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Interim report on the A/H1N1 influenza virus pandemic in Marseille, France, April-November 2009

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Abstract

We report here the results of a 7-month survey of the influenza A/H1N1 pandemic in the Virology laboratory of the public hospitals of Marseille (April–November 2009). In total, 8 587 samples were analysed during this period, of which 1 974 (23%) were positive for the novel influenza variant. The analysis of results obtained using rapid influenza diagnostic tests (RIDTs) revealed a global sensitivity of 49.4% (vs. molecular qRT-PCR detection), strongly correlated with age groups (varying from 30% to 58% for patients >40 age and <10, respectively), indicating that RIDTs can be helpful in accelerating the management of suspected cases. Epidemiological analysis showed that the winter influenza wave began in October in Marseille (i.e. 2 to 3 months earlier than usual seasonal influenza outbreaks) and that the majority of autochthonous cases were observed in patients younger than 20 years old, with a low number of cases in patients over 60 years old. In November 2009, 22.2% (167/754) of patients with a laboratory diagnosis of influenza A/H1N1 infection were hospitalized, of which 9% (15/167) were admitted to an intensive care unit (ICU). Patients in the extreme age groups (>40 years old and <1) were significantly more often hospitalized than others, and 2.4% of hospitalized patients died. During the last 3 weeks of the period, the average number of bed-days attributable to H1N1sw-positive patients was 31.4, of which 5.9 were in ICUs.

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Introduction

In late April 2009, a novel A/H1N1 influenza virus (H1N1sw) was isolated in North America [1]. Rapidly, the World Health Organization increased the alert level from phase 5 to phase 6, defining the first influenza pandemic of the 21st century [2]. The first wave affected Mexico, the USA and Canada with severe cases and deaths [3–5]. The following wave affected the southern hemisphere during the southern winter season [6,7], and once again severe cases were observed; over 3 months, 722 patients were admitted to an Intensive Care Unit (ICU) in Australia or New Zealand, representing 28.7 cases per million inhabitants, with a 14.3% mortality [8].

The first cases in metropolitan France were detected in April 2009 in patients returning from Mexico. Until early July 2009, a systematic surveillance based on laboratory confirmation of suspected cases was implemented in the 'Level A' laboratories of the seven French Defence Zones [9]. During the following months, laboratory confirmation was mainly performed in groups at risk and in hospitalized patients.

We report here data collected by the 'Level A' Virology laboratory of the public hospitals of Marseille (Southern Defence Zone) from 25 April to 29 November 2009; the evolution of the outbreak, the distribution among age groups, the results of rapid influenza diagnostic tests (RIDTs) and the characteristics of hospitalized patients are described and discussed.

Materials and Methods

Clinical samples

All clinical nasal samples were obtained using Virocult swabs (Virocult MW950; Medical Wire and Equipment Co.). Laboratory investigations for diagnostic purposes and epidemiological assessment, warranted by patients' signatures at the hospital entrance office, were performed in accordance with French national regulations (Huriet-Sérusclat law, #881138) and did not require ethical committee approval.

Rapid influenza diagnostic testing (RIDT)

RIDT was performed using the Directigen EZ influenza A+B test (BD EZ Flu A+B, Becton, Dickinson and Company) according to the manufacturer's instructions.

RNA extraction

Samples were spiked with in-house MS2 phage internal control [10] and RNA was extracted using the EZ1 Virus Mini Kit v2 on the EZ1 Biorobot (both from Qiagen).

Quantitative real time PCR assays

Samples were analysed by two qRT-PCR assays: (i) a qRT-PCR assay using SYBR Green technology detecting all influenza A viruses [10], and (ii) a qRT-PCR assay specific for H1N1sw, recommended by the French Influenza Reference National Centre [9] as previously described.

Database of patients infected by H1N1sw

During the last 4 weeks of the study (November 2009) data regarding in-patients and patients presenting at the hospital emergency department with a subsequent laboratory confirmation of H1N1sw infection were collected from the database of the public hospitals of Marseille. The age, sex, arrival and discharge from hospital dates, category of hospitalization unit (paediatric medical unit, adult medical unit, paediatric ICU and adult ICU) and number of deaths were analysed using an anonymized database.

Results

From 25 April to 29 November 2009, we analysed 8 587 specimens for detection of the novel A/H1N1 virus. A total of 5 848 (68.1%) originated from public hospitals in Marseille, the remaining samples being sent by other hospitals from the French South Defence Zone. Until late June the numbers of samples tested remained low but they increased progressively until early September (Fig. 1). During this period, the

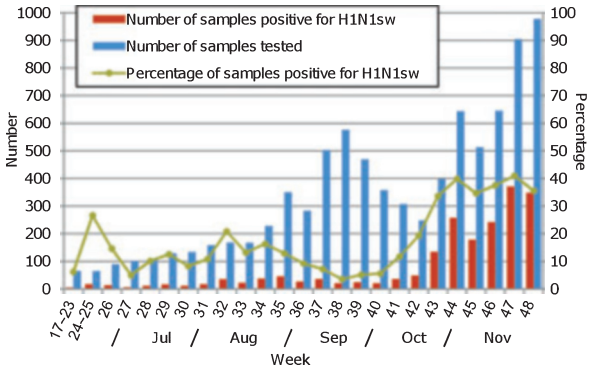

FIG. 1. Time distribution of samples tested and samples positive for H1N1sw during 7 months in the Virology laboratory of the public hospitals of Marseille.

percentage of H1N1sw-positive samples remained stable at *c.* 10%. In September, the number of samples increased suddenly but the percentage of H1N1sw-positive samples decreased (Fig. 1). This episode was associated with the circulation of other respiratory viruses such as rhinoviruses and coronaviruses. The influenza winter wave began effectively in October (weeks 41–42) with a marked increase of both tested and positive samples. From late October, the percentage of H1N1sw-positive samples remained stable (30–40%) but the number of positive samples continued to increase (Fig. 1).

We analysed the distribution among age groups of tested samples (N) and H1N1sw-positive samples (N′) during the study period. Until late August, patients over 20 years old represented 60% of all tested patients and more than 50% of H1N1sw-positive patients. During this period, patients in the 10–19 years age group represented 30% of H1N1sw-positive patients (Fig. 2a,b) but this proportion increased in September (weeks 36–38), to reach more than 40% of infected patients. From mid-September, the proportion of patients younger than 10 years who were tested or infected by H1N1sw increased, representing approximately 40% of both groups and the distribution among age groups remained globally stable afterwards (Fig. 2a,b). The N′/N ratio (allowing standardization of the number of samples in the age groups) showed that the highest proportion of positive patients was observed in the 10–19 years age group (30–60%) and that this proportion fluctuated *c.* 20% for patients over 40 years old (Fig. 2c). During the complete study period the majority of patients infected by H1N1sw were younger than 20 years old.

Among the 8 587 samples tested for influenza virus using qRT-PCR, a total of 7 459 (including 1615 qRT-PCR positive with both systems) were also tested using RIDT. Of the 7 459 samples, 798 (10.7%) gave positive results in the RIDT, all of these being positive for molecular detection of the

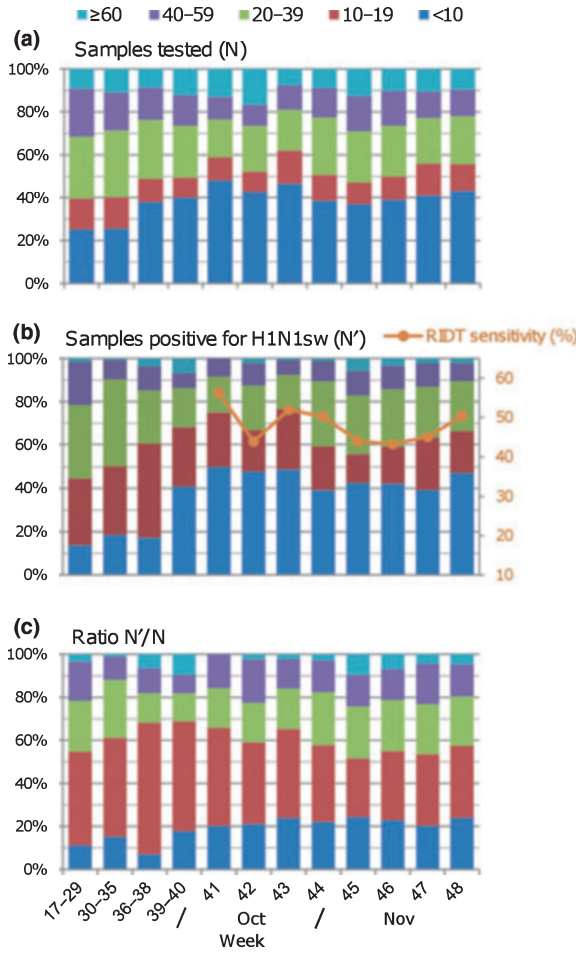

FIG. 2. Distribution among age groups of samples tested (N) (a), samples positive for H1N1sw (N′) (b) and for the N′/N ratio (corresponding to the standardization of number of samples in age groups) (c). The evolution of RIDT sensitivity is indicated for weeks 41–48 (b).

H1N1sw influenza virus (specificity of RIDT = 100%, sensitivity = 49.4%). There was a strong correlation between RIDT sensitivity and age (Fig. 3): the sensitivity varied between 30% and 58% for patients in the ≥40 and <10 years age groups, respectively. Furthermore, from mid-October, the performance of RIDT detection was correlated with the proportion of patients infected who were younger than 20 years (Fig. 2b).

Among the 754 H1N1sw-positive patients admitted to the public hospitals of Marseille during the last 4 weeks of this study (weeks 45–48), 167 (22.2%) were hospitalized and four died (2.4% of hospitalized patients). Comparison of hospitalized and non-hospitalized patients showed comparable sex ratios (M/F, 0.99 and 0.92, respectively) and median ages (14.5, range: 0–98 years, and 11.3, range: 0–99 years, respectively). The age distribution was similar in hospitalized and non-hospitalized patients except for patients younger than 1 year and over 40 years. Overall, 24.0% of in-patients and 10.1% of

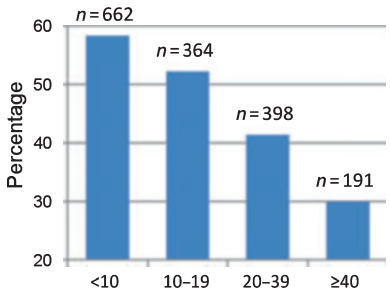

FIG. 3. Sensitivity of RIDT according to age groups amongst 1 615 samples positive for H1N1sw.

non-hospitalized patients were over 40 years (χ^2 test: p <0.0001) and 15.6% of in-patients and 5.7% of non-hospitalized patients were under 1 year (χ^2 test: p <0.0001). Among 167 hospitalized patients, 15 (9.0%, and 2.0% of all 754 infected patients) were admitted to an ICU because of severe clinical conditions. The median age of patients hospitalized in an ICU was 23.7 years (range: 1 month–61 years). Of patients hospitalized in ICUs 40% were over 40 years and 13.3% were under 6 months. From 9 November to 29 November, the average number of bed-days due to H1N1sw-positive patients in the public hospitals of Marseille was 31.4 (95% CI, 29.5 to 33.4 days) of which 5.9 (95% CI, 5.4 to 6.4) were in ICUs. Half and 32.5% of bed-days were attributable to children (under 18 years) in medical units and ICUs, respectively (Fig. 4). Among patients hospitalized in medical units, 21.1% and 61.3% stayed in hospital for 2 days or less and for 4 days or less, respectively (range: 1–23 days), while 12.5% of patients hospitalized in ICUs stayed in hospital 4 days or less and 50% stayed in hospital 13 days or more (range: 4–29 days).

Discussion

The retrospective analysis of the first 7 months (from late April to late November; weeks 17–48) of the wave of

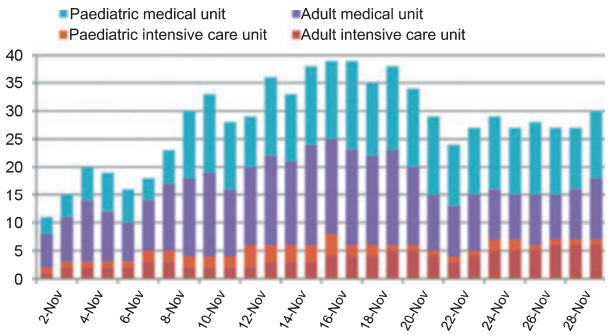

FIG. 4. Daily occupancy of the paediatric and adult medical unit and of the paediatric and adult ICU during 4 weeks in public hospitals of Marseille.

H1N1sw pandemic infections in the region of Marseille (south-eastern France) indicates that three periods with distinct epidemiological characteristics can be distinguished. The very first imported cases were observed from April to August in travellers returning from abroad (mainly Mexico and the USA), and thus with a majority of adults [9]. In the second phase, with indigenous cases, which became predominant during the summer, the number of paediatric cases (<10 years old) increased progressively to reach a stable proportion of approximately 40% of all cases. Consequently, the global distribution among age groups became comparable to that previously observed for seasonal H1N1 outbreaks [11], with a majority of cases in patients younger than 20 years, confirming data from other countries [7,12]) and a low number of patients over 60 years old. The final phase, a 'winter' wave, began in Marseille during October (as was also observed in other regions of metropolitan France [13]) 2–3 months before the classic winter wave of seasonal influenza.

The use of a RIDT is common for the diagnosis of seasonal influenza. Whether 'classical' tests (designed for detection of seasonal A/H3N2, A/H1N1 and B viruses) could be used for the detection of the new A/H1N1sw variant was uncertain and contrasting results had been obtained using various available commercial kits [13–16]. Our results suggest that RIDTs could make a significant contribution to the management of suspected cases. They allowed more rapid delivery of positive diagnosis (<2 h) for 49.4% of the H1N1sw-positive samples, the highest sensitivity (close to 60%) being obtained in children younger than 10 years. Other studies showed a similar sensitivity for the detection of this new variant [13] and an equal analytical sensitivity between seasonal influenza and this new variant [14] using the BD EZ Flu A+B test.

Finally, the follow up organized during November indicated that 22.2% of patients with a documented H1N1sw infection were hospitalized and that 2.4% of hospitalized patients died. Approximately 30 bed-days were attributable to H1N1sw in the public hospitals of Marseille, of which six were in ICUs. Since the public hospitals of Marseille serve a population of approximately one million inhabitants, these numbers are similar to those encountered in surveys previously performed in Australia and New Zealand [8]. Importantly, our results indicate that the duration of hospitalization was short for patients admitted to medical units, but also that the pattern of hospitalization observed (children younger than 1 year and patients over 40 years being significantly more frequently hospitalized than others) was reminiscent of the U-shaped age-distribution curve previously reported for seasonal influenza.

Transparency Declaration

The authors declare that no dual/conflicting interest exists.

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