty box from skin swabs), especially if there was any suggestion of spoilage. Environmental swabs of the areas surrounding the patient bed or other patient

care areas, where the original positive samples were obtained, were also requested. Settle plates for environmental sampling of fungal spores were also positioned conveniently, depending on the respective location, above head-height in the same areas. The environmental samples were sent for molecular typing to verify if the same strain was implicated both in clinical and environmental isolates.

Figure

Schematic outline of the supply chain of hospital equipment, England, 2006



Lessons learned

Environmental investigation was in many cases delayed, because the IMT became aware of the majority of positive isolates only after it had sent out the alerts. However, we recommend keeping the interval between collection of contaminated clinical samples and environmental analysis to a minimum. Environmental sampling in warehouses that supply equipment to affected hospitals and hospital storage areas should also be considered.

Supply chain investigation

When using a traditional case-control study to analyse pseudo-outbreaks it may difficult to select the appropriate controls, because all controls may share the same exposures as the cases (i.e. processed by same technician, using same equipment, etc.) thus making internal comparison inappropriate [21]. Other analytical approaches could be employed that compare sites (hospitals or wards) affected by a particular problem with the unaffected ones [22,23].

Because of the practical constraints discussed above, we decided to take a further investigative approach, analysing the supply chain of consumable equipment to the NHS (National Health Service) Acute Hospital Trusts (hospitals). Supply chain investigations are usually used in outbreaks of foodborne diseases to trace back the affected food items [24,25]. In our investigation we focused on the supply chain of blood taking equipment, but instead of using the standard retrospective approach, we analysed how the supply-chain differed between affected and unaffected hospitals.

In England, hospital equipment is supplied to hospitals via a centralised system, which is managed by the NHS Supply Chain, a subsidiary of DHL (Dalsey, Hillblom and Lynn) express mail services. This agency holds all the information on the equipment supplied to the hospitals in an electronic database, published twice a year. The catalogue has almost 50,000 entries, one for each product supplied. Each product is identified by a unique National Product Code (NPC). The NHS Supply Chain can identify which products are distributed to each hospital, when and how they are transported and in what quantity. The database provides information if specific products were returned to the sender and in what quantity, but information on the reasons for returning is not given. The NHS Supply Chain operates through six different stations

Table

Equipment categories for the analysis of supply chain data from England, Health Protection Agency investigation, 2006

National Health Service (NHS) supply chain category designation	Items included in the category (examples)
Blood collection systems	Blood sample tubes, needles, etc.
Clinical sundries	Kidney dishes, trays
Gloves	Latex or vinyl gloves, with/without powder
Haberdashery	Towels, tapes
Hand washing	Hand towels, paper towels
Intravenous cannulae and accessories	Cannulae, catheters
Laboratory	Blood culture media, blood specimen tubes
Paper and Hygiene	Paper rolls
Sterile services	Mono-use pulp trays or kidney dishes
Syringes needles and associated products	Syringes, needles
Trolley covers	Drapes
Wipes and applicators	Dry wipes, disinfectant wipes

across England; for each of these stations there is a warehouse that stores all the supplies for the area covered. In general each individual hospital is supplied by one warehouse, although goods may be transferred between warehouses (Figure).

Methods

As contamination of blood sampling equipment was suspected, the investigation focused only on the products involved in blood taking listed in the catalogue. We created a short list from the catalogue of products likely to have been used in or around blood sampling procedures (Table).

For each product, information was obtained about the supplying warehouse, the brand, the supplier, the quantity supplied, when it was delivered and to which hospital. It was therefore possible to identify if a product was supplied to a hospital that reported the isolation of Paecilomyces, or to one that did not report this problem. This information was available only for English hospitals since the NHS Supply Chain operates only in England.

We designed a cohort study including all NHS Acute Hospital Trusts (hospitals) in England.

A case was defined as an affected hospital and a non-case as an unaffected hospital. We were interested in measuring the likelihood of a product being supplied to a hospital, so each one of the single entries (products) in the reduced catalogue was multiplied by each hospital to which it was supplied. According to a hierarchical approach, from large categories to smaller ones, we considered the following risk factors: supplying warehouse, product brand, supplier, product category. Due to the size of the database it was not possible to use every single product as a risk factor. We focused on single products if positive associations were found in the broad categories mentioned above.

Univariate analysis was first undertaken, followed by multivariable analysis (a logistic regression model with random components). We also used a log-linear model to investigate the following variables in the NHS product catalogue: supplier (supplying company if different from NHS supply chain), section (equipment category), and storing warehouse. The model included two correlated random effects corresponding to the two versions of the supply data, one created for the period before the contamination problem was first noticed and one for the period after it became evident. This model allows any possible changes in the supply-chain that may have explained the problem.

We used STATA version 8 (Stata, College Station, TX) and SAS Version 9 (Cary, NC: SAS Institute Inc) for analysis.

Lessons learned

With this investigation we discovered that very accurate and comprehensive data on the supplies to hospitals can be obtained in a timely way in England and possibly elsewhere where a central logistic authority exists in the public health system. We did not have any indication of the source of contamination, so we could not use the supply chain data for tracing back any potentially contaminated equipment. Contaminated equipment was, however, not found. In this investigation it took one month between our first enquiries to NHS Supply Chain and obtaining the data in a format suitable for analysis, but this time could be shortened now that we are aware of what kind of data is available and how to process it.

The involvement of the supply chain authority happened when the outbreak was already tailing off. In case of a similar problem occurring again, we recommend earlier involvement by transmitting alerts not only to health professionals but also directly to the supply chain authorities. Even with detailed analysis of the supply chain, it still can be difficult to identify the exact source of contamination because the transfer of goods between warehouses could spread the contaminant throughout the supply chain. Similarly, cross-contamination of equipment that shared the same storing area for a time may occur, creating multiple sources of contamination which are difficult to disentangle through the use of epidemiological analysis.

Discussion and conclusions

We developed an investigation protocol combining microbiological and epidemiological techniques. When more traditional investigative approaches (descriptive epidemiology and environmental sampling) proved to be insufficient to identify the origin of the contamination problem we applied analytical epidemiology to supply chain data. To our knowledge this use of supply chain data is a novel approach to the investigation of such a widespread contamination problem, affecting geographically disparate hospitals at the same time. We used a traditional cohort study, using the supply catalogue in the same way as the food menu would be used in a "classic" wedding food-poisoning outbreak. The large size of the dataset, with almost one thousand different products possibly implicated, and the fact that these data are normally intended for logistical purposes (e.g. ordering of hospital supplies) made this approach unusual. We experienced some methodological challenges investigating this problem, because there was no existing protocol to guide the IMT. We believe that documenting the methodological and organisational aspects of this investigation could inform future investigation of similar problems in the United Kingdom or elsewhere.

Acknowledgements

The authors wish to thank all the microbiologists and the infection control teams who assisted the investigation and the staff of NHS Supply Chain. We also wish to thank Peter Hoffman for his review of the questionnaire and his valuable suggestions and David Powell for his design of the survey database.

References

1. Centers for Disease Control and Prevention (CDC) Outbreaks of pseudo-infection with Cyclospora and Cryptosporidium--Florida and New York City, 1995. MMWR Morb Mortal Wkly Rep. 1997;46:354-8.
2. Beekmann SE, Diekema DJ, Doern GV. Determining the clinical significance of coagulase-negative staphylococci isolated from blood cultures. Infect Control Hosp Epidemiol. 2005;26(6):559-66.
3. Bekeris LG, Tworek JA, Walsh MK, Valenstein PN. Trends in blood culture contamination: a College of American Pathologists Q-Tracks study of 356 institutions. Arch Pathol Lab Med. 2005;129:1222-5.
4. Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev. 2006; 19(4):788-802.
5. Qamruddin A, Khanna N, Orr D. Peripheral blood culture contamination in adults and venepuncture technique: prospective cohort study. J Clin Pathol. 2008;61(4):509-13.
6. Schabrun S, Chipchase L. Healthcare equipment as a source of nosocomial infection: a systematic review. J Hosp Infect. 2006; 63(3):239-45.
7. Freeman J, Rogers K, Roberts S. Pseudo-outbreak of Aspergillus keratitis following construction in an ophthalmology ward. J Hosp Infect 2007; 67(1):104-5.
8. Medeiros EA, Lott TJ, Colombo AL, Godoy P, Coutinho AP, Braga MS, et al. Evidence for a pseudo-outbreak of Candida guilliermondii fungemia in a university hospital in Brazil. J Clin Microbiol. 2007;45(3):942-7.
9. Laurel VL, Meier PA, Astorga A, Dolan D, Brockett R, Rinaldi MG. Pseudoepidemic of Aspergillus niger infections traced to specimen contamination in the microbiology laboratory. J Clin Microbiol.1999;37(5):1612-6.

10. Public Health Laboratory Service. Ochrobactrum anthropi pseudobacteraemias. Commun Dis Rep CDR Wkly [serial online] 2001;11.

11. Lamagni TL, Campbell C, Pezzoli L, Johnson E. Unexplained increase in Paecilomyces variotii blood culture isolates in the UK. Euro Surveill. 2006;11(46):pii=3080. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3080

12. Health Protection Agency. Suspected cases of Paecilomyces variotii pseudofungaemia. Commun Dis Rep CDR Wkly [serial online] 2006; 16(44).

13. Health Protection Agency. Investigation into the increase in Paecilomyces variotii isolates in the UK from blood culture and other sterile sites. Health Protection Report [serial online] 2007;1(16).

14. Castelli MV, Alastruey-Izquierdo A, Cuesta I, Monzon A, Mellado E, Rodriguez-Tudela JL et al. Susceptibility testing and molecular classification of Paecilomyces spp. Antimicrob Agents Chemother. 2008; 52(8):2926-8.

15. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard. Wayne, PA: National Committee for Clinical Laboratory Standards, 2002.

16. Hruszkewycz V, Ruben B, Hypes CM, Bostic GD, Staszkiewicz J, Band JD. A cluster of pseudofungemia associated with hospital renovation adjacent to the microbiology laboratory. Infect Control Hosp Epidemiol. 1992;13(3):147-50.

17. Young VL, Hertl MC, Murray PR, Lambros VS. Paecilomyces variotii contamination in the lumen of a saline-filled breast implant. Plast Reconstr Surg. 1995;96(6):1430-4.

18. Kluczek-Turpeinen B, Tuomela M, Hatakka A, Hofrichter M. Lignin degradation in a compost environment by the deuteromycete Paecilomyces inflatus. Appl Microbiol Biotechnol. 2003; 61(4):374-9.

19. Kapoor KK, Jain MK, Mishra MM, Singh CP. Cellulase activity, degradation of cellulose and lignin and humus formation by cellulolytic fungi. Ann Microbiol (Paris). 1978;129 B(4):613-20.

20. Labombardi VJ, O'brien AM, Kislak JW. Pseudo-outbreak of Mycobacterium fortuitum due to contaminated ice machines. Am J Infect Control. 2002; 30(3):184-6.

21. McNeil MM, Davis BJ, Solomon SL, Anderson RL, Shulman ST, Gardner S, et al. Ewingella americana: recurrent pseudobacteremia from a persistent environmental reservoir. J Clin Microbiol. 1987;25(3):498-500.

22. Yu IT, Xie ZH, Tsoi KK, Chiu YL, Lok SW, Tang XP, et al. Why did outbreaks of severe acute respiratory syndrome occur in some hospital wards but not in others? Clin Infect Dis. 2007; 44(8):1017-25.

23. Kool JL, Carpenter JC, Fields BS. Effect of monochloramine disinfection of municipal drinking water on risk of nosocomial Legionnaires' disease. Lancet.1999; 353(9149):272-7.

24. Ammendrup S, Barcos LO. The implementation of traceability systems. Rev Sci Tech. 2006; 25(2):763-73.

25. Kivi M, Hofhuis A, Notermans DW, Wannet WJ, Heck ME, VAN De Giessen AW, et al. A beef-associated outbreak of Salmonella Typhimurium DT104 in The Netherlands with implications for national and international policy. Epidemiol Infect. 2007 135(6):890-9

This article was published on 11 June 2009.

Citation style for this article: Pezzoli L, Campbell C, Lamagni TL, Johnson E, Saei A, Duckworth G. A methodological approach to investigating a nationwide clinical specimen contamination problem in England. Euro Surveill. 2009;14(23):pii=19234. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19234

Application of RT-PCR for diagnosis of respiratory syncytial virus and human metapneumovirus infections in Bulgaria, 2006-7 and 2007-8

S Pavlova (slavapavlova@ymail.com)[1], T Hadzhiolova[1], P Abadjieva[2], R Kotseva[1]

1. National Laboratory of Influenza and Acute Respiratory Diseases, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
2. Second Multi-Profile Hospital for Active Treatment, Sofia, Bulgaria

We describe here the results of respiratory syncytial virus (RSV) detection by reverse transcription polymerase chain reaction (RT-PCR) during two consecutive seasons, from December 2006 to February 2007 and from October 2007 to March 2008, performed in the National Laboratory of Influenza and Acute Respiratory Diseases, Bulgaria. A total number of 278 nasopharyngeal samples obtained from hospitalised children up to the age of five years were investigated for these two seasons. During the first season, the aetiological role of RSV was confirmed in 56 of 148 samples (37.8%) compared to 11 of 130 samples (8.5%) during the second season. Since the beginning of January 2008, RT-PCR for the detection of the recently identified human metapneumovirus (HMPV) has also been introduced in Bulgaria. This virus has been demonstrated as the aetiological agent in 13 out of 81 samples (16%) from children of the same age group. The use of RT-PCR allows the detection of a broader spectrum of viruses causing respiratory diseases, as well as better discrimination of the aetiological agents in clinically similar cases.

Introduction

Acute respiratory infections (ARIs) represent a considerable health problem in infants and children. Despite the great number of viruses causing ARIs (more than 200), influenza viruses of type A and B, respiratory syncytial viruses (RSV), parainfluenza viruses, and adenoviruses are indicated traditionally among the most important aetiological agents of respiratory system diseases. However, in addition to previously known viruses, a number of respiratory viruses have been recently identified as causative agents of lower respiratory illnesses in children: human metapneumovirus (HMPV), human coronavirus (HCoV-NL63), human bocavirus (HBoV) [1-3].

RSV is the major cause of bronchiolitis and pneumonia during the first years of life. Children with underlying illnesses such as congenital heart disease and bronchopulmonary dysplasia are at increased risk for severe infections due to RSV. In addition, RSV is increasingly recognised as an important pathogen in other groups, including immunocompromised patients and the elderly [4-6].

HMPV was first identified in the Netherlands in 2001 using a PCR designed for the identification of unknown agents multiplying in cell cultures [1]. Together with RSV, HMPV has quickly assumed an important position among the rest of the respiratory pathogens, particularly regarding early childhood diseases [7-9]. Clinical symptoms of HMPV infection seem to be indistinguishable from RSV infections. Major clinical manifestations of the infection caused by these two viruses in infants and young children are bronchiolitis and pneumonia [2,10,11].

Although routine diagnostic methods for respiratory viruses, including virus cultivation on cell culture, are robust, PCR for the detection of viruses in respiratory samples has also been shown to be useful because it offers an enhanced sensitivity combined with rapid detection [12,13]. In the past five years, RT-PCR has been applied as a highly sensitive and specific method for the diagnosis of RSV in the National Laboratory of Influenza and Acute Respiratory Diseases in Bulgaria. Since the beginning of 2008, RT-PCR has been used for HMPV detection in clinical samples as well [14-16].

Table 1

Number of positive initial specimens tested by different methods for RSV in children under five, Bulgaria, 2006-7 (n=148 samples tested)

Number ofspecimens tested	Isolation on cell cultures		RT-PCR- positive specimens
	Specimens positive for CPE	Specimens, confirmed by rapid tests	
148	24 (16.2%)	16 (10.8%)	56 (37.8%)

RSV: respiratory syncytial virus

The purpose of this study was to provide data about the detection of RSV and HMPV by RT-PCR in hospitalised children up to the age of five years during the period from 2006 to 2008 in Bulgaria.

Materials and methods

Clinical material

Nasopharyngeal samples were obtained by gently rubbing the deep nasal turbinate bilaterally with sterile swabs (Viral CulturetteTM system, Becton Dickinson, Fisher Scientific), combined with a third swab from the posterior pharynx. Swabs were transported to the laboratory on the same day, dipped into a vial containing 2 ml saline and divided into aliquots. A fresh aliquot was used to inoculate a cell culture and the remaining aliquots were stored for PCR testing and further investigation. The samples were obtained from hospitalised children up to the age of five years admitted to a paediatric unit of the Second Multi-Profile Hospital for Active Treatment, Sofia, with signs and symptoms of an upper respiratory tract infection, bronchiolitis, or pneumonia. In the 2006-7 season, samples were also taken from Lozenetz Hospital and two orphanages in Sofia. During the two seasons investigated, approximately 1,900 children were covered by these hospitals and by the two orphanages. The following indicators were taken into consideration for children's hospitalisation: infants younger than five years and high risk infants (prematurely born infants, children with bronchopulmonary dysplasia and congenital heart diseases, dystrophia, children born from twin pregnancies).

Cell culture

Samples (0.2 ml) were inoculated on the day of collection onto HEp-2 and MRC-5 cells. The cell cultures were moved to the Cell Cultures Laboratory, National Center of Infectious and Parasitic Diseases (NCIPD). Cultures were observed daily during 10 days for cytophatic effect (CPE). RSV produces a characteristic CPE consisting of syncytia formation. When the CPE had reached 50% or more of the monolayer, the culture supernatant was aspirated for subsequent virus identification. RSV infection was confirmed by enzyme immunoassay (EIA) membrane test for the rapid and qualitative detection of RSV (Directigen RSV, Becton Dickinson) [15].

RNA Extraction

RNA was extracted using Trizol LS reagent (Invitrogen) or RiboSorb (Sacace) kits, according to the manufacturer's instructions.

RT-PCR

RT-PCR for RSV detection was performed by ABGene iT-One Step RT-PCR (Invitrogen), using specific primers directed against a 278 nt fragment in the highly conservative region of the nucleocapsid gene of RSV (position 858-1135) [18].

RT-PCR for HMPV detection was performed by Qiagen® One Step RT-PCR Kit (Qiagen) kit using specific primers directed against a 416 nt fragment of the matrix protein gene of HMPV [17].

RNA extracted from the RSV reference strain, maintained through multiple passaging in a HEp-2 cell line, was used as a positive control for RSV. As positive control for HMPV, extracted RNA received from the Cantacuzino Institute, Romania was used [17]. As negative control distilled water was used.

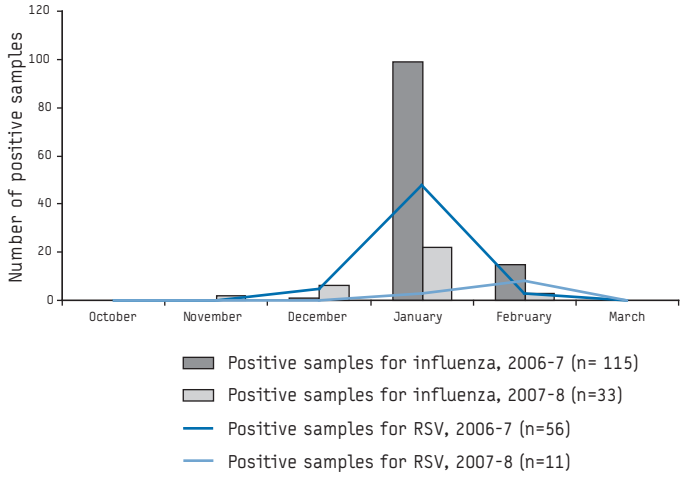
Results

First season (2006-7)

A total of 148 samples from hospitalised children were tested by RT-PCR for RSV during this first period of study. Table 1 demonstrates that 56 (37.8%) samples were RSV-positive. In the parallel testing of all of the samples on cell cultures, 24 samples (16.2%) showed a CPE, expressed mainly in round-cell degeneration of the monolayer without the appearance of the syncytia characteristic for RSV-infected cells. In addition, the culture supernatants were tested by a rapid immuno-chromatographic test assay membrane test (Directigen RSV) as soon as the CPE had spread over at least 50% of the monolayer (between 48 h and 10 days post inoculation).

Figure

Distribution of samples positive for RSV and influenza viruses in children under five, Bulgaria, 2006-7 and 2007-8



RSV: respiratory syncytial virus

Table 2

Distribution by week of the RT-PCR-positive specimens for RSV in children under five, Bulgaria, 2006-7 (n=148 samples tested)

	December 2006 – February 2007							
	Week 49	Week 50-51	Week 52-1	Week 2	Week 3	Week 4	Week 5	Week 6
Number of specimens tested	12	12	5	38	26	28	10	17
Number of positive specimens (percent)	2 (16.6%)	3 (25%)	4 (80%)	32 (84.2%)	7 (26.9%)	5 (17.9%)	1 (10%)	2 (11.8%)

RSV: respiratory syncytial virus

This test was positive in 16 of the samples cultured (10.8% of all tested samples).

Laboratory-confirmed cases of RSV (Figure) showed a peak in January 2007 (48 RSV-positive results). A lower number of positive samples was detected in December 2006 and February 2007 (a total of eight positive samples).

In Table 2, the distribution of the positive specimens is indicated by weeks, which demonstrates that during the peak period, over 84% of samples were RSV-positive.

On the background of the advancing influenza epidemic in the beginning of 2007, RSV infections peaked almost two weeks earlier. It is worth noting that the RSV curve was based on testing of children under the age of five years, received from December 2006 to February 2007, while the influenza curve was based on all clinical specimens (n=732) received in the laboratory during the period from October 2006 to March 2007 in relation to the laboratory follow-up of the epidemic circulation of the seasonal influenza viruses. Nevertheless, the similar course of the two curves is evident, which confirms that both viruses – RSV and influenza A virus - contribute simultaneously to the morbidity rate attributable to influenza and other acute respiratory infections (Figure) [19,20]. The results obtained indicate an increase in RSV infections from the end of December 2006, and a decrease from mid-January 2007 (Table 2).

The age distribution of the 56 positive samples for RSV according to their clinical diagnosis is presented in Table 3. The largest number of RSV positive samples was recovered from the youngest age group from 0 to 12 months (37 positive samples).

Second season (2007-8)

As a continuation of the work done in the first season, RT-PCR was applied as a routine method for confirmation of RSV directly to clinical materials. The successful application of RT-PCR for RSV diagnostics in the season 2006-7 encouraged us to include during the season 2007-8 diagnostics of the newly emerging pathogen HMPV.

A total of 130 nasopharyngeal swabs were tested for RSV in the period from October 2007 to March 2008 (Table 4). The clinical material originated from hospitalised children up to the age of five years. Eighty-one swabs were received after the beginning of 2008 and they were tested in parallel for both RSV and HMPV. Using RT-PCR, the presence of RSV RNA was confirmed in 11 samples (8.5%), and 13 samples (16.1%) were positive for HMPV.

The age distribution of positive RSV and HMPV cases is presented in Table 5. The largest number of HMPV positive samples was recovered from the youngest age group from 0 to 12 months (10 positive samples). RSV confirmation has been comparatively lower in all age groups for the second period than for the first.

Based on the number of laboratory-confirmed RSV cases found, RSV infections were limited to individual cases in the season 2007-8, in contrast to the 2006-7 season, when an RSV epidemic was observed (Figure). In contrast to the CPE caused by the RSV isolated in 2007, which resulted in round cell degeneration of the monolayer, the strains isolated in 2008 caused RSV-typical syncytia in the monolayer. It is possible that this was due to differences in the biological characteristics of the strains isolated in the two seasons, indicating that they may belong to different RSV strains. This will be an object for further investigations.

Superimposed on the RSV results in the Figure, there is a curve showing the number of clinical samples found positive for influenza during the period under discussion. While the peak of the RSV and influenza activity coincided in the season 2006-7, the season 2007-8 saw first a peak in influenza-positive samples and, after the influenza activity had decreased, a peak in RSV-positive samples. Nevertheless, morbidity due to acute respiratory infections during this second season remained constant, indicating that RSV and influenza contribute together to the overall morbidity [20].

Table 3

Distribution of RSV-positive samples in children under five by age group and clinical diagnosis, Bulgaria, 2006-7 (n=56)

Clinical diagnosis	Age groups		
	0 - 12 months	1 - 3 years	4 - 5 years
	RSV	RSV	RSV
Acute bronchiolitis	20	7	0
Bronchitis or acute rhinopharyngitis	15	6	0
Pneumonia	2	3	3

RSV: respiratory syncytial virus

Table 4

Distribution by month of samples positive by RT-PCR for RSV and HMPV in children under five, Bulgaria, 2007-8 (n=130 samples tested)

	October 2007 – March 2008*					
	October 2007	November 2007	December 2007	January 2008	February 2008	March 2008
Number of samples tested for RSV	18	13	18	42	32	7
Number of samples tested for HMPV	0	0	0			
Number of positive samples	0	0	0	3 RSV 2 HMPV	8 RSV 8 HMPV	3 HMPV

*A total of 130 samples were tested for RSV, 81 of which were also tested for HMPV
RSV: respiratory syncytial virus; HMPV: human metapneumovirus

As HMPV testing is new in laboratories in Bulgaria, it seems too early at this stage to draw any conclusions about the epidemic distribution of this virus.

Discussion and conclusion

The development of molecular techniques for diagnosis of respiratory pathogens that cannot be cultured easily by traditional techniques has revolutionised the field of virology and infectious diseases. Even if certain viruses such as RSV can be grown in cell culture, this method is not completely reliable and many scientists have begun to use RT-PCR to identify infection [21]. The diagnosis of HMPV infection is even more problematic, as the virus is difficult to isolate in cell culture [1,22]. RT-PCR examination of respiratory secretions is currently the clinical test chosen for reliable diagnosis of HMPV. This is a reason to start using molecular tests for diagnostics of important respiratory viral causative agents such as RSV and HMPV.

For RSV, we detected 37.8% positive samples by PCR versus 10.8% by cell culture in the first season, and 8.46% versus 1.5% in the second season. This is in accordance to the results of other authors who also report a higher percentage of RSV-positive samples detected by PCR than by cell culture [13]. Some scientists have also tried to use cell lines for the isolation of HMPV [1,23]. As HMPV isolation is difficult to achieve in a cell culture model (non-characteristic cytopathic effect and necessity of prolonged cultivation), RT-PCR remains the single alternative for laboratory confirmation of its aetiological role. In this study, we found 16% HMPV-positive samples by RT-PCR. The largest number of positive samples was recovered from the youngest age group. These data coincide with information published by other researchers [24]. However, due to the limited number of tested samples, it is too early to draw a final conclusion regarding the role of this virus in the ARIs morbidity in Bulgaria.

Keeping in mind the literature data according to which the pathogens described infect preferably the youngest age group, we have focused our diagnostic study on hospitalised children up to the age of five [4,25-26]. We believe that the high percentage of positive results by RT-PCR - more than 84% in the second week during the first season - is an indication that we chose the right age group of children to be tested. This is in accordance with the clinical diagnosis and the intensive circulation of the virus during this period (Table 2, Table 3) [19].

The laboratory confirmation of RSV and HMPV aetiology by RT-PCR gives a prompt response within 24 to 48 hours, which is important for the therapy and critical for effective patient management by focusing appropriate drug treatment, reducing unnecessary use of antibiotics, and preventing nosocomial spread [13,27-29]. Especially because of the necessity of rapid result for the clinician, virus isolation on cell culture is being displaced by molecular biology tests. Nevertheless, the isolation of the viral causative agent remains a gold standard and a basic model for the study of genetic and antigenic changes in the virus population, as well as a means of detection of new respiratory viruses [30].

The clinical picture of HMPV (bronchiolitis or pneumonia among infants and young children) initially resembles the one caused by RSV [24]. A co-infection with both viruses is possible as well, being associated with more severe course of the disease [2,31]. In our study we have not detected such cases. According to data obtained from the second investigated period, there were no clinical criteria for distinguishing both viruses. The highest number of positive samples for both viral agents is in the youngest age group (0-12 months) and they are associated with severe course of the disease, which has also been observed by other authors [32].

In many European countries, investigations were performed of the incidence not only of influenza viruses, but also of RSV as an important pathogen with social and economical impact especially in early childhood [20,33,34]. Increasing circulation of RSV is registered in countries in the European Union: in England, 40.8% of RSV-positive sentinel samples are found in young children aged 0 to 4 years, whereas in Scotland and France this proportion is approximately 11% [20]. The same authors reported that about 92% or more of RSV-positive non-sentinel samples were obtained from 0 to 4 year-old children. In our investigation, we obtained 37.8% positive results for the same age group during the first season. That this percentage is lower than reported in the literature is probably due to limited sample collecting that covered only one hospital in that period. The percentage of laboratory-confirmed RSV cases in Greece for the same season was 5.4%, mainly among children up to the age of three years [35]. Taking into account the fact that the RSV distribution varies in different countries and seasons [34], we consider that the smaller confirmed number of positive results for RSV that we obtained in the season 2007-8 reflects the true situation in Europe.

The epidemic spread of influenza viruses in Bulgaria for the two observed epidemic seasons coincides with the epidemic incidence of influenza viruses in Europe, and influenza was the most commonly detected virus in all European countries [36]. Routine detection of other viral respiratory pathogens yields data which are useful in monitoring general trends in morbidity from ARIs. In current investigations the detection of RSV in clinical samples coincides also with epidemic spread of influenza viruses [37,38]. During the second season, the peak of RSV infections did not coincide with that for influenza virus infections [39].

Many investigators mark the importance of collecting circulation data not only for influenza viruses but also for other causative agents of ARIs, and RSV is one of the most important. It is really necessary to build an RSV surveillance in Europe, in order to broaden and represent the real rate and spectrum of viral respiratory diseases [20,40].

In conclusion, the present results give information about the spread of two respiratory viruses - RSV and HMPV - among hospitalised children, detected by RT-PCR in Bulgaria. Collecting information on the spread of RSV is a requirement from the European Influenza Surveillance Scheme (EISS), which underlines

Table 5

Distribution of RSV- and HMPV-positive samples in children under five by age group and clinical diagnosis, Bulgaria, 2007-8 (n=24)

Clinical diagnosis	Age groups					
	0 - 12 months		1 - 3 years		4 - 5 years	
	RSV	HMPV	RSV	HMPV	RSV	HMPV
Acute bronchiolitis	7	9	2	2	0	0
Pneumonia	2	1	0	0	0	1

RSV: respiratory syncytial virus; HMPV: human metapneumovirus

the necessity of collection of data regarding the incidence of this virus in different European countries. The investigations reported here are a priority for the National Laboratory of Influenza and Acute Respiratory Diseases in Bulgaria in order to confirm the participation of some more widely distributed viruses, as causative agents of ARIs, first and foremost in paediatric pathology.

Acknowledgements

We would like to thank our colleagues from the Laboratory of Influenza, Cantacuzino Institute, Bucharest, Romania, for providing us with primers for HMPV and RT-PCR protocol for the detection of this virus.

References

1. Van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. NatMed 2001;7(6):719–24.
2. Choi EH, Lee HJ, Kim SJ, Eun BV, Kim NH, Lee JA, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. Clin Infect Dis 2006;43(5):585–92.
3. Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H. Detection of human coronavirus NL63 in young children with bronchiolitis. J Med Virol.75(3):463–5.
4. Hall CB. Respiratory syncytial virus and parainfluenza virus. N Engl J Med. 2001;344(25):1917-28.
5. Collins PL, Chanock RB, Murphy BR. Respiratory syncytial virus. In B. N. Fields (ed.), Virology. Forth edition. Philadelphia: Lippincott Williams and Wilkins, Philadelphia. 2001;1443-85.
6. Walsh EE, Falsey AR, Hennesey PA. Respiratory syncytial virus and other infections in persons with chronic cardiopulmonary disease. Am J Respir Crit Care Med. 1999;160(3):791-5.
7. Dollner H, Risnes K, Radtke A, Nordbo SA. Outbreak of human metapneumovirus infection in norwegian children. Pediatr Infect Dis J. 2004;23(5):436-40.
8. Van den Hoogen BG, van Doornum GJ, Fockens JC, Cornelissen JJ, Beyer WE, de Groot R, et al. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. J Infect Dis. 2003;188(10):1571-7.
9. McIntosh K, McAdam AJ. Human metapneumovirus-an important new respiratory virus. N Engl J Med. 2004;350(5): 431-3.
10. Falsey AR, Erdman D, Anderson LJ, Walsh EE. Human metapneumovirus infections in young and elderly adults. J Infect Dis. 2003;187(5):785-90.
11. Wolf DG, Greenberg D, Kalkstein D, Shemer-Avni Y, Givon-Lavi N, Saleh N, et al. Comparison of human metapneumovirus, respiratory syncytial virus and influenza A virus lower respiratory tract infections in hospitalized young children. Pediatr Infect Dis J. 2006;25(4):320-4.
12. Stockton J, Ellis JS, Saville M, Clewley JP, Zambon MC. Multiplex PCR for typing and subtyping influenza and respiratory syncytial viruses. J Clin Microbiol. 1998;36(10): 2990-5.
13. Falsey AR, Formica MA, Walsh EE. Diagnosis of respiratory syncytial virus infection: comparison of reverse transcription PCR to viral culture and serology in adults with respiratory illness. J Clin Microbiol. 2002;40(3):817-20.
14. Hadzhiolova T, Kotseva R, Kalvachev Z, Dundarova D, Mladenova Z. Application of a complex of laboratory methods for identification of Influenza viruses and RSV in participation of Laboratory of Influenza and ARD in Second European Qualitative Control. Infectology. 2004; XLI: 21-26.
15. Hadzhiolova T, Pavlova S, Kotseva R. Possibilities for laboratory diagnosis of Respiratory Syncytial Virus. Biotechnology. 2005;2(19):67-7111.
16. Pavlova S, Hadzhiolova T, Kotseva R, Abadgieva P. Application of RT-PCR for detection of RSV in hospitalized children. Infectology. 2007;1:119-22.
17. ecu C, Orasanu D, Sima A, Mihai M, Alexandrescu V, Matei D, et al.: First detection of human metapneumovirus in children with respiratory infections in Romania. Roum Arch Microbiol Immunol. 2007;66(1-2):37-40.
18. Valdivia A, Savón C, Chacón D, Sarmiento L, Morier L, Otero A, et al. Analysis of respiratory syncytial virus in clinical samples by reverse transciptase-polymerase chain reaction restriction mapping. Mem Inst Oswaldo Cruz.1997;92(3):389-93.
19. Meury S, Zeller S, Heininger U. Comparison of clinical characteristics of influenza and respiratory syncytial virus infection in hospitalised children and adolescents. Eur J Pediatr. 2004;163(7):359–63.
20. Meerhoff TJ, Fleming D, Smith A, Mosnier A, van Gageldonk-Lafeber AB, Paget WJ, et al. Surveillance recommendations based on an exploratory analysis of respiratory syncytial virus reports derived from the European Influenza Surveillance System. BMC Infect Dis.2006;6:128.
21. Zambon MC, Stockton JD, Clewley JP, Fleming DM. Contribution of influenza and respiratory syncytial virus to community cases of influenza-like illness: an observational study. Lancet. 2001;358(9291):1410–6.
22. Van den Hoogen BG, Osterhaus DM, Fouchier RA. Clinical impact and diagnosis of human metapneumovirus infection. Pediatr Infect Dis J. 2004;23(1 Suppl): S25-32.
23. Abiko C, Mizuta K, Itagaki T, Katsushima N, Ito S, Matsuzaki Y, et al. Outbreak of human metapneumovirus detected by use of the Vero E6 cell line in isolates collected in Yamagata, Japan, in 2004 and 2005. J Clin Microbiol. 2007;45(6):1912–9.
24. Garcia-Carcia ML, Calvo C, Martin F, et al. Human metapneumovcirus infections in hospitalized infants in Spain. Archives of Disease in Childhood. 2006; 91:290-295
25. Selwyn BJ. The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. Coordinated Data Group of BOSTID Researchers. Rev Infect Dis. 1990;12 Suppl 8:870-88
26. Garcia-Garcia ML, Calvo C, Perez-Brena P, De Cea JM, Acosta B, Casas I. Prevalence and clinical characteristics of human metapneumovirus infections in hospitalized infants in Spain. Pediatr Pulmonol. 2006;41(9):863-71.
27. Wihley DM, Syrmis MW, Mackay IM, Sloots TP. Detection of human respiratory syncytial virus in respiratory samples by LightCycler reverse transcriptase PCR. J Clin Microbiol. 2002;40(12): 4418-22.
28. Groothuis JR, Wooden KA, Katz R, Robertson AD, McBride JT, Hall CB, et al. Early ribavirin treatment of respiratory syncytial viral infection in high-risk children. J. Pediatr. 1990;117(5):792-8.
29. O`Callaghan CA. Prevention of nosocomial respiratory syncytial virus infection. Lancet. 1993;341(8838):182.
30. Nunes IF, Moura FE. Isolation of respiratory syncytial virus from nasopharyngeal aspirates stored at 20 degrees C from one to fifteen months after collection. Mem Inst Oswaldo Cruz. 2006;101(4):451-3.
31. Greensill J, McNamara PS, Dove W, Flanagan B, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis. 2003;9(3):372-5.
32. Ginocchio CC, Manji R, Lotlikar M, Zhang F. Clinical evaluation of the NucliSENS analyte-specific reagents for the detection of human metapneumovirus in pediatric respiratory specimens. J Clin Microbiol. 2008;46(4):1274-80. Epub 2008 Feb 13.
33. Domegan L, O'Donnell J, Cunney R, O'Kelly E, Dooley S. Increased respiratory syncytial virus activity in Ireland. Euro Surveill. 2002;6(49):pii=1980. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1980
34. Terletskaia-Ladwig E, Enders G, Schalasta G, Enders M. Defiding the timing of respiratory syncytial virus (RSV) outbreaks: an epidemiological study. BMC Infect Dis. 2005;5(1):20.
35. Gioula G, Melidou A, Exindari M, Chatzidimitriou D, Kyriazopoulou-Dalaina V. Contribution of RSV and hMPV to patients with influenza-like infections during 2006-2007 season in northern Greece, 2008; Abstract. 18th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain
36. Arkema JM, Meijer A, Meerhoff TJ, Van Der Velden J, Paget WJ, European Influenza Surveillance Scheme EISS). Epidemiological and virological assessment of influenza activity in Europe, during the 2006-2007 winter. Euro Surveill. 2008;13(34):pii=18958. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18958
37. Kojouharova M, Kurchatova A, Vladimirova N, et al. News Bulletin of the National Center of Infectious and Parasitic Diseases (NCIPD) [in Bulgarian]. 2008;6:4-40.
38. Izurieta HS, Thomson WW, Kramarz P, Shay DK, Davis RL, DeStefano F, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. N Engl J Med. 2000;342(4):232-9.
39. Gasparini R, Ansaldi F, Banfi F, Ferro G, et al. The impact of influenza and respiratory syncytial virus in a 0–14-year children cohort: a comparison with the national network and between methods for influenza virological surveillance. International Congress Series. 2004; 329-33.
40. Meerhoff TJ, Paget WJ, Aguilera JF, van der Velden J. Harmonising the virological surveillance of influenza in Europe: results of an 18-country survey. Virus Res. 2004;103(1-2):31-3.

This article was published on 11 June 2009.

Citation style for this article: Pavlova S, Hadzhiolova T, Abadjieva P, Kotseva R. Application of RT-PCR for diagnosis of respiratory syncytial virus and human metapneumovirus infections in Bulgaria, 2006-7 and 2007-8. Euro Surveill. 2009;14(23):pii=19233. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19233

Pandemic alert level 6: Scientific criteria for an influenza pandemic fulfilled*

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control, Stockholm, Sweden

The World Health Organization (WHO) today announced the decision to raise the level of influenza pandemic alert from phase 5 to phase 6 [1]. This makes the current influenza A(H1N1)v outbreak officially a pandemic.

In its press conference at 18:00 CETS, the WHO emphasised that the severity of the pandemic is, at least currently, moderate. Countries are advised to concentrate on mitigation measures as appropriate for their specific situation. They are not advised to close borders or restrict international traffic and trade as there is no evidence that these measures stop the spread of the disease and are unnecessarily disruptive for international traffic and trade.

At the same time, vaccine manufacturers are working on the production of influenza vaccine specific for the pandemic strain.

Criteria

The pandemic alert phases 5 and 6 reflect the global spread of the outbreak. They do not necessarily reflect a graduation of the severity of the disease in the individual.

While phase 5 is defined as sustained community level outbreaks in at least two countries within a single WHO region, phase 6, the pandemic, is declared when sustained, community-wide human-to human transmission occurs in at least one additional WHO region [2].

The move to pandemic phase 6 is a response to the fact that according to WHO the spread of influenza A(H1N1)v in several countries can no longer be traced to clearly-defined chains of human-to-human transmission and that the scientific criteria for an influenza pandemic have been met.*

As of 11 June, the number of laboratory-confirmed cases of influenza A(H1N1)v worldwide amounts to 28,774, with 144 deaths [3].

Implications

In response to the WHO declaring a pandemic, all countries affected by influenza A(H1N1)v should consider activating national pandemic plans and to implement the measures detailed in them. They include monitoring and reporting of cases, monitoring of resources and compliance, ensuring the availability of vaccine and antiviral drugs, potential limitations on travel and mass gatherings, insuring business continuity, and informing and educating the public.

This has economical implications and puts a strain on staff involved in the pandemic in many areas. However, many of the above measures have already been in place since the declaration of pandemic phase 5. For the individual country, the real change is the moment when it becomes affected, i.e. when it starts seeing significant transmission in the community, whereas the formal move to phase 6 may not imply large changes to the way it is dealing with the situation. According to the ECDC, the declaration of pandemic phase 6, which can be expected to last for several months, does not change the present ECDC risk assessment [4].

Phase 6 is the highest level of pandemic alert. It can be difficult to understand for non-experts why this is triggered in response to a disease that, at least at this stage, is mild in the majority of people, with a low mortality rate published for North America [5]. This can lead to confusion and uncertainty in the population, especially with the tendency of parts of the media to over-emphasise the sensational aspect of such news. This is why the WHO today emphasised once more that the term 'pandemic' describes the geographic spread of the disease rather than its severity and is a means of coordinating world-wide preventive measures.

*Erratum: The following changes were made on 12 June 2009. The title was changed to "Pandemic alert level 6: Scientific criteria for an influenza pandemic fulfilled" and the third paragraph under the heading "Criteria" to "The move to pandemic phase 6 is a response to the fact that according to WHO the spread of influenza A(H1N1)v in several countries can no longer be traced to clearly-defined chains of human-to-human transmission and that the scientific criteria for an influenza pandemic have been met."

References

1. World Health Organization. Current WHO phase of pandemic alert. [Accessed 11 June 2009]. Available from: http://www.who.int/csr/disease/avian_influenza/phase/en/
2. World Health Organization. Pandemic Influenza Preparedness and Response. Geneva: World Health Organisation; 2009.
3. World Health Organization. Influenza A(H1N1) - update 47. June 11, 2009. Available from: http://www.who.int/csr/don/2009_06_11/en/index.html
4. European Centre for Disease Prevention and Control. ECDC situation report. Influenza A(H1N1)v infection. Update 11 June 2009, 17:00 hours CEST. Available from: http://www.ecdc.europa.eu/en/files/pdf/Health_topics/Situation_Report_090611_1700hrs.pdf
5. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, et al. Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings. Science. 2009 May 14. [Epub ahead of print].

This article was published on 11 June 2009.

Citation style for this article: Eurosurveillance editorial team. Pandemic alert level 6: Scientific criteria for an influenza pandemic fulfilled. Euro Surveill. 2009;14(23):pii=19237. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19237

News

Updated risk areas for tick borne encephalitis in Germany

Editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control, Stockholm, Sweden

In its issue on 4 May 2009, the Epidemiologisches Bulletin of the Robert Koch-Institut (RKI), Germany, published an updated map of the risk areas for tick borne encephalitis (TBE) in Germany. TBE risk areas are areas in which TBE is endemic and where, in agreement with experts, a risk exists for people exposed to ticks that justify preventive measures. Such measures include vaccination for locals exposed to ticks, for tourists travelling to risk areas and people with occupational exposure.

The updated map shows that there is a risk of contracting the TBE virus through tick bites in the southern parts of Germany, in particular in the states Baden-Württemberg and Bavaria,

The TBE risk map is based on cases mandatory notification of TBE cases reported to the RKI in the period from 2002 – 2008. In this period there were 1,917 TBE cases notified in total with the annual number ranging from 238 to 546 cases and 288 cases notified in 2008. Five year reporting periods (2002 – 2006, 2003 – 2007, 2004 – 2008), were used to generate five year TBE incidences at the district level. To minimize the probability of underestimating the risk of infection in a particular district with increasing vaccination coverage, the number of reported cases in the so called district region, consisting of the district in question plus the adjacent districts, was created. A district was defined as a risk area if the incidence in the district or the district region was significantly (p-value < 0.05) higher than 1 per 100,000 inhabitants over a five year period.

In 2008, 136 districts were classified as TBE risk areas, four of them for the first time:

- 42 districts in Baden-Württemberg (1 additional district);
- 78 districts in Bavaria (3 additional districts);
- 8 districts in Hesse (unchanged);
- 7 districts in Thuringia (unchanged); and
- 1 district in Rhineland-Palatinate (unchanged).

The accompanying report summarises the TBE risk according to federal state as follows:

- federal states with defined TBE risk areas: Baden-Württemberg, Bavaria, Hesse, Rhineland-Palatinate, Thuringia;
- federal states with isolated cases of autochtonous TBE, in which no district fulfils the criteria of a TBE risk area: Brandenburg, Mecklenburg-West Pomerania, Lower Saxony North Rhine-Westpfalia, Saarland, Saxony, Saxony-Anhalt
- federal states in which no TBE cases have been diagnosed: Schleswig-Holstein, Hamburg, Bremen, Berlin.

Furthermore, it mentions that a slow but steady extension of the TBE risk areas in the past years is mainly limited to the southern federal states.

Surveillance and reporting of TBE cases are seen as the most efficient and cost effective basis on which to base preventive measures. However, should the incidence of cases drop due to the increase in vaccination, supplementary indicators for the risk of capturing TBE may become necessary in the future according to RKI. In this context, data on vaccination coverage, establishment of systematic surveillance of the number of ticks, the ratio of ticks carrying the TBE virus or the number of animals infected, become important.

Based on the above mentioned epidemiological criteria, the German Standing Vaccination Committee (STIKO) recommends TBE vaccination for people who live in districts where the risk of exposure to ticks is established. Vaccination against TBE is recommended by the local health authorities in Baden-Württemberg, regardless of district (only two districts are not classified as risk areas).

References

1. Robert Koch-Institut. [TBE: Risk Areas in Germany] [In German] Epid Bull 2009;18:165-72

This article was published on 11 June 2009.

Citation style for this article: Editorial team. Updated Risk areas for tick borne encephalitis in Germany. Euro Surveill. 2009;14(23):pii=19236. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19236