A seroepidemiological study of pandemic A/H1N1(2009) influenza in a rural population of Mali

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Abstract

The swine-origin H1N1 influenza A virus (pH1N1(2009)) started to circulate worldwide in 2009, and cases were notified in a number of sub-Saharan African countries. However, no epidemiological data allowing estimation of the epidemic burden were available in this region, preventing comprehensive comparisons with other parts of the world. The CoPanFlu-Mali programme studied a cohort of 202 individuals living in the rural commune of Dioro (southern central Mali). Pre-pandemic and post-pandemic paired sera (sampled in 2006 and April 2010, respectively) were tested by the haemagglutination inhibition (HI) method. Different estimates of pH1N1(2009) infection during the 2009 first epidemic wave were used (increased prevalence of HI titre of $\geq 1/40$ or $\geq 1/80$, seroconversions) and provided convergent attack rate values (12.4–14.9%), the highest values being observed in the 0–19-year age group (16.0–18.4%). In all age groups, pre-pandemic HI titres of $\geq 1/40$ were associated with complete absence of seroconversion; and geometric mean titres were $<15$ in individuals who seroconverted and $>20$ in others. Important variations in seroconversion rate existed among the different villages investigated. Despite limitations resulting from the size and composition of the sample analysed, this study provides strong evidence that the impact of the pH1N1(2009) first wave was more important than previously believed, and that the determinants of the epidemic spread in sub-Saharan populations were quite different from those observed in developed countries.

Keywords: Epidemiology, H1N1, haemagglutination inhibition, influenza, Mali, pandemic, sub-Saharan Africa

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Introduction

A new swine-origin variant of H1N1 influenza A virus (pH1N1(2009), referred to here as pH1N1) was first identified in Mexico and the USA in March and early April 2009, and secondarily circulated worldwide, reaching pandemic alert level 6 on 11 June 2009. A global estimate of the number of cases is difficult to provide, given the number of mild or asymptomatic cases and the absence of epidemiological data from many regions of the world. It was probably in the order of magnitude of several hundreds of million cases in 2009 and early 2010. Whereas data regarding the spread of the virus in the Americas, Europe and a number of Asian countries have been documented [1], the impact of the pandemic in sub-Saharan Africa remains poorly assessed. During the first epidemic wave, the circulation of the new variant was reported in the literature in Kenya and South Africa [2–4], and by May 2010 cases had been notified in more than 35 countries by the
WHO Pandemic (H1N1) 2009 surveillance system in the African Region [5]. In the sub-Saharan region, the highest numbers of confirmed cases were in South Africa (12 640), Tanzania (770), Ghana (720), Rwanda (524), Kenya (417), and Senegal (325). Forty cases were reported in Mali.

However, no epidemiological data allowing the estimation of the epidemic burden in sub-Saharan Africa were available. Incomplete epidemiological data and the limited recourse to specific influenza diagnosis currently available make any comparison with other parts of the world extremely difficult.

As part of the CoPanFlu-Mali programme, we carried out the first seroepidemiological evaluation of the pH1N1 pandemic in sub-Saharan Africa by studying a rural area located in the southern central part of Mali.

**Materials and Methods**

**Ethics statement**
Study protocol collection was approved by the Institut National de Recherche en Santé Publique (Ministry of Health, Bamako, Mali) ethical committee. The approved informed consent document was translated into the Bambara local language and recorded on audiotape. The tape was played for each participant before written consent was obtained (fingerprint).

**Study population and data collection**
The Malian Millennium village project provided sera from a cohort of 202 subjects followed since 2006, before the epidemic started. Fig. 1 shows the locations of the different villages investigated in the Commune of Dioro (Ségou Region), located 240 km from Bamako, the capital city of Mali. Sampling was performed from eight research villages (marked with yellow triangles in Fig. 1). The distribution in the different villages is indicated in Table 3.

This sampling was dependent on the previous Malian Millennium village project study and on the concrete possibility of obtaining paired sera from the same individuals. It should be noted that the number of individuals in the 10–19-year age group who could be sampled during the 2010 campaign was low (4%), explaining why numbers were lower than expected in the 0–19-year age group and slightly higher in the 20–29-year and 30–39-year age groups, in comparison with the general Malian population and that of the villages included in the study. The collection of sera sampled in 2006 was matched with a second set of sera from the same subjects obtained in April 2010 under similar conditions. The 202 individuals recruited were aged 4–53 years in 2010 (male/female sex ratio = 0.96), matching the Malian age distribution, in which c. 95% of individuals are younger than 60 years and the sex ratio is 0.95. The global distribution of age groups is shown in Table 1.

**Laboratory methods**
Venous blood samples were centrifuged within 2 h. Serum was aliquoted in cryotubes and immediately stored in liquid nitrogen containers until the end of the sampling campaign, and finally stored at −80 °C.

These paired samples were used to estimate the seroconversion rate between 2006 and 2010 and examine the circulation of pH1N1 according to age group. They were tested with a standard haemagglutination inhibition (HI) technique adapted to the detection and quantification of antibodies against pH1N1 [6]. Briefly, the antigen was made of 5.3 haemagglutinating units of non-inactivated virus, strain OPYFLU-1 (a pH1N1 strain isolated from a young adult male patient returning from Mexico in early May 2009 [7]). This strain is...
TABLE 1. Distribution of the population studied based on age group

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>N</th>
<th>0–19 (%)</th>
<th>20–39 (%)</th>
<th>40–59 (%)</th>
<th>&gt;59 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mali, c. 14 million</td>
<td>58.44</td>
<td>23.18</td>
<td>12.76</td>
<td>5.62</td>
<td></td>
</tr>
<tr>
<td>Villages investigated</td>
<td>15,602</td>
<td>54.92</td>
<td>28.57</td>
<td>11.41</td>
<td>5.11</td>
</tr>
<tr>
<td>Population studied</td>
<td>202</td>
<td>43.07</td>
<td>35.15</td>
<td>21.78</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis

The seroprevalence of pH1N1 antibodies was calculated as the percentage with antibody titres of ≥1/40 or ≥1/80. The increase in the prevalence of HI titres of ≥1/40 and ≥1/80 between 2006 and 2010 was indicated as Δ ≥ 1/40 and Δ ≥ 1/80, respectively. The increase in geometric mean titres (GMTs) between 2006 and 2010 was indicated as Δ GMT. Seroconversion was defined as a four-fold or greater increase in antibody titres.

For comparison of seroprevalence between 2006 and 2010, the McNemar chi-square for matched pairs was used. For comparison of the GMTs between 2006 and 2010, the Wilcoxon matched-pairs signed ranks test was used. For comparison of the GMTs between seroconverters and non-seroconverters, the Wilcoxon signed rank test was used.

GMTs were calculated by assigning a titre of 5 to specimens in which no HI antibody was detected. The log transformation used was as follows: log HI titre = log2 (HI titre/5). An unbiased estimate of the GMT can be obtained by a statistical method that originates from the analysis of survival data (maximum likelihood estimation for censored observations). The maximum likelihood estimate of the GMT of truncated HI titres and its confidence interval were obtained.

TABLE 2. Seroprevalence and seroconversion rates in age groups based on pH1N1 HI analysis

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>N</th>
<th>HI ≥ 1/40</th>
<th>2006 (% (95% CI))</th>
<th>2010 (% (95% CI))</th>
<th>Δ ≥ 1/40</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>All age groups</td>
<td>202</td>
<td>16.8 (11.9–22.6)</td>
<td>29.2 (22.9–35.8)</td>
<td>12.4</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>4–19</td>
<td>87</td>
<td>12.6 (6.5–21.5)</td>
<td>31.0 (21.6–41.9)</td>
<td>18.4</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>20–53</td>
<td>115</td>
<td>20.0 (13.1–28.5)</td>
<td>27.8 (19.9–37.0)</td>
<td>7.8</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>N</th>
<th>HI ≥ 1/80</th>
<th>2006 (% (95% CI))</th>
<th>2010 (% (95% CI))</th>
<th>Δ ≥ 1/80</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>All age groups</td>
<td>202</td>
<td>3.5 (1.4–7.0)</td>
<td>16.3 (11.5–22.2)</td>
<td>12.8</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>4–19</td>
<td>87</td>
<td>3.5 (0.7–9.8)</td>
<td>19.5 (11.8–29.4)</td>
<td>16.0</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>20–53</td>
<td>115</td>
<td>3.5 (0.9–8.7)</td>
<td>13.9 (8.5–21.6)</td>
<td>10.4</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>N</th>
<th>GMT</th>
<th>2006 (% (95% CI))</th>
<th>2010 (% (95% CI))</th>
<th>Δ GMT</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>All age groups</td>
<td>202</td>
<td>21.5 (19.6–23.5)</td>
<td>28.6 (25.7–31.6)</td>
<td>7.1</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>4–19</td>
<td>87</td>
<td>20.7 (18.2–23.5)</td>
<td>30.5 (25.8–35.5)</td>
<td>9.8</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>20–53</td>
<td>115</td>
<td>22.1 (19.4–24.9)</td>
<td>27.2 (23.7–31.1)</td>
<td>5.1</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Individuals with 2006 HI titre of &lt;1/40 (% (95% CI))</th>
<th>Individuals with 2006 HI titre of ≥1/40 (%)</th>
<th>All groups (% (95% CI))</th>
<th>Seroconverters (% (95% CI))</th>
<th>Others (% (95% CI))</th>
<th>p-valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>All age groups</td>
<td>17.9 (12.4–24.5)</td>
<td>0.0</td>
<td>14.9 (10.3–20.5)</td>
<td>12.0 (11.5–12.4)</td>
<td>22.5 (20.3–24.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>4–19</td>
<td>21.1 (12.5–31.9)</td>
<td>0.0</td>
<td>18.4 (10.9–28.1)</td>
<td>12.3 (11.6–13.0)</td>
<td>21.1 (18.0–24.5)</td>
<td>0.6</td>
</tr>
<tr>
<td>20–53</td>
<td>15.2 (8.6–24.3)</td>
<td>0.0</td>
<td>12.2 (6.8–19.6)</td>
<td>12.0 (undetermined)</td>
<td>23.5 (20.6–26.4)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

GMT, geometric mean titre; HI, haemagglutination inhibition.

aMcNemar’s test for matched data.
bWilcoxon matched-pairs signed ranks test.
cWilcoxon signed ranks test.
using the PROC LIFEREG in the statistical software package SAS [8].

All data analyses were performed with the statistical software package SAS version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Different estimates of the number of pH1N1 infections could be established from the available dataset:

1. A first ‘standard’ estimate was based on the comparison of HI titres of ≥1/40 in 2006 and 2010 (Δ ≥ 1/40; Table 2A). It indicated a significant increase in the seroprevalence of 12.4%, which was greater in the <20-year age group than in the ≥20-year age group (18.4% and 7.8%, respectively, p 0.02). Fig. 2 shows the seroprevalence by age group and for the overall population.

2. Titres of >1/40 were infrequent in the pre-epidemic period (3.5%, 95% CI 1.4–7.0), but reached 16.3% (95% CI 11.5–22.2) (p <0.0001, McNemar test) in the post-epidemic panel, providing direct evidence for the circulation of pH1N1. The Δ ≥ 1/80 value (which represents the increase in prevalence of HI titres of ≥1/80 between 2006 and 2010, i.e. 12.8%) was used as a second estimate of the number of pH1N1 infections: it was in the same order of magnitude as the Δ ≥ 1/40, and reached significant values in the general population (p 0.001 and p 0.002 in the 4–19-year and 20–53-year age groups, respectively; see Table 2B).

3. The impact of pH1N1 infection was further evaluated by a third method, i.e. by analysing the rate of documented strict seroconversions (a minimum four-fold increase in HI titre between pre-epidemic and post-epidemic samples) in the studied population (Table 2D). In agreement with the results presented above, it was higher in individuals under the age of 20 years (18.4%) than in those over the age of 20 years (12.2%, with similar values for the 20–39-year and 40–53-year age groups; data not shown).

Accordingly, all methods used (Δ ≥ 1/40, Δ ≥ 1/80, and seroconversion rate) provided results in the same order of magnitude, and therefore robust estimates of the infection rates. The increase in the uncensored GMT faithfully followed that of seroconversion rates in the age groups, and indicated a significant increase in all groups between 2006 and 2010 (Table 2C). Pre-epidemic GMT values were lower in seroconverters than in others (Table 2D).

Important variations in seroconversion rate existed among the different villages investigated (0–50%), with a lack of independence between location and seroconversion (exact Fisher test, p 0.046). These are detailed in Table 3.

Discussion

To date, there is little information available regarding the spread of pH1N1 in populations living in rural areas, and even less regarding sub-Saharan Africa. By October 2011, more than 30 seroepidemiological studies of the pandemic had been published in peer-reviewed scientific journals, including studies performed in North America, Europe, Asia,
and Oceania. No study was performed in Africa, very limited information was made available from rural populations, and only a few studies were based on paired sera sampled before and after the pandemic. In addition, the comparability of studies is limited by differences in technical HI protocols and endpoint analysis methods [9]. Accordingly, studying a rural area of southern central Mali, with the same HI protocol as in previously reported studies in other regions of the world (metropolitan France and the southeast Indian Ocean [6,10]), was of great epidemiological interest.

The population investigated has obvious limitations in terms of epidemiological representativeness: it is limited (202 individuals), the 10–19-year age group is under-represented, and, given that the prevalence differed from village to village, the extrapolation of results to larger populations is delicate. However, obtaining paired sera sampled in 2006 and April 2010 in a programme providing strong guarantees in terms of ethical procedures as well as quality of biological samples and collected information constituted a unique opportunity to perform, in a sub-Saharan rural population, a seroepidemiological investigation of the first pH1N1 wave, which occurred in 2009. In Mali, the first case was officially reported by the WHO on 9 January 2010 [11]. As of April 2010, the date corresponding to the beginning of our study, 29 cases had been reported by the WHO [12]. The final count for infected cases was 40 in the country [5] (3 May 2010). No deaths were reported.

HI analyses showed that, in the pre-epidemic period, antibodies cross-reacting with pH1N1 antigen could be detected at titres of ≥1/40 in a significant proportion of individuals (16.8%; Table 2A). Given the age groups examined, it is unlikely that such antibodies were acquired following previous contact with strains related to the 1918 Spanish flu original virus (as proposed in the case of individuals over 60 years of age). It is more likely that they denote cross-reactivity with recent seasonal H1N1 influenza strains, as previously observed in the younger age classes of Western countries (see [13,14] for reviews). Importantly, high pre-pandemic titres (≥1/80) were rare (<4%; Table 2B); that is, cross-reactivity with seasonal H1N1 resulted in low-titre (1/40) pH1N1 HI results. This implies, in accordance with previous observations in metropolitan France [6], that populations commonly exposed to seasonal H1N1 influenza did not develop, in the pre-epidemic period, high-titre cross-reactive antibody responses to pH1N1. Accordingly, the increased prevalence of pH1N1 HI titres of ≥1/80 between the pre-epidemic and post-epidemic periods ($\Delta \geq 1/80 = 12.8\%$; Table 2B) is expected to provide a specific picture of the impact of recent pH1N1 infections in this population. In addition, this value is similar to other estimates of the attack rate based on the $\Delta \geq 1/40$ value or the number of strict seroconversions in paired sera (12.4% and 14.9%, respectively; Table 2).

Where pH1N1 infection occurred, the most susceptible parts of the population identified by our three methods were (Table 2): (i) the 0–19-year age group, as previously reported in other parts of the world; and (ii) individuals with a low titre of cross-reacting antibodies in the pre-pandemic period. Regarding the latter item, serological data revealed that, in all age groups, pre-epidemic GMT values were <15 in individuals who seroconverted and >20 in others (Table 2D). This result is corroborated by the observation that, in all age classes, individuals with HI titres of ≥1/40 in the pre-epidemic period had complete protection against infection (as estimated by seroconversion), whereas the seroconversion rates reached 21.1% and 15.2% in patients with titres of <1/40 belonging to the 0–19-year and 20–53-year age groups, respectively. These findings are in agreement with those from a study performed in Reunion Island [10], using the same HI protocol, and suggest that acquisition of a sufficient titre of cross-reacting antibodies against seasonal influenza viral strains may have been protective against pH1N1 infection. They also confirm that some epidemiological determinants of infection are specific to the youngest age class, as pre-pandemic HI titres of <1/40 were associated with quite different seroconversion rates in the 0–19-year and 20–53-year age groups.

The population studied in the commune of Dioro mainly comprised members of the farming community. No relationship was identified between pH1N1 seroconversion and sex or occupation. This population was extremely stable, with a very few individuals travelling outside their region of origin during the year preceding the 2010 investigation. The proportion of individuals who experienced seroconversion ranged from 0% to 50% according to the different villages included in the analysis, with the highest seroconversion rate being encountered in the village of Tiby Wëre (Table 3), where the only two individuals (one adult and one 4-year-old child) who had travelled significantly over the last 12 months (i.e. to Niono, a village located 200 km away) lived. The child presumably contaminated four individuals in the same family, possibly initiating the outbreak.

This heterogeneous distribution of cases illustrates the specific epidemiological pattern observed in such rural areas. In contrast with what has previously been observed in most developed countries (and in urban populations in general), which are exposed to multiple viral entries because of important population exchanges allowing rapid progression of the pandemic, the geographical stability of the population studied obviously limited the spread of the disease, and some villages were not exposed to the epidemic wave.
This also constitutes, in conjunction with the size of the population studied, a serious limitation to the extrapolation of these results. However, given the specific shape of the Malian age distribution (nearly 60% of the population are younger than 20 years), it is likely that the vast majority of cases occurred in the 0–19-year age group (our data provide estimates of >70%). In conclusion, the most careful estimates indicate that pH1N1 circulated in this rural area in 2009, and that the 40 confirmed cases previously reported in Mali [5] provide a very limited picture of the actual impact of the epidemic. Presumably, the attack rate in the rural area studied was of an order of magnitude comparable with that of Western countries (e.g. c. 1.2% in a French population, using the same HI method [6]).

As the conditions in Mali are quite different from those encountered in most regions of the world investigated previously, being characterized by a rural setting, a limited number of viral introductions, the probable importance of intrafamilial spread, and the absence of specific preventive or curative countermeasures, this study highlights the absolute need to improve our knowledge of the epidemiology of viral respiratory infections in sub-Saharan African countries.

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Transparency Declaration

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