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Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in winter 2010/11

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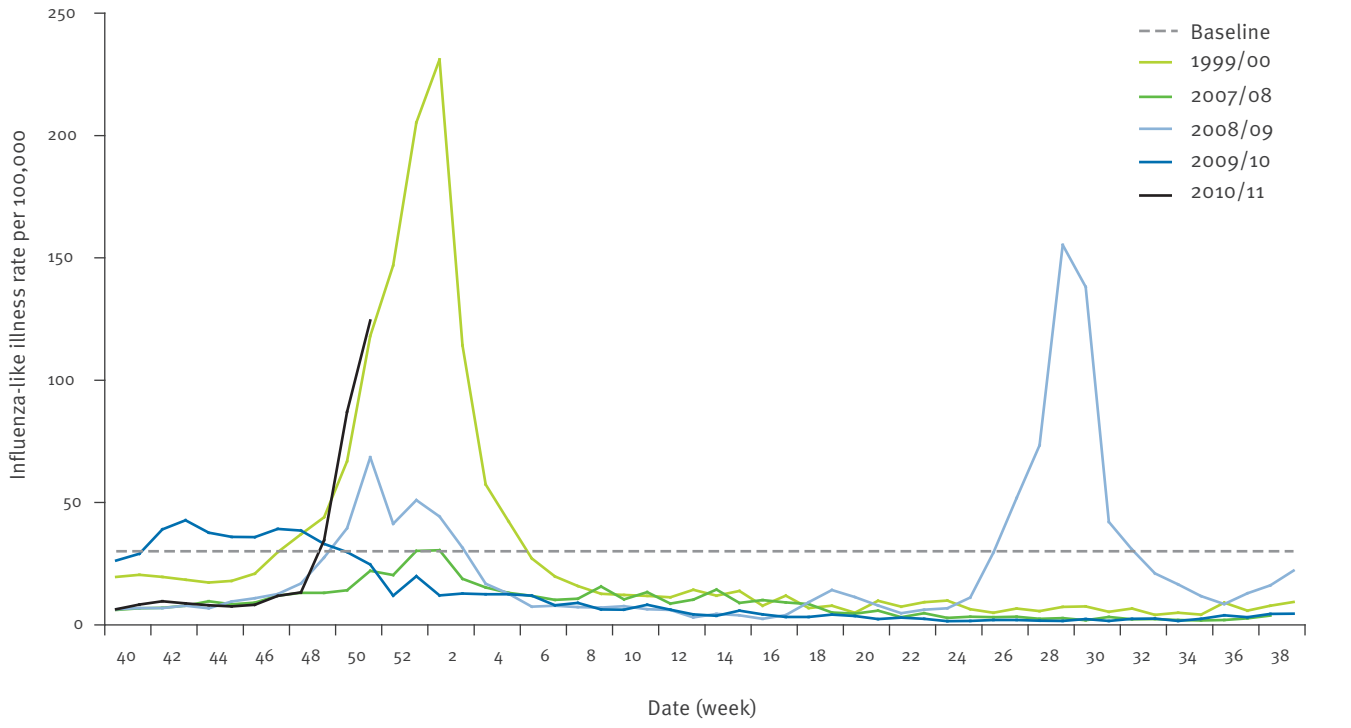
The 2010/11 winter influenza season is underway in the United Kingdom, with co-circulation of influenza A(H1N1)2009 (antigenically similar to the current 2010/11 vaccine strain), influenza B (mainly B/Victoria/2/87 lineage, similar to the 2010/11 vaccine strain) and a few sporadic influenza A(H3N2) viruses. Clinical influenza activity has been increasing. Severe illness, resulting in hospitalisation and deaths, has occurred in children and young adults and has predominantly been associated with influenza A(H1N1)2009, but also influenza B viruses.

Introduction

The onset of this winter season in the northern hemisphere is associated with more uncertainty than usual about which influenza viruses are likely to circulate and predominate, given the varying proportions of different virus strains circulating in the southern hemisphere between June and September 2010 [1]. Notably, influenza A(H3N2) predominated over influenza A(H1N1)2009 in several countries, e.g. South Africa and Chile. The second wave of the pandemic in the United Kingdom (UK) during the winter season of 2009/10 was almost exclusively associated with circulation of influenza A(H1N1)2009 [2]. Serological evaluation in the UK of population immunity to the pandemic strain after the second wave suggested that susceptibility was lowest in younger age groups (‹15 years), with significant remaining susceptibility in the age group of 15–44 year-olds [3]. In view of the importance of children in the transmission of influenza A(H1N1)2009 [4], and the limited remaining susceptibility within this group, the

Figure 1

Royal College of General Practitioners influenza like illness consultation rates, England and Wales, current and past seasons



probability of extensive morbidity in this age group associated with this strain in winter 2010/11 was considered unlikely in the absence of significant antigenic change in the pandemic virus. The extent, however, to which influenza A(H1N1)2009 would predominate over influenza A(H3N2) and cause illness in the remaining susceptible children and younger adults was unknown.

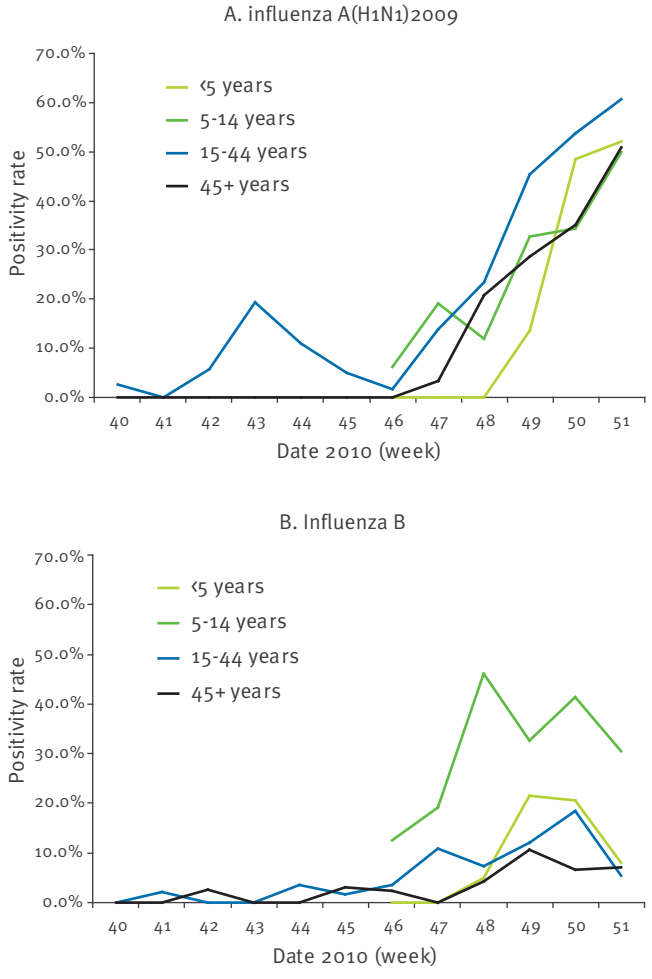
Investigations

Virological surveillance in the UK operates through hospital laboratories in secondary care and community-based schemes. Specimens containing influenza virus from community, hospitalised and fatal cases are forwarded to the UK National Influenza Centre for further characterisation. Samples are also received directly from sentinel primary care physicians participating in virological surveillance schemes in the community [5]. An antigenic typing profile is developed for each virus isolate and compared with influenza vaccine and reference strains. Genotypic and, where appropriate, phenotypic antiviral susceptibility analyses are performed on influenza-positive clinical material and/or virus isolates. Genetic characterisation is performed by targeted haemagglutinin (HA) sequence analysis and/or whole genome sequencing for a subset of isolates (primer sequences available on request).

We describe here observations undertaken as part of routine national surveillance. These are carried out under National Health Service (NHS) Act 2006 (section 251), which provides statutory support for disclosure of such data by the NHS, and their processing by the Health Protection Agency (HPA) for communicable disease control [6].

Figure 2

Influenza positivity rates from community sentinel virological surveillance in England by age, 4 October-26 December 2010



Rates with sample number less than 10 are not presented. Recent weeks' data may not be complete due to reporting time lag.

Epidemiological findings

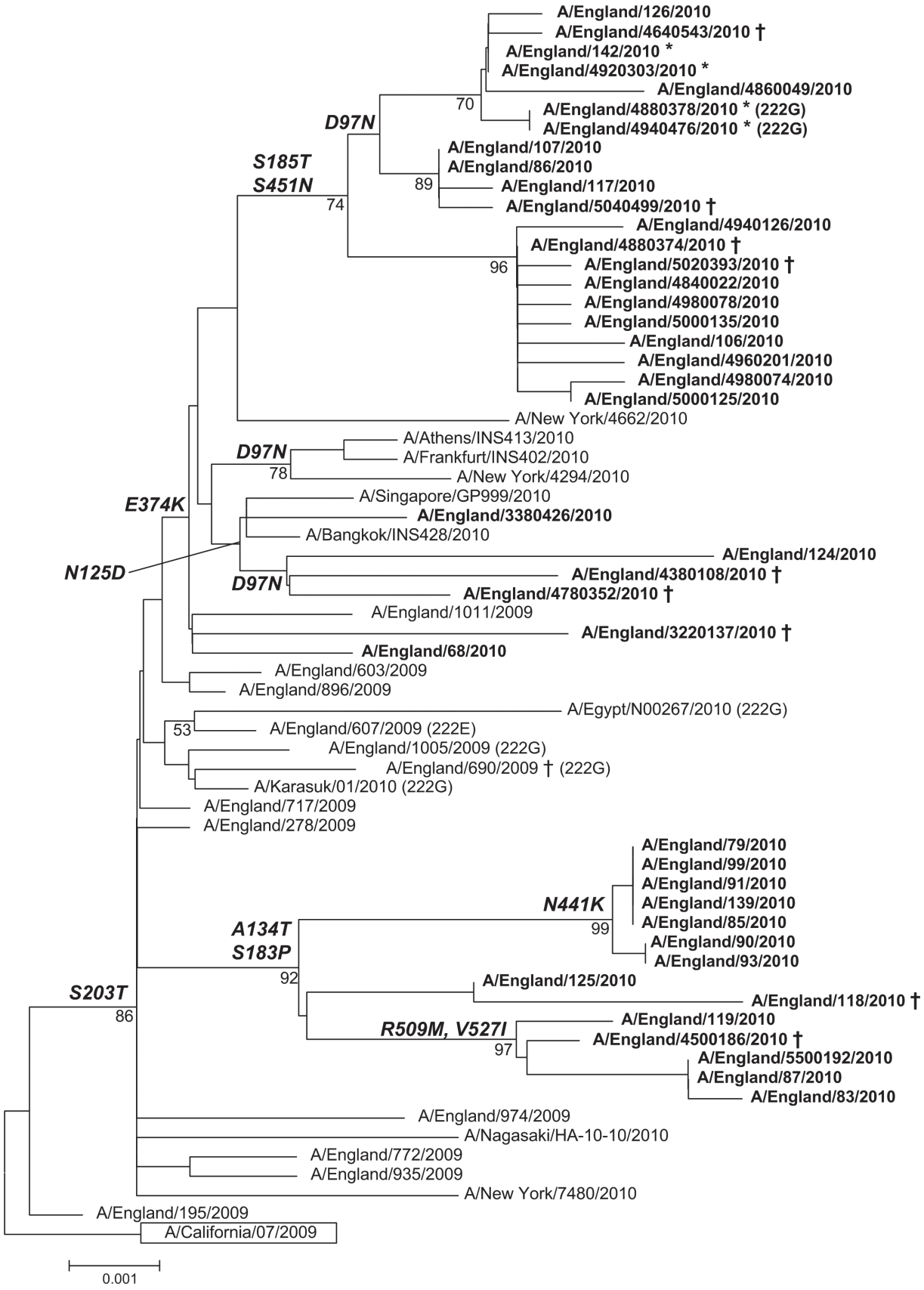
Early detections of influenza A(H1N1)2009 virus were first reported in weeks 40–42 from cases in the community. Indicators of clinical activity began to rise in week 47 crossing the traditional baseline threshold level indicating generalised influenza activity in the community in week 49, and have continued to increase up to week 52 (Figure 1).

Influenza A(H1N1)2009 viruses, followed by influenza B, have been the predominant influenza viruses circulating in the community in the period from October to the end of December 2010. Of 3,959 respiratory specimens reported to the English Data Mart system as taken in week 51, 1,711 (43.2%, increased from 38.9% in week 50) were positive for influenza, namely 1,402 influenza A(H1N1)2009, 41 not subtyped influenza A and 268 influenza B [7]. Since the beginning of the season, over 120 institutional outbreaks of respiratory illness have been reported, primarily from schools: 112 (93%) outbreaks from schools, four from care homes, two from hospitals, one from a military base, one from a nursery and two from prisons. Both influenza B and influenza A(H1N1)2009 have been detected in the few outbreaks that have been virologically investigated and confirmed: 22 outbreaks (44%) with influenza A(H1N1)2009 detected, 16 with influenza B, four with a mixture of influenza A(H1N1)2009 and influenza B, one with influenza A(H3N2) and seven with other respiratory viruses.

Admissions to hospital with severe illness have been reported. As of 30 December 2010, there were 738 patients with confirmed or suspected influenza in NHS critical care beds in England (42 cases under five years of age, 24 cases between five and 15 years, 586 cases between 16 and 64 years, and 86 cases 65 years and above) [8]. Thirty-nine deaths were reported between weeks 36 and 52 associated with confirmed influenza infection [7]. Four of the fatal cases were under five years of age, seven were 5-14 years of age, 27 cases were 15-64 years of age, and one fatal case was older than 64 years. The majority (36/39) of these deaths were associated with influenza A(H1N1)2009 infection, and three with influenza B infection. Underlying chronic conditions were reported in 23 of the 38 fatal cases for whom this information was available, with neurological disease such as cerebral palsy (n=9) and asthma

Figure 3

Phylogenetic relationship of full-length HA sequences of influenza A(H1N1)2009 viruses from fatal, severe and mild cases in the United Kingdom during 2010



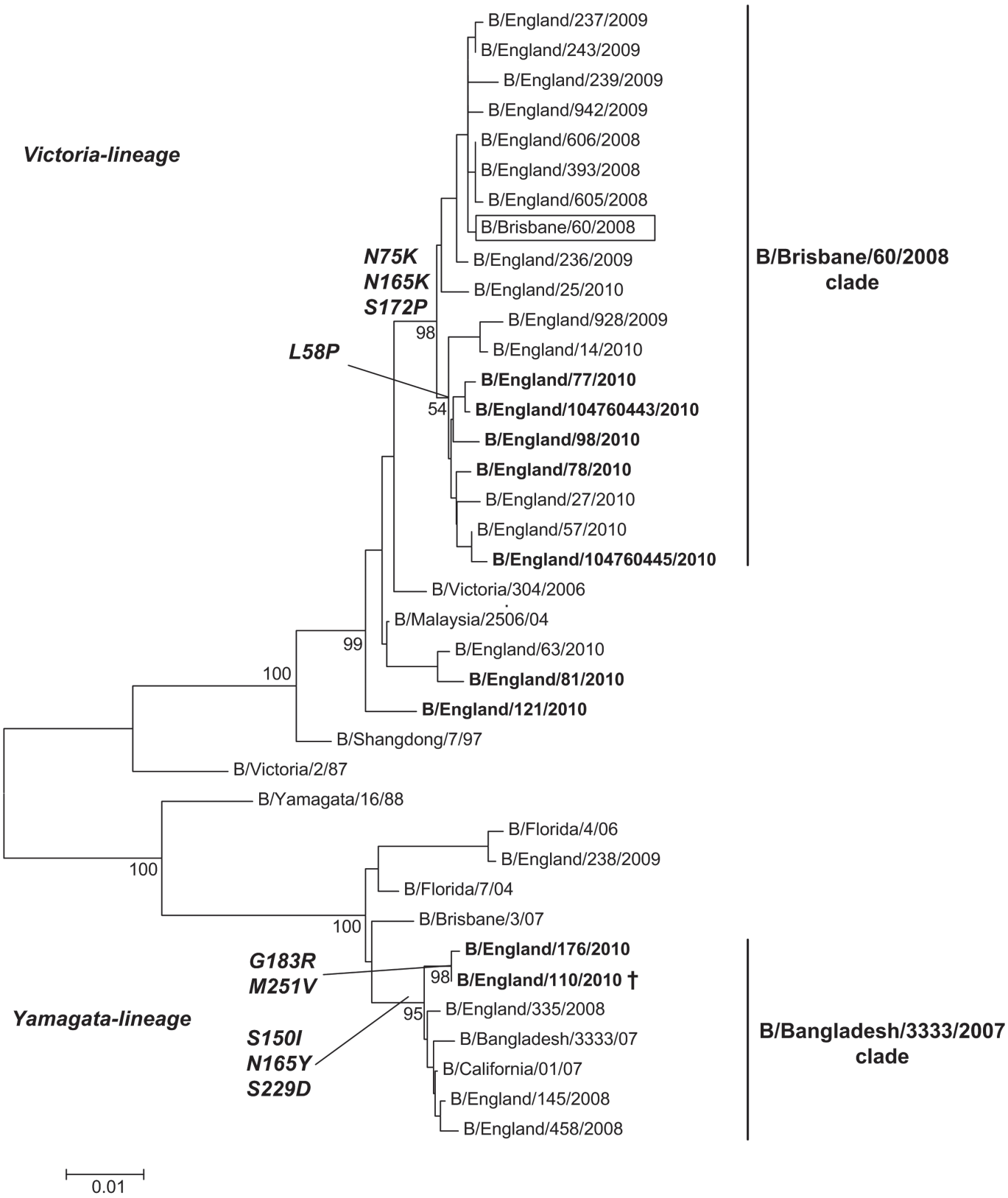
HA: haemagglutinin.

† Fatal case; * severe case.

Sequences downloaded from the NCBI Influenza Virus Resource (http://www.ncbi.nlm.nih.gov/genomes/FLU/) representative of globally circulating viruses during 2010 and, in bold, UK sequences from the first and second pandemic wave of 2009 were included. The tree was rooted with the vaccine strain A/California/07/2009 (boxed) as outgroup. Branch lengths are drawn to scale. Signature amino acid changes (H1 numbering) are annotated at the nodes of each cluster. Viruses with 222G or 222E changes are marked in the tree.

Figure 4

Phylogenetic relationship of HA1 sequences of influenza B viruses from fatal and mild cases in the United Kingdom during 2010



HA: haemagglutinin.

† Fatal case.

Branch lengths are drawn to scale. Amino acid changes characteristic of clades are marked in the tree. Sequences from UK 2010 viruses are in bold, and the 2010/11 vaccine strain is boxed.

(n=8) the most frequently reported underlying risk factors for vaccination [9]. Very few of the fatal cases (2/33) had received the 2010/11 trivalent influenza vaccine. A third of the cases (8/22) had not received antiviral therapy.

The proportion of samples from patients with influenza-like illness in sentinel general practitioner surveillance schemes in the community reported positive for influenza virus (A(H1N1)2009 or B) has risen rapidly to over 50% in week 49. The proportion of samples positive for influenza A(H1N1)2009 virus was highest in young adults (15-44 years), and for influenza B in children aged 5-14 years (Figure 2).

By week 50, the proportion of the population in England aged under 65 years in a risk group who had received the 2010/11 influenza vaccine was 43% [7].

Virological investigations

Influenza A(H1N1)2009 isolates characterised to date, in samples from the community, hospitalised patients and fatal cases, are antigenically homogeneous and similar to the A(H1N1)2009 virus included in the 2010/11 seasonal influenza vaccine, A/California/7/2009. Only minor genetic drift has been noted in influenza A(H1N1)2009 viruses circulating in 2010 compared with the earliest isolates in April 2009, and this observed genetic diversity has been consistent with expected patterns of virus evolution (Figure 3). Phylogenetic analysis shows that HA sequences from nine fatal and four severe cases in the UK in 2010 were interspersed with sequences from mild cases in 2010 from the UK and elsewhere. All UK 2010 viruses cluster in two main branches, characterised by either E374K with additional mutations in minor subclusters such as D97N, S185T, S451N and N125D, some of which have been recently described [10], or by A134T and S183P, with additional substitutions such as N441K, R509M and V527I. Almost all viruses from winter 2010 analysed to date from fatal and non-fatal cases had 222D in the HA gene (39/41).

Preliminary analyses from a limited number of whole genome sequences including some from fatal cases, indicate that these are consistent with observations from seasonal influenza and from the first and second waves of the recent pandemic: so far no unique mutations have been associated with severe or fatal cases of influenza A(H1N1)2009, but further comprehensive analysis is required.

Between October and December 2010, antiviral resistance monitoring was undertaken on 156 community and 159 hospital isolates. Six cases of oseltamivir resistance associated with the H275Y mutation in the neuraminidase (NA) gene have been detected.Only one of these cases has had known exposure to oseltamivir, Two of them have been identified from community surveillance of uncomplicated infections, three cases have been detected before treatment in individuals hospitalised with underlying risk factors, and the sixth case has been detected after oseltamivir treatment in a hospitalised individual.

Over 98% of influenza B viruses isolated in the UK since week 40 in 2010 have been from the B/Victoria/2/87 lineage, with most showing good reactivity to antisera raised against reference viruses from this lineage. The HA sequences group within the genetic clade represented by the current vaccine strain, B/Brisbane/60/2008, characterised by amino acid substitutions L58P N75K, N165K and S172P (Figure 4). A separate small cluster of three viruses from the antigenically distinct B/Yamagata/16/88 lineage have also been detected in one region of England: one fatal case and two hospitalised cases. The three known fatal influenza B cases were distributed across both lineages. The HA segment of the influenza B/Yamagata lineage virus isolated from a fatal case in week 46 belonged to a clade represented by influenza B/Bangladesh/3333/2007, with amino acid substitutions S150I, N165Y and S229D relative to a previous vaccine strain, B/Florida/4/06. This HA sequence contained two additional substitutions, G183R and M251V, which had been sporadically detected in influenza B viruses isolated in several countries in 2009/10.

Antigenic characterisation of the few influenza A(H3N2) viruses detected since week 38 indicates that these viruses are closely related to A/Perth/16/2009, the influenza A(H3N2) 2010/11 vaccine strain.

Conclusions

Influenza virus circulation is underway in the UK and is contributing to seasonal winter pressures in the health system. The circulation of other winter viruses such as respiratory syncytial virus (RSV) and the particularly cold weather are also contributing. The virological picture is complex, with many strains of influenza virus circulating but no antigenic change in the influenza A(H1N1)2009 virus, and no immediately obvious genetic differences between viruses recovered from fatal cases and those causing mild illness. The picture of the illness associated with influenza A(H1N1)2009 infection is consistent with what was seen in the 2009 pandemic, with a similar demographic impact, particularly affecting children and young adults. Whilst young age groups have the least experience of influenza and are recognised as important in the transmission of influenza, it is also possible that propensity to consult a doctor is greatest in younger age groups. Although the remaining susceptibles in the age group under 15 year account for high rates of positivity in peak weeks in community samples (as is often the case during seasonal influenza), it is notable that overall, sustained high rates of positivity are most marked in the age group between 15 and 44 years. This is in contrast to earlier pandemic waves in 2009 when highest rates of positivity in the community were observed in the 5-14 year-olds. The age group of 15-44 year-olds is also clearly the major group contributing to hospital admissions and deaths. The increase in requirement for

critical care in the current season reflects the impact of influenza A(H1N1)2009 illness in the remaining susceptible young adults (15-44 years) and risk groups in the population.

Most of those with severe illness, and those dying, have not previously been vaccinated against influenza and have not had the benefit of the early use of antiviral drugs. Countries in Europe yet to experience substantial influenza activity this winter may wish to take all reasonable measures to increase the uptake of seasonal influenza vaccine in those at high risk of the complications of influenza and to ensure that antiviral drugs are readily available for those who are either severely ill or at increased risk of severe illness from influenza.

Further analysis of the antigenic and genetic properties of all influenza viruses from hospitalised patients, outbreaks and community cases is ongoing.

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Fatal acute melioidosis in a tourist returning from Martinique Island, November 2010

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We report the fatal case of acute melioidosis in a patient returning from Martinique with fever in November 2010. Gram-negative rods were isolated from a blood culture and *Burkholderia pseudomallei* identified within 24 hours after first medical contact. The patient died two days after admission to hospital despite intravenous therapy with high doses of imipenem/cilastatin and intensive care. Clinicians seeing travellers returning from the subtropics or tropics with severe pneumonia or septicaemia should consider the possibility of acute melioidosis.

Introduction

Melioidosis is an infectious disease of public health importance that is endemic in southeast Asia and northern Australia. However, cases are increasingly recognised in other parts of the world, including Africa, Latin America, and the Middle East. In the Caribbean, few cases of melioidosis have been reported: in Puerto Rico during flood waters, in Guadeloupe and a single case 15 years ago from an inhabitant of Martinique [1-3]. Melioidosis is occasionally reported in travellers returning from Asia, including in three Finnish tourists after the tsunami of 2004 [4-10]. Melioidosis in travellers from other regions is rarely reported: a tourist who presented with acute fatal septicaemic pneumonia due to melioidosis after a visit to Brazil was diagnosed in the Netherlands [11].

Burkholderia pseudomallei, the causative organism, is a saprophyte living in soil and surface water [12]. Although the exact mode of transmission of this disease remains unknown, percutaneous inoculation and especially exposure of non-intact skin to contaminated soil or surface water is mostly reported in the literature [13]. Other modes of transmission include inhalation of contaminated water or soil dust, laboratory hazards and, rarely, person-to-person through contact with blood or body fluids [14].

The clinical presentation and the spectrum of severity range from fulminant sepsis to chronic disease. The most common manifestations are pneumonia, sepsis and intra-abdominal suppuration. Other common manifestations include parotitis, abscesses of the prostatic gland and of other organs as well as encephalomyelitis [12]. The incubation period can be as short as one day; however, years may lapse between presumed exposure and appearance of the chronic clinical disease [14]. Despite optimal antibiotic treatment, acute melioidosis is still associated with significant mortality, between 30% and 50% in cases with severe sepsis [12].

Case report

A healthy man in his 30s living in Switzerland visited the east coast of Martinique 10 days in November 2010. He suffered from a few episodes of mild watery diarrhoea for some days during this period and became febrile on the last day of his stay, with chills, headache, abdominal cramps and lower back pain, without articular or neurological complaints. He did not seek medical advice until two days later, following his return home, when he was admitted to the Geneva University Hospitals on 25 November.

Biological findings showed systemic inflammatory response syndrome with a leukocyte count of $10x10^9$/L, 97% of neutrophils and 39% band forms (immature white blood cells), elevated C-reactive protein (315mg/L) and elevated transaminases: aspartate aminotransferase 257 IU/L (norm: 14-50) and alanine aminotransferase 128 IU/L (norm: 12-50). Gram-negative rods grew from blood cultures after 24 hours of incubation. Mass spectrometry (MS) was performed according to standard practise in our laboratory [15].

Burkholderia sp. was identified on a very thin layer of bacteria grown on blood agar two hours after subculture. The identification score from the MS was 1.8, corresponding to secure identification at the gender level (according to the manufacturer). Initially medical staff suspected typhoid fever, and the patient was treated with ceftriaxone. Once the first bacteriological results were available, this was changed to high-dose imipenem-cilastatin therapy. The presence of *Burkholderia* sp. in the blood of a previously healthy patient returning from subtropical area, and developing rapidly progressive respiratory failure led to the presumption of melioidosis. MS was performed again the same day, 26 November, on a single well-grown colony and a score >2 of *B. thailandensis* was identified. Hereafter, sequencing of the 16S rDNA confirmed the presence of *B. pseudomallei* in all blood cultures.

Despite the absence of respiratory symptoms and a normal initial chest X-ray on 25 November, the patient developed respiratory failure the following day, due to severe bilateral abscess-forming pneumonia. An X-ray and a computed tomography at 36 hours after admission showed bilateral infiltrates with nodular lesions probably corresponding to micro-abscesses. Dysuria with pyuria appeared 36 hours after admission. The patient died on the second day of admission from septic shock with multi-organ failure and acute respiratory distress syndrome, in spite of additional therapy with granulocyte colony-stimulating factor (G-CSF), low-dose steroids and intensive care including extracorporeal membrane oxygenation.

Discussion

This is the first reported case, to our knowledge, of melioidosis in a traveller returning from Martinique. Clusters of cases are known to occur mainly in hyperendemic countries. But following natural disasters (storms, typhoons, tsunamis) they are also seen in areas of lower endemicity [16-19]. At the end of October 2010, Martinique was affected by hurricane Tomas. Two weeks later, following three days of heavy rain, the areas visited by our patient experienced flooding. Severe melioidosis is strongly correlated with heavy rain, considered an independent risk factor for a pneumonic presentation, septic shock, and death [19].

The majority of cases are seen in patients with a predisposing medical condition such as diabetes, cirrhosis, alcoholism or renal failure. One third of infections affect healthy subjects, as in the case of our patient [14]. As soon as the diagnosis of melioidosis was suspected, more details of his medical history were recorded: risk factors such as walking in the mud in a forest and across recently flooded areas with multiple scratched mosquito bites on his legs were identified. Information about suspected contaminated areas in Martinique where the patient had walked in the mud and names of other tourists exposed to the mud (the patient participated in a surf camp with several French tourists) was communicated within two days following the patient's hospital admission to the local clinicians in Martinique, international travel medicine networks (EuroTravNet, TropNetEurop and GeoSentinel), as well as to Swiss and French national health authorities. Following our informing the local hospitals in Martinique, two additional cases were suspected and antibiotic therapy immediately adapted. Local authorities will investigate areas considered as suspicious for potential transmission of the pathogen.

Rapid diagnostic methods are critical as the clinical presentation of melioidosis is non-specific and treatment requires specific antibiotics. Conventional techniques, including Gram staining and culture remain the mainstay of diagnosis for melioidosis, but require 24 hours for isolation and an additional 48 hours for species identification [12]. In our case, MS initially detected *B. thailandensis*. *B. thailandensis* cannot be reliably distinguished from *B. mallei* or *B.pseudomallei* by MS. *B thailandensis* and *B. pseudomallei* are very closely related species with similar susceptibility to antibiotics, but with a completely different clinical spectrum. Infections caused by the former are usually asymptomatic or cause mild disease, whereas infections caused by the latter are always highly pathogenic and cause acute or chronic manifestations [20].

B. pseudomallei has been classified as a potential bioterrorism agent and should be handled with biosafety level 3 in laboratories. Few cases of laboratory-acquired melioidosis have been reported. The level of risk exposure in the laboratory determines the need for post-exposure prophylaxis [21]. In our case, two laboratory assistants were exposed to aerosols of *B. pseudomallei* cultures and another two were exposed to patient specimens (urine and faeces). Exposure to the latter was considered low-risk and post-exposure prophylaxis (PEP) was stopped after 72 hours (culture negative). Exposure to positive culture aerosols was considered to be high-risk and PEP using the 3-weeks co-trimoxazole scheme proposed by Peacock *et al*. was performed [21].

Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) MS provides rapid identification of the agent of melioidosis, with the potential to improve the prognosis of fulminant melioidosis [15,22]. However, the availability of this new technology in developing countries is still limited. Identification techniques such as indirect haemagglutination and other serological tests have limited utility for clinical case management [13]. The gold standard for identification still remains 16S rDNA sequencing, but this is time-consuming and costly.

All cases of melioidosis, even mild disease, should be treated with initial intensive therapy (two to four weeks of intravenous antibiotics). Recommended intravenous therapy is ceftazidime, imipenem or meropenem [13]. G-CSF is sometimes proposed as adjuvant therapy and low-dose steroids are suggested as beneficial in

refractory shock [12]. Intensive therapy should always be followed by at least 12 weeks of oral eradication therapy, using typically trimethoprim–sulfamethoxazole with doxycycline [12].

Conclusion

As clusters of melioidosis are known to occur, local medical authorities and international travel medicine networks should be informed quickly when a case is diagnosed. This could be particularly critical when the contaminated area is a touristic location where the disease is rarely reported. Prompt reporting facilitates the investigation of possible additional cases, the intensification of security measures in laboratories, and the realisation of a field study to analyse the soil in suspect areas.

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Cluster of Legionnaires' disease in a newly built block of flats, Denmark, December 2008 – January 2009

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During December 2008 to January 2009, two persons contracted Legionnaires' disease in a newly built block of flats in a suburb of Copenhagen in Denmark. Polymerase chain reaction and culture was used to diagnose Legionnaires' disease in this cluster. Isolates from both patients tested positive for *Legionella pneumophila* serogroup 1 subgroup Philadelphia sequence type 1 and the same strain was detected in hot water samples taken from the residential area indicating that the hot water supply system was the most likely source of infection. *Legionella* was not detected in the cold water. Two interventions were conducted to limit the *Legionella* colonisation of the piping and storage tanks and the effect was monitored by investigating water samples from various sites in the block of flats. Only the second intervention had a sufficient effect on the *Legionella* colonisation. The cluster described here points to several risk factors regarding growth of *Legionella* in hot water systems: (i) stagnancy of water from when the building is constructed and piping installed and until residents move in, (ii) stagnancy and low temperature (from room temperature to approximately 38 °C) of water in shower hoses and (iii) failure in operation of and control measures for the hot water system.

Introduction

Legionnaires' disease (LD) is a severe pneumonia with high mortality caused by the inhalation of aerosolised *Legionella* bacteria. *Legionella* occurs naturally in water sources but the bacteria multiply to high numbers at temperatures between 20 °C and 45 °C [1]. The multiplication of *Legionella* is, associated with several other factors apart from water temperature such as water being stagnant, type of pipe material used, the presence of a biofilm (a micro-environment between surface and water) and amoebae [1,2]. These factors are the reasons why man-made water systems, often harbour *Legionella* in high numbers. Outbreaks of LD are often associated with aerosols from cooling towers [3,4], spas [1,5,6], and hot and cold water systems at hospitals [7,8] and hotels [9].Studies have shown that domestic hot water systems are often colonised with *Legionella* [10,11] but outbreaks are rarely associated with potable water distribution systems.

Each year 100-130 cases of LD are notified in Denmark (approximately 20 per million) which is a rather high incidence compared to other European countries [12]. Most of the cases are sporadic and only few outbreaks have been identified. This study was conducted to investigate factors associated with risk of *Legionella* colonisation in new buildings and to monitor and investigate the effect of control measures.

Cluster description

A cluster of two culture-confirmed LD cases was identified during December 2008 to January 2009 in a suburb of Copenhagen in Denmark. Neither case had any recent history of travel. On 11 November 2008, the first case (Case 1), a man in his early forties with an underlying condition, was hospitalised 250 km away from the building that was later found to be the source of infection. Case 1 was linked to the block of flats only after the second case (Case 2) was diagnosed, since he had only spent a few days in the newly built block of flats, in an apartment which had not been used before. On 30 December the second patient (Case 2), a man in his mid-sixties who had been treated for an underlying condition, was hospitalised and on 5 January 2009 he was diagnosed with LD. He died 20 days after admission. Case 2 lived in the building later found to be the source of infection. His family had earlier complained about the low temperature of the hot water in the apartment and the hot water of this apartment was therefore the first to be investigated.

Both cases were positive for *L. pneumophila* by polymerase chain reaction (PCR) on samples from tracheal secretions and *L. pneumophila* was subsequently isolated by culture by standard techniques. Isolates were identified by agglutination test (*Legionella* latex test DR0800M, Oxoid); and sero- and subgrouping were performed with monoclonal antibodies [13]. Extracted DNA was analysed by sequence-based typing (SBT) according to the European Working Group for *Legionella* Infections (EWGLI) standard

procedure [14]. Both cases were found to be infected with *L. pneumophila* serogroup 1 subgroup Philadelphia sequence type (ST) 1.

All residents of the block of flats, as well as visitors, were informed about the outbreak and advised to go and see their general practitioner and take blood samples if showing symptoms of LD and 16 of these chose to do so. These 16 samples were tested for *Legionella* antibodies. Three of them were also tested for *Legionella* urinary antigen but none of the samples were positive. None had pneumonia but some may have had Pontiac fever based on clinical symptoms (influenza-like illness caused by *Legionella* infection), although this was not confirmed by laboratory tests.

Methods

The water system

The building identified as the most probable source of contamination had 225 apartments distributed in six blocks. Of these 225 apartments, 210 were inhabited at the time the cluster was detected. The hot water system had two boilers in use and a circulation pump in place to circulate the hot water. In each stairway, proportional thermostatic adjusting valves set at 50 °C regulated the flow of hot water. The water supply in the area was based on ground water, which was not disinfected and distributed without disinfection residuals.

Sampling and analysis

Water was collected from the building on eight occasions from 9 January to 7 September 2009. Two types of water samples were collected each time from kitchen and bathroom taps as well as from shower hoses: A-samples – the first litre of water (first flush) – and B-samples – one litre collected after flushing until constant water temperature (warm or cold) was reached. Apartments located as distant from and as close to the boiler as possible and apartments with no, low or normal levels of water consumption, as well as apartments associated with the cases, were sampled. B-samples from shower hoses all had a temperature of 38 °C due to the thermostatic mixing valves installed on shower fixtures in all apartments.

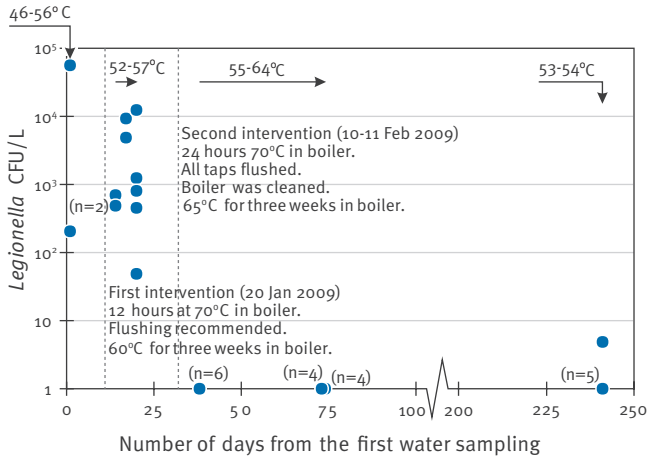
The temperature ranges in the samples in the different apartments are given at the top of the figure. The first water samples were collected on 9 January 2009. Unless otherwise indicated, each dot represents one sample. The dotted lines indicate the two interventions.

The first water samples were collected on 9 January 2009. Unless otherwise indicated, each dot represents one sample. The dotted lines indicate the two interventions.

Testing of water samples for the detection of environmental *Legionella* by cultivation was done according

Figure 1

Concentration of *Legionella* spp. in the hot water system (B-samples[a]), data collected from seven different apartments, Copenhagen, Denmark, January – September 2009



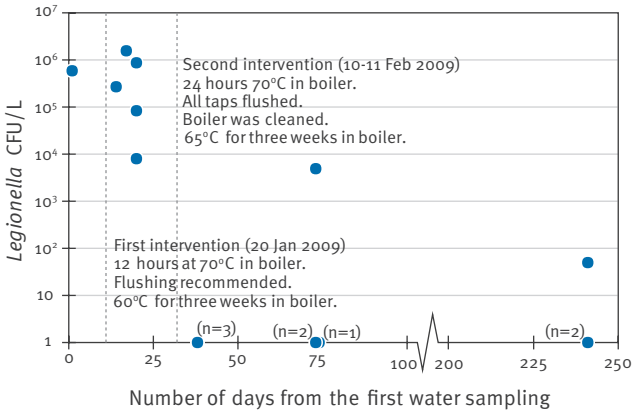
CFU: colony forming unit.

[a] One litre samples collected after flushing until constant water temperature (warm or cold) was reached.

Concentration of *Legionella* spp over time in the hot circulation water in the block of flats. The water samples were collected after flushing until constant temperature (B-samples), and the temperature interval in the samples in the different apartments are given at the top of the figure. Each dot represents one sample but there are two samples with 800 CFU/L on day 20, six samples with no *Legionella* detected on day 38, four samples with no Legionella detected on day 73, four samples with no *Legionella* detected on day 74 and five samples with no *Legionella* detected on day 241. The dotted lines indicate the first and the second intervention, respectively. The first water samples were collected on 9 January 2009.

Figure 2

Concentration of *Legionella* spp. in the first litre of water sampled (A-samples[a]), data collected from seven different apartments, Copenhagen, Denmark, January – September 2009



CFU: colony forming unit.

[a] A-samples are the first litre of water from the shower hose (first flush).

Concentration of *Legionella* spp over time in the first litre of water sampled (A-samples) from different shower hoses. There are 3 samples on day 38, where no *Legionella* could be detected. Each dot represents one sample but there are two samples on day 73 and one sample on day 74 where no Legionella could be detected. At day 241, there are two samples with no *Legionella* detected. The dotted lines indicate the first and the second intervention, respectively. The first water samples were collected 9 January 2009.

to the ISO standard 11731:1998 [26]. One-litre water samples were concentrated 10-fold and 100-fold respectively by 0.2 µm membrane filtration and subsequent centrifugation. Modified Wadowsky Yee and Glycin,Vacomycin, Polymyxin, Cycloheximide agar plates were both seeded with 0.5 ml water directly from the sample before concentration, 0.1 ml after filtration and 0.1 ml after centrifugation Plates were incubated at 37 °C for seven to 10 days before colonies were counted. The highest colony count from any of the three steps was considered the result and expressed as colony-forming units (CFU) per litre.

Isolates from water were analysed as isolates from clinical samples, but only two environmental isolates of *Legionella pneumophila* serogroup 1 subgroup Philadelphia from two different samples were selected for further DNA typing as described above. Isolates (>10) identified as *Legionella* species by OXOID where identified to species level by sequencing of the *Legionella mip* gene [15].

Control measures

To control the *Legionella* contamination of the hot water system, two interventions were conducted. The first was initiated on 16 January 2009 (11 days after the diagnosis of Case 2) when the temperature and the flow of the water system were increased. On 20 January, water in the boiler was heat-treated at 70°C for 12 hours together, after which all residents were requested to flush their taps for five minutes. Subsequently, the water in the boilers was completely replaced with fresh water and the temperature was reduced to 60 °C for three weeks. Circulation pumps were set at maximum flow.

The second intervention was performed on 10–11 February 2009. For 24 hours the water in the boilers was heated to approximately 70°C and all taps were flushed for five minutes. The hot water temperature in the taps was kept at a minimum of 65 °C. The boilers were hyperchlorinated and the temperature was set at 65°C. All shower hoses in all apartments were replaced with new ones and over the next month the boiler temperature was regulated to ensure the water in the most distant taps was kept at 50°C. To monitor how the second heat treatment affected the *Legionella* level in the long term, samples were collected one week, six weeks and seven months after the intervention.

The design, dimensions and regulation of the hot water system, including boilers, pumps, valves and control procedures were evaluated by consulting engineers.

Information to residents

The residents were informed about the *Legionella* colonisation of the water system by letters delivered to each apartment on 15, 20 and 21 January 2009. In addition, posters were displayed on the entrance doors of the building, and an information meeting for residents and visitors was organised on 4 February 2009 by the administration . Residents and visitors to the block of flats who had symptoms compatible with *Legionella* infection (influenza-like symptoms and/or respiratory symptoms) were asked to contact their general practitioner for consultation and collection of samples for laboratory testing.

Results

Water samples

The sample collected after flushing (B-samples) from the tap in the apartment of Case 2 revealed a hot water temperature below 50 °C (46 °C after 15 minutes of flushing) and 5.5 x 10^4 *Legionella* CFU/L by culture (Table 1) whereas the temperature should be above 50 °C as a minimum.

The apartment of Case 2 was situated far from the boilers (only two apartments were placed further away in that direction). The first flush samples (A-samples) collected from shower hose in the apartment of Case 2 showed more than 6 x10^5 CFU/L. This shower hose had rarely been in use, so water had been stagnant for several days. *L. pneumophila* serogroup 1 subgroup Philadelphia was found in both the A-sample and in the B-sample tapped after 15 minutes. One B-sample was collected from a tap in an apartment very close to the boilers; the temperature measured 56 °C and only 2 x 10^2 CFU/L were detected in that sample. Only *L. pneumophila* serogroup 3 was found in the sample. The subgroup Philadelphia isolated from the patient's apartment was also found to be ST 1.

The water system

During the investigation to reveal the cause of the low hot water temperature in the apartment of Case 2, operational problems were detected. These problems were caused by a combination of low flow in the hot water system and inadequate temperature in the boilers. The circulation pump was adjusted to low capacity, which made the circulation slow. The slow circulation was also caused by small pipe dimensions. In fact the resistance in the pipes was so high that the water was prevented from circulating at the required flow. The slow circulation caused heat loss, and despite the thermostatic adjusting valves being opened, a circulation speed high enough to compensate for the heat loss could not be obtained. Thus, the temperature of the water leaving the boilers was not high enough to compensate for the low flow and the heat reduction throughout the water system. The monitoring arrangements were problematic as well, since a thermometer installed to manually control the water temperature of the hot water return, was found not to function as it showed too high a temperature.

The high concentration of *Legionella* in some parts of the hot water system resulted in the first intervention which unfortunately failed to eradicate *Legionella* from the hot water system. The concentration of *Legionella* decreased in the hot water B-samples (taps), but they remained present, with an average of more than 3x10^3

Table 1

Effect of heat treatments on the number and species, serogroups, strains of *Legionella*, data collected from seven different apartments, Copenhagen, Denmark, January – September 2009

Timing of the sampling	Sampling site	Type of sample[a]	Number of samples	Number of positive samples	Temperature of water tested (°C)	*Legionella* concentration CFU/litre CFU/L Median		Type of *Legionella* identified
Before the first intervention (9/1/09)	Shower hose	A	1	1	not measured	› 6 *10^5	–	*L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3
	Tap	A	1	1	not measured	1,4 *10^5	–	*L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3
	Tap (hot water)	B	1	1	46	5,5*10^4	–	*L. pneumophila* serogroup 1 subgroup Philadelphia
	Kitchen tap (hot water)	B	1	1	56	2,0*10^2	–	Sg 3
After the first intervention	Shower hose	A	5	5	not measured	8,0*10^2– 1,6*10^6	266000	Sg 1. *L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14. Sg 4 subgroup Portland.
	Shower hose 38 °C[b]	B	4	4		2,0*10^2 –1,2 *10^4	9000	Sg 1. *L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Bathroom tap (hot water)	A	5	5	not measured	5,0*10^3 –1,2*10^5	20000	Sg 1. *L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Bathroom tap (hot water)	B	5	5	51.5–56	4,5*1+ ^{2}–1,2*10 ^{4}	800	*L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14.
	Kitchen tap (hot water)	A	5	5	not measured	7*10 ^{2}–3,3*10 ^{5}	31000	*L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Kitchen tap (hot water)	B	5	5	52–57	5 *10^1–5*10^3	800	Sg 3. Sg 2-14
	Bathroom tap (cold water)	B	4	0	8.5–16	BD	BD	–
After the second intervention	Shower hose	A	7	1	not measured	BD–5*10^3	BD	spp. *L. anisa*
	Shower hose 38 °C[b]	B	7	3		BD–1*10^2	BD	*L. anisa*
	Bathroom tap (hot water)	A	7	0	not measured	BD	BD	–
	Bathroom tap (hot water)	B	7	0	55.3–64	BD	BD	–
	Kitchen tap (hot water)	A	7	3	not measured	BD–1*10^3	BD	spp. *L. anisa*
	Kitchen tap (hot water)	B	7	0	56.7–64	BD	BD	–
	Bathroom tap (cold water)	B	3	0	7.3–16.7	BD	BD	–
Seven months after the second intervention	Shower hose	A	3	1	not measured	BD – 5*10 ^{1}	BD	Sg 3. Sg 2-14
	Shower hose 38 °C[b]	B	3	2		BD –1*10 ^{2}	5	spp.
	Bathroom tap (hot water)	A	3	2	not measured	BD – 2*10 ^{1}	5	*L. pneumophila* serogroup 1 subgroup Philadelphia. Sg 3. Sg 2-14
	Bathroom tap (hot water)	B	3	0	53–54	BD	BD	–
	Kitchen tap (hot water)	A	3	1	not measured	BD – 5*10^1	BD	Sg 3. Sg 2-14
	Kitchen tap (hot water)	B	3	1	54	BD – 5	BD	Sg 3

BD: below detection by culture; CFU: colony-forming unit; Sg: serogroup.

[a] A-samples are the first litre of water from the tap or shower hose (first flush); B-samples are one-litre samples collected after flushing to reach constant water temperature (warm or cold) was reached.

[b] Samples of taps are collected after flushing until constant temperature. B-samples from shower hoses were collected when the thermostats were mixing cold and warm water to 38 °C. If B-samples are not referred to in the text as being from shower hoses, B-samples are from samples collected at constant temperature from taps.

Table 2

Distribution of the different *Legionella* species serogroups and strains before and after the two interventions, data collected from seven different apartments, Copenhagen, Denmark, January – September 2009

Type of *Legionella* identified	Tap samples						Shower samples[a]					
	Before the first intervention		After the first intervention		After the second intervention		Seven months after the second intervention		Before the first intervention		After the first intervention		After the second intervention		Seven months after the second intervention	
	A	B	A	B	A	B	A	B	A		A	B	A	B	A	B
L. pneumophila serogroup 1 subgroup Philadelphia	+	+	+	+	–	–	–	–	+		+	+	–	–	–	–
Sg 1	–	–	+	–	–	–	+	–	–		+	+	–	–	–	–
Sg 3	+	+	+	+	–	–	+	+	+		+	+	–	–	+	-
Sg 4 portland	–	–	–	–	–	–	–	–	–		+	-	–	–	–	–
Sg 2-14	–	–	+	+	–	–	+	–	–		–	+	–	–	+	–
L. anisa	–	–	–	–	+	–	–	–	–		–	–	+	+	–	–
Any *Legionnella* species	–	–	–	–	+	–	–	–	–		–	–	+	–	–	+

[a] Includes shower samples 38 °C ('B' samples), as in Table 1

+: detection by cultivation, agglutination test, monoclonal antibodies and DNA typing.

–: not detected.

CFU/L (Figure 1). In the A-samples (first flush) from shower hoses, the number of *Legionella* was high: 8.0 x 10^3 to 8.8 x 10^6 CFU/L (Figure 2).

Since *Legionella* remained present in the water system after the first intervention, a second intervention was conducted. Samples were collected one week (day 38) and six weeks after the second heat treatment (day 73 and 74) and revealed none or only very few *Legionella* in samples collected from the taps after flushing to constant temperature (B-samples). This indicated that the increased temperature suppressed *Legionella* growth in the circulating water. However, *Legionella* remained present in some A-samples although in low numbers. Of seven samples from shower hoses, only one contained *Legionella* (5 x10^3 CFU/L) (Table 1). The detection of *Legionella* in A-samples but not in B-samples, from the same tap or shower hose, indicated local growth. Local growth can be established when the most distant parts of the pipework (shower hose or tap) have not been effectively included in an intervention or if this habitat is particularly favoured for rapid regrowth. *Legionella* was not detected in the cold water system probably because water temperatures (less than 20 °C) were outside the optimal growth temperature for *Legionella* (Table 1).

Seven months after the second heat treatment, only a few *Legionella* (five *Legionella* CFU/L in one sample) were detected by culture of B-samples from taps. Only 50 CFU/L were detected in one of three A-samples from shower hoses. The second heat treatment and the continuing flow and temperature regulation seemed to control the number of *Legionella* in this newly built block of flats.

The composition of *Legionella* species and serogroups changed during the course of the treatments (Table 2).

Before and after the first heat treatment only *L. pneumophila* was detected (dominated by serogroup 3 and serogroup 1 subgroup Philadelphia) in both A- and B-samples but after the second heat treatment only *L. anisa* was detected, and mainly in A-samples. Seven months after the second heat treatment, *L. pneumophila* serogroup 3 and serogroup 1 subgroup Philadelphia were found again, but serogroup 1 Philadelphia was only detected in one A-sample, at a very low (5 CFU/L) concentration.

Discussion and conclusion

The hot water in the building was not kept at temperatures outside the range within which *Legionella* can multiply. Two interventions were conducted to eradicate the *Legionella* contamination but only the second intervention (water in the boilers heated to 70 °C for 24 hours followed by 65 °C for three weeks) followed by a generally increased temperature of the whole warm water system compared to before any of the interventions.was effective with only very limited regrowth after seven months. Other studies [16-18]

have investigated different kinds of heat treatments but none of them have proven to be effective over a longer period of time. These studies showed that an important factor common to all treatments was that the normal day-to-day operation of the water systems was not adjusted after the different interventions. In this case permanent changes were made in the functioning of the water system after the second heat treatment (higher circulation speed and flushing in unoccupied apartments).

Bacterial biofilms are important for the survival of *Legionella* and may limit the effectiveness of any intermittent systemic disinfection regime [18]. If not totally erased they constitute a serious factor for potential regrowth. The change we found in the composition of *Legionella* species before and after the different heat treatments indicated a higher heat tolerance for *L. anisa* than for *L. pneumophila* as only *L. anisa* was cultured immediately after the second heat treatment. Another explanation for this change could be a faster colonisation of *L. anisa* than other *Legionella* species. When investigating the composition of species it was also shown that *L. pneumophila* either had survived in the biofilm or had been supplied with the water from the waterworks since this species was detected seven months after the second heat treatment when the temperature was lowered. The finding of *L. pneumophila* serogroup1 subgroup Philadelphia emphasises the importance of keeping the water system under strict temperature control. *L. pneumophila* serogroup 1 subgroup Philadelphia was not found in water with temperatures above 55 °C.

L. anisa, which was detected right after the second heat treatment, is common in Danish residential water systems [19], but has only very rarely been associated with infections in humans) [20]. . To get a real picture of the risk of a given water system, it is important to be able to discriminate between different species and serogroups. In this specific cluster, we would probably not have seen any LD cases had *L. pneumophila* serogroup 1 subgroup Philadelphia not been part of the *Legionella* flora in the residential area. The subgroup Philadelphia belongs to a virulent subgroup of *L. pneumophila* serogroup 1 (called Pontiac or MAb 3/1 positive) [21], which is seldom cultured from hot water systems (< 5% in Denmark) [20].

L. pneumophila serogroup 1 subgroup Philadelphia ST 1 is uncommon in hot water systems. The finding of this particular strain in both patients and in the water system of the new block of flats where both cases had lived or spent time during the incubation period, clearly points to the water as the infectious route.

Legionella is often found in private houses and apartments [11,23,24]. In old buildings with old water installations, the risk of *Legionella* contamination is normally considered to be larger compared with newer buildings with newly established water systems [10,11,25]. However, this cluster demonstrated that newly built blocks of flats can present a risk of *Legionella* infection. From when a building is finished and water is let into the system until all apartments are inhabited, water may be stagnant in the pipes at ambient temperature, and a biofilm with *Legionella* can be established in the system. This was probably the situation in the apartment of Case 1. In order to prevent high levels of *Legionella* in the water pipe systems in new buildings, standard procedures to clean the systems should be applied before occupation. Treatment with biocides could be a solution. In a newly built residential area with many unoccupied apartments, it should also be taken into consideration that the water consumption (both cold and hot water) is lower than the consumption the system is designed for. Hence the water system should be designed to accommodate varying levels of water consumption.

Shower hoses were found to be important risk factors in this study since we found a high number of *Legionella* in them. This may be due to the material of the hose, temperature and flow of the water. If not regularly flushed with hot water, the low temperature and stagnancy of water in them could pose a risk for infection.

Another risk factor – obvious but nevertheless often overlooked, as in this newly built block of flats – is the control and regulation of the water system. Thermostatic heating systems should be properly controlled and correctly sized, including adequate boilers and pumps to run the system optimally. Circulation pumps should have the capacity to keep the water circulating sufficiently also during periods of low water consumption, when the circulation pumps provide the main force in circulating the water. Water should leave the boiler at a temperature hot enough to maintain the temperature above 50 °C even at the most distant tap and in the return water. Pipe dimensions should be scaled according to the size of the building and flow should be adjustable according to the water consumption.

In the building described in this study, some of the pipes have been changed in order to reduce the resistance. The water system has now two separate re-circulation systems each with a pump, and taps in the apartments that are not occupied are flushed once a week.

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Beyond the influenza-like illness surveillance: The need for real-time virological data

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To the editor: We read with great interest your special issue on the Experiences with the pandemic in Europe (Vol. 15, issue 49, 9 December 2010). The reports in that issue clearly highlight the importance of surveillance and monitoring of both emergence and spread of influenza outbreaks through syndromic and laboratory surveillance networks [1]. We would however like to highlight that, in medical practice, it is hardly possible to determine the aetiology of viral respiratory infections by using only clinical symptoms as a basis for diagnosis. For example the correlation between the influenza and influenza-like illnesses (ILI) presentation and the diagnosis of influenza may vary considerably depending on the definition of ILI, the accuracy of the clinician, the epidemiological context, and the presence of co-circulating confounding respiratory viruses. Collecting virological data is mandatory for such networks.

The early phase of the A(H1N1)2009 pandemic in France is an interesting example of this risk of confusion. In France, two independant surveillance networks are involved in influenza surveillance: the 'réseau sentinelle' or sentinel network and the Groupes Régionaux d'Observation de la Grippe (GROG). The sentinel network [2] declared the A(H1N1)2009 pandemic in France the first week of September 2009 (week 36), based on the increase in ILI reports. At the same time, GROG [3] and the laboratory network linked to the National Influenza Centre reported a low incidence of pandemic influenza A(H1N1)2009 [4]. From week 36 to week 43, the GROG network reported a limited pandemic influenza A(H1N1)2009 activity. The pandemic started only mid-October (week 44), according to clinical and virological data. This discrepancy is explained by the difference in the surveillance methods of the two networks. The sentinel network uses clinical surveillance

Table

Percentage of clinical symptoms observed in paediatric patients with a positive influenza or rhinovirus laboratory-confirmed nasal sample, week 36 to 46, France 2009[a] (n=415)

Symptom	Influenza A(H1N1)	Rhinovirus	Odds ratio, 95% confidence interval	p
Cough	87,9	59,2	(0,12;0,32)	p<0,001
Asthenia	24,6	13,8	(0,29;0,81)	p<0,001
Myalgia	22,2	6,4	(0,11;0,47)	p<0,001
Diarrhoea	9,8	5,3	(0,90;4,10)	Not significant
Vomiting	21,8	15,7	(0,40;1,10)	Not significant
Hyperthermia	81,7	79,2	(0,50;1,38)	Not significant
Temperature ≥ 39,5°C	28	25,3	(0,53;1,51)	Not significant
Swollen lymph nodes	12,1	11,8	(0,53;1,76)	Not significant
Nasal secretion	36,1	45,1	(0,98;2,16)	p<0,05
Bronchitis	3,4	5,4	(0,62;4,30)	Not significant
Dyspnoea	8,2	24,1	(17,7;41,30)	p<0,001
Otitis	8,2	2,5	(0,10;0,78)	p<0,01
Pharyngitis	32,2	8,7	(0,11;0,35)	p<0,001
Cutaneous rash	7,8	4,9	(0,27;1,39)	Not significant
ILI diagnosed	36,5	35	(0,62;1,39)	Not significant

[a] Results are presented as the likelihood of the presence of symptoms and rhinovirus detection (Odds ratio, 95% confidence interval)

of ILI based on reports from general practitioners (GPs), whereas the GROG network associates virological diagnoses to the clinical surveillance of ILI reported by GPs. The latter network could ascertain that non-influenza respiratory viruses, mainly rhinoviruses and other respiratory viruses such as parainfluenza viruses, were responsible for the increase in reported ILI from week 36 to week 43 [4,5].

To investigate this point further, we reviewed 415 emergency paediatric medical records collected between week 36 and week 46 (mean age 4.8 years +/- 7.1 standard deviation). We compared the clinical symptoms of 208 laboratory-confirmed A(H1N1)2009 influenza virus-positive and 207 rhinovirus-positive patients (Table). It was clear that there were differences between the clinical presentations. Cough, asthenia, myalgia, pharyngitis and otitis were more frequent in the A(H1N1)2009 influenza group whereas nasal secretion and dyspnea were more frequent in the rhinovirus group. However, all these symptoms were noticed in both groups. Temperature did not differ significantly between the A(H1N1)2009 influenza and rhinovirus groups. The conclusion of ILI in the emergency paediatric medical report was not predictive for either laboratory-confirmed influenza or rhinovirus cases. The pandemic context, the expectation of influenza to spread with the start of the school year in September, massive media coverage of the pandemic and the general level of anxiety made the presumptive clinical diagnosis of influenza a real challenge in the early pandemic phase.

These data highlight the fact that viral respiratory infections can easily be clinically confused. It is important to keep in mind these limitations and that ILI and other respiratory symptoms can account for the presence of different respiratory viruses. As reported by Thomson and Nicoll [1], clinical surveillance of upper respiratory tract infection is required but the link of non-specific surveillance data (including surveillance of ILI, schools or work absenteeism, analysis of search engine query data) with a reliable virus surveillance system is mandatory for optimal surveillance and epidemic or pandemic management

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