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Abstract
The new influenza A/H1N1 (2009) virus spread worldwide and Niger was one of the last countries affected. This work aimed to depict the transmission dynamics of the A/H1N1 (2009) epidemic in Niamey.

A sentinel surveillance system for influenza was set up in Niamey. Subjects with symptoms of influenza-like illness or severe acute respiratory infection attending 10 health care facilities were enrolled in the surveillance network system. When subjects met one of the case definitions, a nasopharyngeal swab was sampled and influenza viruses A/H1N1 (2009), A/H3N2 and B were detected and typed by reverse transcription-PCR (RT-PCR). Several factors were measured through a questionnaire. $R_0$ was estimated according to two methods using two parameters: the observed value of growth rate and the mean generation interval.

The A/H1N1 (2009) epidemic lasted 10 weeks; between 2 February and 8 April 2010. Forty-six cases of A/H1N1 (2009) were detected with 11 additional cases of influenza B. The epidemic curve showed an increase in cases from the 5th to the 8th week. The mean age of A/H1N1 (2009) cases was 13.6 years without any gender predominance. Three main symptoms were found among all subjects: fever, cough and sore throat. Maximal values of $R_0$ were estimated at 1.6 and 1.9.

Strengthening sentinel surveillance all around in the world is of particular importance to understand future influenza epidemics.

Keywords: Influenza; H1N1 Virus; Basic Reproduction Number; Outbreak; Transmission

Introduction
In April 2009, a sentinel surveillance system for flu was set up at Niamey.

Three influenza pandemics have threatened the world during the 20th century, leading to millions of deaths and improved case management aimed at reducing their socioeconomic consequences [1]. Circulating influenza strains are less known in Africa than in other continents. Indeed, laboratory capacity for influenza diagnosis is available in only 18 of the 46 countries located in the WHO African region [2]. A better knowledge of influenza in Africa is of particular interest as it will enable clinicians to empirically treat their patients and prevent non-negligible consequences of failures in therapeutic choices or development of antimalarial/antibiotic resistance.

The role played by climatic factors is being studied. However, temperature and humidity have the most impact on influenza transmission both in temperate and tropical countries [3]. Some diseases like meningitis have a well-established marked seasonality but this information is lacking for influenza in Africa.

In March 2009, the newly identified A/H1N1 (2009) virus was first detected in Mexico and quickly spread worldwide leading to a new pandemic [4]. First cases in West Africa were reported from 24 June 2009 in Côte d’Ivoire. Niger was one of the last Sahelian and African

countries to report A/H1N1 (2009) in February 2010, after Mali and Senegal. As soon as A/H1N1 (2009) emerged in Mexico, the reproductive base number \( R_0 \) was estimated to anticipate the severity of the pandemic and make decisions on control measures like travel restrictions, closure of schools or hygienic measures in hospitals and communities \[5,6\]. In temperate countries, estimations led to a \( R_0 \) between 1.7 and 2.1 at the peak of influenza epidemics \[7\]. During the summer wave of the 1918 influenza pandemic, \( R_0 \) as high as 3.10 - 6.74 were observed in England \[8\]. In Africa, this parameter is poorly estimated and no data is available in Niger.

This work aimed to study the transmission dynamics of influenza through a sentinel network in Niamey, Niger.

**Methods**

**Background**

Niger is a country situated in the Sahelian region of Africa where climate is characterized by a long dry period with elevated temperatures and a very short rainy season.

In April 2009, a surveillance network for influenza was set up by the CERMES (Centre de Recherche Médicale et Sanitaire) in Niamey, the capital of Niger. The surveillance was aimed to estimate the proportion of influenza cases among patients presenting with either influenza like illness or severe acute respiratory infection, and to detect circulating subtypes of influenza viruses in the population \[9\]. The network consisted of 10 sentinel sites and a laboratory dedicated to influenza detection and typing.

These sites were all located at Niamey: two emergency wards and one paediatric unit of the three general hospitals, four private clinics, two health care centres, and a private medical cabinet. Children under five years were most expected to consult at public health care facilities because of free medical care. For older children and adults, self-medication through informal vendors is more developed.

**Study Population**

Patients with influenza like illness or severe acute respiratory infection symptoms attending the sentinel sites were recruited. The study onset was one month before the epidemic and ended one month after i.e. between 1\(^{st}\) January – 31 May 2010. This period comprised the cold season (January – March) and the very hot season (April - May). The only criterion of exclusion was refusal to participate in the study.

**Case Definition**

Case definitions of influenza like illness and severe acute respiratory infection were those used by the influenza surveillance worldwide \[10\]. An influenza like illness was defined as a patient with fever ≥ 38°C and suffering from cough or sore throat. A severe acute respiratory infection had the same clinical presentation as an influenza like illness, but required hospitalisation accompanied with a dyspnoea for patients aged 5 years and more. A severe acute respiratory infection in patients below 5 years was defined as cough or dyspnoea, requiring hospitalisation and at least a symptom of severity. The symptoms began less than 7 days before consultation for all patients. Patients who declared having been in contact with known infected patients or who had travelled to a pandemic country were nonetheless sampled early in the investigation.

For all the patients meeting the inclusion criteria, a nasopharyngeal swab was sampled.

**Laboratory Testing**

The nasopharyngeal samples collected into a standard virus transport medium (Copan Diagnostic Inc., Italy) were referred to CERMES within 72 hours at 4°C. Total RNA was extracted from 140 µl of each primary sample using the QIAamp viral RNA minikit (QIAGen, Courtaboeuf, France) in accordance with the manufacturer’s protocol. Purified RNA was frozen at -80°C in aliquots. One aliquot of RNA was first separately tested for the different influenza A (M gene) and B viruses by a one-step real-time reverse transcription-polymerase chain reaction (RT-PCR) using the protocol of the National Influenza Centre, Unit of Molecular Genetics of Respiratory Viruses, Pasteur Institute.

Paris with slight modifications. In case of positivity for influenza A the sample was then subtyped into the seasonal A(H1N1) and A(H3N2) and pandemic A(H1N1)pdm09 [11]. Primers and fluorescent probes are described in Table 1.

<table>
<thead>
<tr>
<th>Type/subtype</th>
<th>Gene</th>
<th>Name</th>
<th>Sequences</th>
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<tr>
<td>Influenza type A</td>
<td>GRAM/7Fw</td>
<td>CTTCTAACCAGCTGAAACGTA</td>
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<tr>
<td></td>
<td>GRAM/161Rv</td>
<td>GGTGACAGATTGCTTTGCTTTTA</td>
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<td>GRAM probe/52/+</td>
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<td>Influenza type B</td>
<td>NA(B)-916Fw</td>
<td>TACACAgCAAAAAgACCC</td>
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<td></td>
<td>NA(B)-1069Rv</td>
<td>TCCACKCCCCCTRTGCC</td>
<td></td>
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<tr>
<td></td>
<td>NA(B)-1001Probe(+)</td>
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<tr>
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<td>H3h-306Probe</td>
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<td>N2h-1344 Rv</td>
<td>GCCACAAAAACACAAATAC</td>
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<td>N2h-1290 probe</td>
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<td>N1h-1134 Fw</td>
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<tr>
<td></td>
<td>N1h-1275 Rv</td>
<td>CTTCAACCGAAGAAAGTC</td>
<td></td>
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<td>N1h-1206 probe</td>
<td>5′-Fam-CAGCGGAAGTTGCTTACCAT-3′-BHQ-1</td>
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</tr>
<tr>
<td>Pandemic H1N1</td>
<td>GRswN1-975Fw</td>
<td>TCCACGGCCTTATGATAAA</td>
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<tr>
<td>Pandemic H1N1</td>
<td>GRswN1-1084Rv</td>
<td>TTCTCCCTATCCAAACAC</td>
<td></td>
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<tr>
<td>Pandemic H1N1</td>
<td>GRswN1-1045bProbe(-)</td>
<td>5′-Fam-ATCCCTTTACTCCATTGCTCC-3′-TAMRA</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1:** Primers and probes used for the typing and subtyping of the different influenza A (M gene) and B viruses.

The laboratory participates in the WHO external quality assessment project (EQAP).

**Factors Measured**

Several factors were measured through a questionnaire completed by the nurse or the physician participating in the sentinel network and who had received prior training on sample collection and questionnaire completion. The following variables were collected on the day of consultation: body temperature, cough, sore throat, dyspnoea, age, sex, date of symptom onset, history of travel in the month before the date of consultation. By computing the difference between the date of detection of the positive sample by RT-PCR and the date of symptom onset, the minimal duration of shedding was calculated.

The proportion of positive patients according to their status of influenza infection was estimated weekly during the study period.

Meteorological factors such as measures of minimal and maximal temperature, minimal and maximal relative humidity, wind speed and visibility were collected from the meteo station located at Niamey’s airport.

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**Statistical Analysis**

The analysis distinguished two periods:

1. The A/H1N1 epidemic period between 2nd February and 8 April: the distribution of studied factors according to the status of infection was performed using chi-square test for qualitative variables and Kruskal-Wallis test for quantitative variables.
2. Between the 1st January and 31 May 2010: to study the link between meteorological factors and daily counts of confirmed influenza cases. Student t-test was used to compare means of meteorological factors before and after 8 April 2010 when the influenza epidemic ended.

Several formulas were used to compute $R_0$. It was considered that $R_0$ represented the maximum potential of the infection. The period to perform the estimations of $R_0$ was chosen after inspecting the daily epidemic curve. The $R_0$ was calculated according to Wallinga and Lipsitch and needs to know the observed value of growth rate and the mean generation interval [12].

The generation interval is defined as the duration between onset of symptoms in a primary case and a second case. The values of generation interval mean and its extremes, 3.6 (2.9 - 4.3) days, were chosen according to Cowling, et al [13].

The observed value of growth rate was estimated by regression using an exponential model in two ways: 1°) Model I representing the best fit for a given period, 2°) Model II fitted for the longest period with a statistically significant coefficient. The fit of the model was checked with the multiple R-squared and the F-statistic.

All statistical analysis were performed with the software R version 2.11.1 [14].

**Ethical Aspects**

This sentinel network was authorized by the National Ethics Committee of Niger and supported by the Ministry of Public Health. Nominate data were collected to allow clinical investigation of A/H1N1 (2009) secondary cases.

Feedback information to sentinels and public health authorities was performed weekly during the course of the A/H1N1 (2009) epidemic in Niger.

**Results**

**Description of the A/H1N1 (2009) epidemic period**

The A/H1N1 (2009) epidemic lasted 10 weeks (2nd February - 8 April). During that period, 239 patients were recruited and 46 cases of A/H1N1 (2009) were detected. One case was imported from Senegal. Additionally, 11 cases of influenza B were detected during the same period, representing 78.6% of all type B cases detected since April 2009. One hundred and eighty-two patients were negative. More than 50% of nasopharyngeal swabs came from paediatric consultations. The mean age of A/H1N1 (2009) cases was 13.6 years (SD = 16 years), 12.1 for B cases and 15 for negative cases. The median age was 7 years for all groups. The male:female ratio was 0.9:1 for A/H1N1 (2009) cases, and 0.7:1 for negative patients, whereas B cases showed a male predominance (2:1). A travel history was more frequently reported among B cases, approximately three times more than in A/H1N1 (2009) and negative patients. Cough was the most frequent symptom concerning at least 86% of the patients. Table 2 depicts some potential risk factors for the A/H1N1 (2009) cases versus the B cases and the negative patients. None of these factors showed a statistically significant difference between the three kinds of infectious status.

The epidemic curve depicted one main peak occurring at week 8 (Figure 1 Weekly distribution of A(H1N1)2009, B and negative patients during the epidemic: number and proportion of A(H1N1)2009 patients). The daily and weekly numbers of A/H1N1 (2009) cases were respectively 0.7/day and 4.6/week. The onset of symptoms for the first case occurred on the 28th January 2010. The average minimal duration of shedding was 2.8 days (SD = 1.8 day, range = 1 - 14 days). The proportion of patients positive for A/H1N1 (2009) among all patients was initially 100% at week 5 and decreased sharply to 7.1% at week 13.
Table 2: Distribution of some potential risk factors according to the status of influenza infection.

*aAfter grouping A/H1N1(2009) and B subjects*
Estimation of the basic reproduction number $R_0$ during the A/H1N1 (2009) epidemic

Assuming a $\delta$ distribution for the mean generation interval, $R_0$ reached 1.9 (range = 1.6 - 2.1) for a mean generation interval of 3.6 days. According to model II, $R_0$ was around 1.3 with extreme values between 1.2 and 1.3.

Change in daily counts of influenza confirmed cases and meteorological factors

Sixty-one influenza cases, consisting of 46 A/H1N1 (2009), 4 A/H3N2 and 11 B were detected between the 1st January and 31 May 2010. Figure 2 (Figure 2 Daily count of confirmed flu cases according to daily measures of temperature, relative humidity, wind speed, and visibility) shows that all the climatic factors increased significantly after the epidemic than before. Mean difference of the maximal and minimal temperatures reached +3.5 ($t$-test = 7.23, df = 128.4, $p < 0.001$) and +7.9°C ($t$-test = 14.1, df = 149, $p < 0.001$) of magnitude respectively (Table 3).

The influenza epidemic stopped suddenly when maximal and minimal relative humidity increased significantly, +30% ($t$-test = 12.3, df = 65, $p < 0.001$) and +11.4% ($t$-test = 10.9, df = 53, $p < 0.001$), respectively. During the A/H1N1 (2009) epidemic, means of maximal and minimal relative humidity were respectively 22 and 4.7%.

Wind speed and visibility also increased significantly after the end of epidemic (respectively $t$-test = 5.48, df = 100, $p < 0.001$ and $t$-test = 2.21, df = 140, $p < 0.03$).


Figure 2: Daily count of confirmed flu cases according to daily measures of: a) temperature, b) relative humidity, c) wind speed, and d) visibility.
Solid line = daily count of confirmed flu cases; dashed line = minimal value for temperature and relative humidity and observed values for wind speed and visibility; dotted line = maximal value for temperature and relative humidity

<table>
<thead>
<tr>
<th>Meteorological factor</th>
<th>End of epidemic (08/04/2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (01/01 – 08/04)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Maximal 37.8 (3.2)</td>
</tr>
<tr>
<td></td>
<td>Minimal 21.3 (4.4)</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>Maximal 22 (8.0)</td>
</tr>
<tr>
<td></td>
<td>Minimal 4.7 (1.2)</td>
</tr>
<tr>
<td>Wind speed (Km/h)</td>
<td>8.8 (2.7)</td>
</tr>
<tr>
<td>Visibility (m)</td>
<td>6 446 (2738)</td>
</tr>
</tbody>
</table>

Table 3: Variations in meteorological measurements before and after the end of the influenza epidemic, Niamey, Niger.

“SD: standard deviation

Discussion

In this study, the $R_0$ was estimated at 1.3 and 1.9 for the A/H1N1 (2009) epidemic in Niger. The values are lower than those found during the two influenza pandemics in 1918 and 1889 ($R_0 > 2$) [8,15,16]. Using the same method of calculation, these values are however closer to those retrieved in Mexico and United Kingdom, 1.4 – 1.6 and 1.2 – 1.5, respectively [17,18]. In New Zealand, the $R_0$ reached 1.55 using a different method [19]. $R_0$ of the 2009 influenza pandemic was generally more than that of the seasonal influenza estimated in France, USA, Australia, and Brazil, between 1 and 1.4 [20,21]. To the best of our knowledge, no such data have yet been published for
Africa. Based on the value of $R_0 = 1.9$, 47% of this non-immunised population should benefit from protective measures (vaccination, chemoprophylaxis, isolation...).

The A/H1N1 (2009) epidemic ended before mid-April 2010. This was followed by a concomitant increase in humidity, temperatures, wind speed and visibility. At 30°C, the transmission of aerosols has been shown to be absent [22]. It has also been shown that humidity is another meteorological factor involved in influenza transmission. Indeed, an increase in absolute and specific humidity was correlated with a decrease of influenza virus transmission and survival in five states of USA [23]. In Nigeria, major outbreaks of influenza-like illness or respiratory infections are observed when the dry and dusty Harmattan wind blows from November to March. During this period, temperatures and humidity are at the lowest [24]. On the contrary, an increase in relative humidity was correlated with the increase in incidence of influenza A virus in Argentina and Hong Kong; similarly observed in other humid tropical countries such India during rainy season [25, 26]. These apparently contradictory results could be explained by the work of Hanley and Borup that has shown that the transmission of influenza viruses depends both on temperature and relative humidity [3]. At high temperature levels, the transmission is almost zero irrespective of the relative humidity levels. At lower temperature levels, the transmission is maximal for relative humidity levels between 20 and 50%. Below 20% humidity, the transmission of influenza virus is poorly estimated. The evolution of the H1N1 epidemic at a very low relative humidity shows that transmission is possible at the minimum temperatures (50% were between 13°C and 25°C during the study period) observed in Niamey with $R_0$ comparable to other countries [27].

It is not clear whether influenza is seasonal in Niger and even less in Africa. This is probably due to the varied climatic regions on this continent. These preliminary study results encourage further investigation.

On average, A/H1N1 (2009) patients consulted at a sentinel site within 2.8 days after the onset of their symptoms. This could correspond to the average minimal duration of shedding. This result is in accordance with a meta-analysis on volunteer challenge studies showing the maximum symptom scores related to influenza virus infection at day 2 or 3 [28].

Limited information on the A/H1N1 (2009) pandemic is available in Africa. In this study, the patients were younger with a median age of 7 years compared to at least 12 years in a systematic review of 31 studies by Khandaker, et al [29]. Such a difference in age group could be explained by the free medical care in the public health system in Niger for children under 5 years and the existence of few imported cases or low frequency of travel history among A/H1N1 (2009) patients in Niger. It has also been shown in the United Kingdom that age under 16 were more susceptible to A/H1N1 (2009) infection in the household [18].

Travel history was less frequent probably due to fewer movements in Niamey compared to other capitals. Nevertheless, the role of the 2009 Hajj return on the onset of the epidemic, as previously pointed out by Ebrahim, et al. is unravelled. Nevertheless, a quota of 9 000 pilgrims from Niger was allowed entry into Mecca, Saudi Arabia [30]. During the first 15 days of December 2009, about 500 travellers returning from Mecca were expected each day at the Niamey international airport. For a $R_e$ equal to 1.5, it has been shown that the median delay to import an influenza epidemic from the source region could reach 57 days, i.e. 2 months [31]. In the Niger context, the epidemic should have started by mid-February. Low vaccination coverage has been reported among UK pilgrims; this way of prevention should probably be more strongly recommended [32].

The studied population was not representative of the entire population of Niamey since 10 sentinel sites participated in the surveillance among a total of 60 health care facilities in the city. In addition, clinical presentation of influenza could be similar to malaria and people frequently treat themselves at home, but consult the health care facilities only in case of serious disease. Nevertheless, the 10 sentinel sites involved in the surveillance were the biggest health care facilities in Niamey.

Conclusions

To conclude, this study shows similar values of $R_0$ in Niger as with other countries concerned by A/H1N1 (2009). No standard preventive measures other than those implemented in other countries need to be applied. The Vaccination coverage required for influenza

should not be as high as that required for measles, representing an important economic savings for poor countries like Niger. Nevertheless, during the return of pilgrims, reinforcing information and sensitization of these particular populations is relevant, especially as they return under climatic conditions favourable for transmission. Vaccination could be proposed in a more systematic way to pilgrims. Another particularity of this epidemic was its short duration that could be explained by climatic factors unfavourable to influenza transmission in Niger, especially during the very hot season where temperatures are very high and relative humidity is at its lowest level. No clinical symptoms could distinguish positive from negative RT-PCR patients. Laboratory testing is entirely justified for the syndromic surveillance and remains of particular importance in the diagnosis. Because pandemic and seasonal influenza virus continues to be transmitted in Sub-Saharan Africa, strengthening the sentinel surveillance in Niger is of particular importance to detect and anticipate intensity of future influenza epidemics, as demonstrated with the A/H1N1 (2009) outbreak. Furthermore, the impact of vaccination campaigns against influenza could be better assessed.

**Bibliography**


