

Epidemiological and Virological Characterization of 2009 Pandemic Influenza A Virus Subtype H1N1 in Madagascar

Arnaud Orelle,^{1,a} Norosoa Harline Razanajatovo,^{1,a} Soatiana Rajatonirina,² Jonathan Hoffmann,^{1,3} Laurence Randrianasolo,² Girard Marcellin Razafitrimo,¹ Dhamari Naidoo,⁴ Vincent Richard,^{2,b} and Jean-Michel Heraud¹

¹National Influenza Center, Virology Unit, and ²Epidemiology Unit, Institut Pasteur de Madagascar, and ³Centre d'Infectiologie Charles Mérieux, Faculté de Médecine, Université d'Antanarivo, Antananarivo, Madagascar; and ⁴National Influenza Unit, National Institute for Communicable Diseases of the National Health Laboratory Service, Sandringham, South Africa

Background. Madagascar was one of the first African countries to be affected by the 2009 pandemic of influenza A virus subtype H1N1 [A(H1N1)pdm2009] infection. The outbreak started in the capital city, Antananarivo, and then spread throughout the country from October 2009 through February 2010.

Methods. Specimens from patients presenting with influenza-like illness were collected and shipped to the National Influenza Center in Madagascar for analyses, together with forms containing patient demographic and clinical information.

Results. Of the 2303 specimens tested, 1016 (44.1%) and 131 (5.7%) yielded A(H1N1)pdm09 and seasonal influenza virus, respectively. Most specimens (42.0%) received were collected from patients <10 years old. Patients <20 years old were more likely than patients >50 years old to be infected with A(H1N1)pdm09 (odds ratio, 2.1; 95% confidence interval, 1.7–2.6; $P < .01$). Although phylogenetic analyses of A(H1N1)pdm09 suggested multiple introductions of the virus into Madagascar, no antigenic differences between A(H1N1)pdm09 viruses recovered in Madagascar and those that circulated worldwide were observed.

Conclusions. The high proportion of respiratory specimens positive for A(H1N1)pdm09 is consistent with a widespread transmission of the pandemic in Madagascar. The age distribution of cases of A(H1N1)pdm09 infection suggests that children and young adults could be targeted for interventions that aim to reduce transmission during an influenza pandemic.

Influenza pandemics are widespread outbreaks of highly contagious respiratory disease that appear suddenly, infrequently, and at irregular intervals [1]. Influenza pandemics are characterized by the rapid worldwide spread of a novel influenza virus to which

humans have had no previous exposure [2]. Influenza symptoms during a pandemic are nonspecific, and therefore laboratory confirmation is required to positively identify a case.

On 11 June 2009, 2 months after the first case of swine-origin 2009 pandemic influenza A virus subtype H1N1 [A(H1N1)pdm2009] infection was detected in Mexico [3, 4], the World Health Organization (WHO) declared a level 6 pandemic alert (defined as sustained, community-wide, human-to-human transmission occurring in at least 2 WHO regions) [5, 6], because of the rapid global spread of the virus [7].

In Madagascar, influenza surveillance has been conducted for several decades. A WHO National Influenza Center was officially recognized in 1978. In September 2007, the Malagasy Ministry of Health, with the support of the Institut Pasteur de Madagascar, established a sentinel surveillance network (SSN)

Presented in part: Options for the Control of Influenza, Hong Kong, SAR, 3–7 September 2010 (Poster P-368); Global National Influenza Centers Meeting, Hammamet, Tunisia, 30 November–3 December 2010.

^aA. O. and N. H. R. contributed equally to the study.

^bPresent affiliation: Epidemiology Unit, Institut Pasteur de Dakar, Dakar, Senegal.

Correspondence: Jean-Michel Heraud, PhD, National Influenza Center, Virology Unit, Institut Pasteur de Madagascar, Ambatofotsikely, BP 1274, Antananarivo 101, Madagascar (jmheraud@pasteur.mg).

The Journal of Infectious Diseases 2012;206(S1):S140–7

© The Author 2012. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/infdis/jis533

to monitor febrile illnesses on the basis of daily reports of epidemiological data and weekly virological surveillance [8]. The aim of the network was to facilitate rapid detection of an outbreak and to identify the circulating virus (eg, influenza viruses or arboviruses) responsible for the outbreak.

In this study, we describe the epidemiological and virological characteristics of the 2009 pandemic of A(H1N1)pdm09 infection in Madagascar during the first wave of disease, from August 2009 through February 2010.

MATERIALS AND METHODS

National SSN

The National SSN encompasses private and public primary healthcare centers as part of a global National Integrated Surveillance System that monitors fever syndromes [8]. In August 2009, SSN included 24 sites located in 17 of the 22 health regions in the country. The sites send daily epidemiological reports on influenza-like illness (ILI) cases, while 9 sites ship specimens from up to 5 suspected cases per week.

Surveillance of Study Subjects and Specimen Collection

Samples were received through the SSN and the private health sector, which encompasses general practitioners, private clinics, and hospitals. During the pre-pandemic period in Madagascar (from April through October 2009), a suspected case of A(H1N1)pdm09 infection was defined as a patient with ILI who met at least one of the following criteria from the WHO case definition [9]: (1) returned from a country or region with an epidemic of A(H1N1)pdm09 infection within the past 7 days, (2) was in close contact with a persons with a confirmed case of A(H1N1)pdm09 infection within the past 7 days, or (3) handled samples suspected of containing A(H1N1)pdm09 in a laboratory or other setting within the past 7 days. When the pandemic was declared by the Malagasy Ministry of Health in October 2009 (calendar week 43), the case definition for suspected A(H1N1)pdm09 infection was changed to the WHO standard case definition for ILI (ie, fever [temperature, $\geq 38^{\circ}\text{C}$] with cough and/or sore throat). Patients with suspected cases were sampled as previously described [10].

Oral and/or nasopharyngeal specimens were placed in universal transport medium (Copan, Italy) and shipped at 4°C to the National Influenza Center at the Institut Pasteur de Madagascar. Specimens were either processed upon reception or stored at -80°C . Demographic and clinical information was collected from each patient with a suspected case.

Virus Detection

Patients with suspected cases were tested using the Centers for Disease Control and Prevention (CDC) real-time reverse-transcription polymerase chain reaction protocol for detection and characterization of human and swine influenza virus [11].

This detection kit includes panels of oligonucleotide primers and dual-labeled hydrolysis (Taqman[®]) probes for the identification of influenza A virus (subtype H3N2 A(H3N2)s, seasonal subtype H1N1 A(H1N1)s, and A(H1N1)pdm09) and influenza B virus (B).

Phylogenetic Analyses

A(H1N1)pdm09 isolates were sent to the WHO Collaborative Centre (London, United Kingdom) and to the National Institute for Communicable Diseases (Johannesburg, South Africa) for sequencing. Sequences of hemagglutinin (HA) and neuraminidase (NA) genes from a subset of Malagasy viruses were aligned by the ClustalW program, using MEGA 4 software [12]. Phylogenetic trees were reconstructed using the neighbor-joining method of MEGA 4 software. Sequences were deposited into the Global Initiative on Sharing Avian Influenza Database (available at: <http://platform.gisaid.org>) and GenBank JQ733142 to JQ733145; JQ733151 to JQ733153; JQ733161; JQ733185; JQ733186; JQ733189 to JQ733193 and JQ733196.

Data Analysis

We described the demographic and clinical characteristics of patients with suspected and confirmed cases of A(H1N1)pdm09 infection. We compared the clinical characteristics of A(H1N1)pdm09-positive patients to those of uninfected patients and those of patients infected with seasonal influenza viruses. The χ^2 test and the Fisher exact test were used for univariate analysis. *P* values of $<.05$ were considered to be statistically significant. Variables with a *P* value of $<.20$ from univariate analysis were included in multivariate logistic regression analysis. A backward stepwise analysis was performed, and at each step, the new model was compared with the previous one. To confirm whether the model fit, a maximum-likelihood χ^2 test was used. Analyses were performed using R software, version 2.12.0 (available at: <http://www.r-project.org>).

RESULTS

Characteristics of Patients and Specimens

In Madagascar, the first imported case of A(H1N1)pdm09 infection was confirmed on 12 August 2009 in a patient returning from India (Figure 1). Thereafter, several imported cases were detected until October 2009. The first laboratory-confirmed cases of A(H1N1)pdm09 infection in individuals without a history of travel were detected on 8 October 2009. For this study, we selected a period that covered the entire first wave of the pandemic (ie, from 1 August 2009 through 28 February 2010), from the time of the first detected case of A(H1N1)pdm09 infection to the Ministry of Health's declaration, after 4 consecutive weeks without a new case, that the first wave had ended.

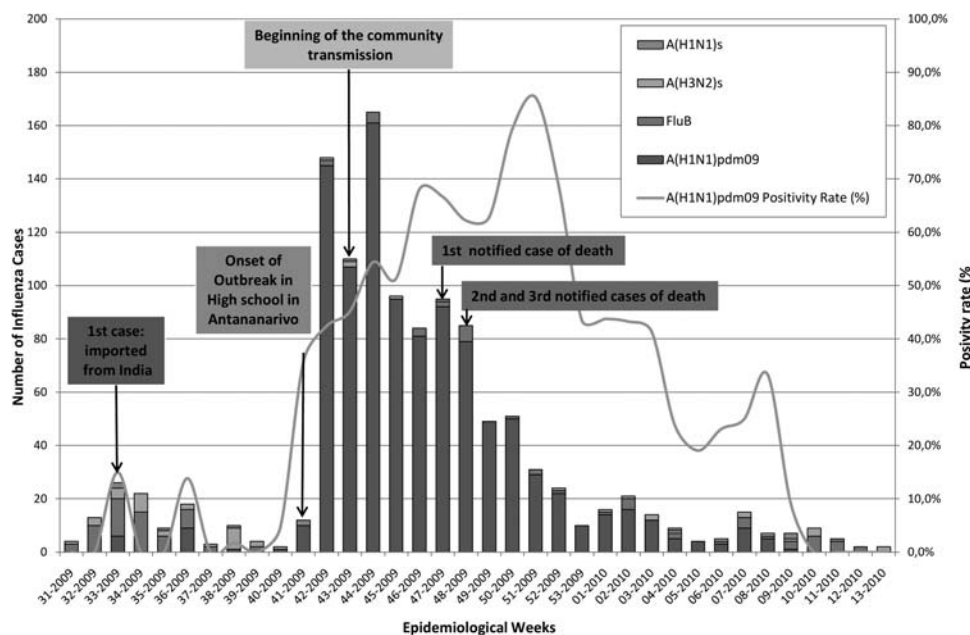


Figure 1. Distribution of infections due to 2009 pandemic influenza A virus subtype H1N1 [A(H1N1)pdm2009] and seasonal influenza virus (influenza A virus subtypes H1N1 [A(H1N1)s] and H3N2 [A(H3N2)s] and influenza B virus [FluB], as well as the rate of A(H1N1)pdm09 positivity among tested specimens, 2009–2010, Madagascar.

A total of 2353 specimens were received at the National Influenza Center, of which 40.9% (963 of 2353) were collected (Table 1). From the 24 established sentinel sites, specimens were received from 20 sites, and A(H1N1)pdm09 cases were confirmed at 16 sites. The number of samples collected at the sentinel sites ranged from 1 to 182 (median number, 24 samples), and positivity rates ranged from 0% to 76% (median rate, 43%). Specimens received from sites located in the

capital, Antananarivo, represented 41.2% of total specimens received from the SSN. Interestingly, of the 59.1% of specimens (1390 of 2353) received from outside of the SSN (eg, from hospitals and private clinics), most came from the capital city during the first weeks of the epidemic.

The age distribution of patients from whom specimens were collected was different from the age distribution of the overall Malagasy population. Indeed, more specimens were collected from patients <20 years old than from patients >20 years old (1608 and 706 specimens, respectively). Overall, we tested 97.9% of specimens (2303) received. A total of 1016 of 2303 patients (44.1%) were confirmed to be infected with A(H1N1)pdm09. Seasonal influenza viruses (A(H1N1)s, A(H3N2)s, and B) were detected in 131 of 2303 patients (5.7%), and influenza A viruses (unsubtyped) were detected in 88 of 2303 (3.8%) (Figure 1 and Table 2).

Among patients with confirmed pandemic influenza, 505 (49.7%) were male (Table 2). The median age was 11 years and ranged from 5 weeks to 79 years. Overall, the majority of specimens received (56.2%; 1323 of 2353) and confirmed case of A(H1N1)pdm09 infection (64.5%) involved children <15 years old. While most specimens tested (26.4%) were from patients <5 years old, patients aged 5–9 years, 10–14 years, and 15–19 years had the highest positivity rates for A(H1N1)pdm09 (61.0%, 60.9%, and 49.3%, respectively). Young people and children aged <20 years were significantly more affected than other age groups (odds ratio [OR], 2.1; 95% confidence

Table 1. Laboratory Results for Specimens Collected in Our Sentinel Surveillance Network (SSN) and From Other Areas Between 1 August 2009 and 28 February 2010, Madagascar.

Region ^a	Specimens Received, No.	Specimens Tested, No.	Positive for A (H1N1)pdm09, No. (%)
Total SSN (24) ^b	963	955	416 (43.6)
Antananarivo (4) ^b	397	393	166 (42.2)
Provinces (20) ^b	566	562	250 (44.5)
Other ^c	1390	1348	600 (44.5)
Total	2353	2303	1016 (44.1)

Abbreviation: A(H1N1)pdm09, 2009 pandemic influenza A virus subtype H1N1.

^a For clarity, we split results into 2 regions, Antananarivo, the main capital city that represent 10% of the Malagasy population, and provinces that encompass all main cities of all administrative districts.

^b Number of sites.

^c Other means specimens received outside our SSN (ie, hospital, private clinics, etc).

Table 2. Sampling Criteria, Age Distribution, and Clinical Symptoms Among Patients With or Without Infection Due to 2009 Pandemic Influenza A Virus Subtype H1N1 A(H1N1)pdm09 or Another Influenza Virus (Seasonal), Madagascar

Variable	A(H1N1)pdm09 (n = 1016)		Other Influenza ^a (n = 131)		Negative (n = 1068)		A(H1N1)pdm09 vs Negative			A(H1N1)pdm09 vs Other Influenza ^a		
	No.	%	No.	%	No.	%	OR	95% CI	<i>P</i> ^b	OR	95% CI	<i>P</i> ^b
Sampling criteria (case definition) ^c												
ILI	785	77.3	100	76.3	662	62.0	1.9	1.6–2.4	<.01	1.1	.7–1.7	<.01
Non-ILI	177	17.4	25	19.1	293	27.4	Ref	...		Ref	...	
Missing ^f	54	5.3	6	4.6	113	10.6	
Age, y												
0–4	230	22.6	28	21.4	324	30.3	1.8	1.1–3.0	<.01	3.9	1.7–8.6	.02
5–9	224	22.0	26	19.8	109	10.2	5.3	3.2–8.9	<.01	4.1	1.8–9.1	<.01
10–14	201	19.8	14	10.7	110	10.3	4.6	2.8–7.9	<.01	6.9	2.8–16.6	<.01
15–19	138	13.6	13	9.9	121	11.3	2.9	1.7–4.9	<.01	5.1	2.1–12.5	<.01
20–29	81	8.0	17	13.0	122	11.4	1.7	.9–2.9	.06	2.3	.9–5.4	.06
30–39	57	5.6	11	8.4	120	11.2	1.2	.7–2.1	.49	2.7	1.0–7.3	.04
40–49	49	4.8	9	6.9	76	7.1	1.6	.9–2.9	.09	2.3	.9–6.3	.08
≥50	25	2.5	12	9.2	64	6.0	Ref	...		Ref	...	
Missing	11	1.1	1	0.8	22	2.1	
Sex												
Male	505	49.7	57	43.5	496	46.4	Ref	...		Ref	...	
Female	492	48.4	74	56.5	550	51.5	0.9	.7–1.1	.14	0.8	.5–1.1	.12
Missing	19	1.9	0	0.0	22	2.1	
Clinical symptom												
Fever	793	78.1	108	82.4	699	65.4	1.7	1.4–2.1	<.01	0.8	.4–1.3	.36
Cough	954	93.9	114	87.0	899	84.2	4.4	3.1–6.3	<.01	3.3	1.8–6.0	<.01
Sore throat	586	57.7	84	64.1	588	55.1	1.1	.9–1.3	.09	0.8	.5–1.1	.38
Asthenia	625	61.5	55	42.0	603	56.5	1.3	1.1–1.5	<.01	2.2	1.5–3.2	<.01
Headache	697	68.6	72	55.0	608	56.9	1.7	1.4–2.0	<.01	1.8	1.3–2.7	<.01
Rhinorrhea	711	70.0	91	69.5	677	63.4	1.4	1.1–1.7	<.01	1.0	.7–1.5	.9
Dyspnea	178	17.5	12	9.2	196	18.4	0.9	.7–1.1	.17	1.2	.7–2.4	.5
Muscular pain	404	39.8	32	24.4	352	33.0	1.2	.9–1.4	.06	1.0	.7–1.7	.9
Diarrhea	104	10.2	11	8.4	124	11.6	0.8	.6–1.0	.10	0.7	.4–1.5	.3

Abbreviations: CI, confidence interval; ILI, influenza-like illness; OR, odds ratio; Ref, reference.

^a Other influenza are seasonal influenza viruses (ie, influenza A virus subtypes H1N1 and H3N2 and influenza B virus). Some influenza A viruses (88) were detected but untyped because of a high cycle threshold value but were not include in the table.

^b Considered statistically significant when $P < .01$.

^c "ILI" was defined as fever and cough and/or sore throat. "Non-ILI" was defined as nonsatisfaction of the ILI case definition and presence of 1 or 2 of the following signs: fever, cough, and sore throat. "Missing" was defined as the absence of information about fever, cough, and sore throat.

interval [CI], 1.7–2.6; $P < .01$), and individuals aged 5–19 years were at higher risk of getting infected with A(H1N1)pdm09 (OR range, 2.9 [95% CI, 1.7–4.9] to 5.3 [95% CI, 3.2–8.9]). No significant difference was observed between the 50 nonanalyzed specimens and the analyzed specimens with regard to sex or age group (data not shown).

Clinical Symptoms of Patients

To compare clinical symptoms according to the different subtypes of influenza viruses, all 88 patients with untyped influenza A virus were excluded. Complete or partial clinical information was available for the 1016 confirmed pandemic

cases. The most frequent symptoms reported were cough (93.9%), fever (78.1%), and rhinorrhea (70.0%) (Table 2). Only 178 patients (17.5%) reported dyspnea and 104 (10.2%) had diarrhea. Overall, 1547 patients who were sampled (69.8%) met the ILI case definition, and influenza infection with A(H1N1)pdm09 was confirmed in 50.7% (Table 2). Among the 1016 patients with confirmed A(H1N1)pdm09 infection, 785 (77.3%) met the ILI case definition, but 177 (17.4%) did not (Table 2). Among all analyzed samples, the positivity rate among specimens from patients who met the ILI case definition was higher (57.2%) than for samples from those who did not meet the case definition (40.9%) ($P < .01$).

A total of 552 specimens (23.4%) were received from hospitalized patients. Of these patients, 190 (34.4%) had A(H1N1)pdm09 infection. A total of 53.7% of infected patients were male, 45.3% were children aged <10 years, and 10 (5.3%) were aged >50 years. During the study period, 3 deaths of patients with confirmed A(H1N1)pdm09 occurred. One patient had a history of tuberculosis, a second was a heavy smoker with a history of alcoholism, and a third had congestive heart failure.

When comparing patients with A(H1N1)pdm09 infection to uninfected patients, fever (OR, 1.7; 95% CI, 1.4–2.1), cough (OR, 4.4; 95% CI, 3.1–6.3), asthenia (OR, 1.3; 95% CI, 1.1–1.5), headache (OR, 1.7; 95% CI, 1.4–2.0), and rhinorrhea (OR, 1.4; 95% CI, 1.1–1.7) were more frequent among persons with pandemic influenza (Table 2). In a multivariate analysis that adjusted for age group and clinical symptoms, cough (OR, 4.6; 95% CI, 3.0–7.5), headache (OR, 1.8; 95% CI, 1.5–2.2), fever (OR, 1.6; 95% CI, 1.3–2.0), and asthenia (OR, 1.4; 95% CI, 1.1–1.7) remained associated with pandemic influenza.

When comparing patients with A(H1N1)pdm09 infection to patients with seasonal influenza virus infection, cough (OR, 3.3; 95% CI, 1.8–6.0), asthenia (OR, 2.2; 95% CI, 1.5–3.2), and headache (OR, 1.8; 95% CI, 1.3–2.7) were more common among patients with pandemic influenza (Table 2). In a multivariate analysis that adjusted for age and clinical symptoms, cough (OR, 4.8; 95% CI, 2.2–10.3), asthenia (OR, 3.2; 95% CI, 2.1–4.9), and headache (OR, 1.7; 95% CI, 1.1–2.6) remained associated with A(H1N1)pdm09 infection.

Virological Characteristics of A(H1N1)pdm09 in Madagascar Antigenic Characteristics of Malagasy Isolates

Malagasy isolates were antigenically closely related to the vaccine strain A/California/7/2009 virus and showed good reactivity against antisera from ferrets immunized with the vaccine strain (J. McCauley, National Institute for Medical Research, personal communication). A set of viruses were analyzed with a wider panel of antisera that included sera raised against viruses from later on in the pandemic (Bayern/69/2009 and A/Lviv/N6/2009). Again, the viruses were homogeneous to these sera, as well as to the older sera. Analyses with different panel of antisera showed that the Malagasy viruses were all homogeneous, except for A/Madagascar/95/2010, which showed a lower level of reactivity with several sera of the panel (J. McCauley, personal communication).

Genetic Characterization of HA Genes From Malagasy Isolates

Sequencing data for the HA gene were available for 26 A(H1N1)pdm09 viruses identified between 2009 and 2010. Phylogenetic analysis revealed 3 amino acid changes in the HA gene, I321V, P83S, and S203T, which were identified in 20 viruses. These changes are similar to the changes identified in

viruses isolated worldwide between 2009 and 2010 (Supplementary Figure 1). Among these viruses, 2 subgroups are predominant. The first subgroup showed a unique change (A256T), whereas the second subgroup showed a unique change (D86G). The A256T change is on the head of the HA away from the receptor-binding site in the globular head of the trimer. In the D86G subgroup, we found one isolate, A/Madagascar/95/2010, that showed a lower reactivity against some antisera from immunized ferrets mentioned earlier.

Genetic Characterization of NA Genes From Malagasy Isolates

Phylogenetic analysis of 11 A(H1N1)pdm09 Malagasy viruses isolated between 2009 and 2011 showed 2 amino acid changes, V67I and E398K, in viruses identified between October and December 2009 (Supplementary Figure 2). However, in 2010, different amino acid changes, G77E and R257K, were identified, clustering with viruses isolated in Hong Kong in 2010. Again, these changes were not retained because isolates from 2011 showed different changes (V241I and N369K) as compared to the 2010 isolates from La Reunion Island. The Malagasy viruses did not contain the amino acid change at H275Y associated with oseltamivir resistance (H275Y).

Sensitivity to Sialidase Inhibitors

A subset of 22 A(H1N1)pdm09 viruses were tested for resistance to the sialidase inhibitors oseltamivir and zanamivir, and all were sensitive to both inhibitors (J. McCauley, personal communication).

DISCUSSION

During a 7-month period of the 2009 influenza pandemic, the National Influenza Center received and analyzed 2303 specimens, compared with an average of 432 specimens for the same period during the previous 3 years. The high rate of positivity for A(H1N1)pdm09 among specimens analyzed (45.9%), compared with the average positivity rate for seasonal influenza during the last 3 previous years (38.8%; J. M. Heraud, personal communication), is likely due to the lack of immunity toward this new reassortant virus among the general population. Similar results were observed in several countries [13, 14]. Moreover, data from serological studies suggested a large diffusion of A(H1N1)pdm09 virus among the population [15]. Nevertheless, this rate is quite similar to the rates in some years with active circulation of some seasonal influenza viruses (eg, 45.3% from August 2007 through February 2008) (J. M. Heraud, personal communication). The high number of specimens received might reflect increased media attention and the fear of the population toward this new virus. The real impact of the pandemic within the Malagasy

population might be similar to that of seasonal influenza. Despite some sporadic cases since February 2010, no second wave of A(H1N1)pdm09 infection occurred in Madagascar. As a result, a national serosurvey will be conducted to estimate the incidence of A(H1N1)pdm09 infection in the Malagasy population.

As previously reported, individuals aged 0–4 years accounted for the highest number of cases of A(H1N1)pdm09 infection worldwide [16]. This is probably because mothers are more likely to visit a healthcare center when their children are ill, compared with young adults and adults, who prefer to stay at home and use self-administered medication. Interestingly, our analysis showed that young people and children aged <20 years were significantly more affected than other age groups and that individuals aged 5–19 years had a higher risk of infection with A(H1N1)pdm09. These results are supported by previous studies showing that the highest cumulative incidence of infection with influenza virus occurred in people aged 5–14 years, because of their behavior and because of exposure at school [17, 18]. Thus, to reduce transmission during a pandemic, this population might be a key target for vaccination, both for their own protection (particularly for young children between 6 months and 5 years of age) and to maintain herd immunity. Among people born before 1989 (ie, those ≥ 20 years old), the percentage of A(H1N1)pdm09-positive individuals decreased globally with age. This age distribution in Madagascar is in accordance with a recently published serological study in England, which showed that older age groups seem to be more protected against A(H1N1)pdm09 because of preexisting antibodies directed against other influenza virus subtypes that could induce cross-protection [19]. Among patients who required hospitalization, 10 (5.3%) were aged ≥ 50 years. This rate is more than the global rate among patients with confirmed A(H1N1)pdm09 infection for the same age groups (2.5%) but similar to recently published data that demonstrated that highest risk of death per capita was among individuals aged ≥ 50 years [20].

The establishment of the SSN helped in the early detection and efficient monitoring of the pandemic. The network was used as a backbone for other health districts to ship specimens from patients with suspected cases, allowing the National Influenza Center to confirm cases from health districts not directly covered by the network. Moreover, the comparison of positivity rates between specimens collected within and outside of the SSN (43.6% and 44.5%, respectively) indicates that the SSN is representative of the population. In Madagascar, after the first imported case of A(H1N1)pdm09 infection was detected in August 2009, several imported cases were detected until October 2009, similar to other countries [21]. The first laboratory-confirmed A(H1N1)pdm09 cases without a travel history were detected on 8 October 2009 in 3 teenagers from one of the largest schools in Antananarivo [22]. Some of the

specimens received did not strictly meet the ILI case definition. Because of the controversy surrounding the ILI case definition, we took the opportunity to study whether the ILI criteria were sensitive and/or specific enough to capture a good proportion of pandemic cases. [23–25]. On the basis of our results, use of “fever” as the only criterion for ILI during the pandemic, which has sometimes been suggested, does not seem to be specific enough to detect true ILI. Indeed, among all analyzed samples, we noticed that the positivity rate among specimens that met the ILI case definition was higher than for samples that did not meet the ILI definition. Interestingly, absence of at least 1 criterion from the ILI case definition (ie, fever, cough, or sore throat) is linked to the lowest detection rate for influenza (34.7%). Consequently, for low-resources countries with limited laboratory capacities, sampling and testing specimens only from individuals who meet the ILI case definition should be considered. We noticed that general practitioners (GPs) outside our SSN collected many specimens from patients who did not meet the ILI case definition. This finding highlights the need to train GPs to use appropriate case definitions when sampling patients for surveillance purposes. Multivariate analysis showed that only fever, cough, and headache were significantly more common among patients with A(H1N1)pdm09 infection, suggesting that these clinical symptoms are more predictive of pandemic infection.

The typical seasonal pattern of influenza activity in Madagascar is January–March and June–October [10, 26, 27]. Thus, the circulation of A(H1N1)pdm09 occurred during the typical period for seasonal influenza within the community in Antananarivo. This may in part explain the intensity and spread of the epidemic. The pandemic virus circulated in Madagascar for approximately 6 months. The pattern of spread of the virus and the observation of epidemic peaks at different times in different regions (data not shown) were probably due to differences in the introduction of viruses, population mobility factors, population density, and bioclimatic factors. Indeed, the capital city is located in a more temperate region of Madagascar, while coastal areas have a tropical, equatorial, or semi-arid climate.

During the epidemic, the majority of strains that circulated in Madagascar did not show any antigenic differences from viruses that circulated in other regions of the world, suggesting that a vaccination campaign could have reduced morbidity. Results of phylogenetic analyses of the HA and NA genes of viruses isolated during the study period, especially comparisons of the 2 groups that showed different unique changes (A256T or D86G), favors multiple introductions of A(H1N1)pdm09 strains within the country. Interestingly, sequences of recent 2011 isolates showed that they are more closely related with viruses isolated in La Reunion. This result confirmed that 2009–2010 viruses in Madagascar did not continue to circulate in the country and were replaced in 2011 with new lineages.

Our study has a number of limitations. During the beginning of the epidemic, a large number of specimens received were collected in patients <20 years old, because of outbreaks in schools. Little information was available regarding underlying comorbidities, intensive care unit admissions, and deaths of infected patients. Because only 3 deaths were confirmed, we could not address the severity of or risk factors for pandemic influenza in Madagascar. Another limitation is the lack of sequencing data to better define each season. If we had more viruses sequenced in 2009 and 2010, we might have seen more lineages for the HA gene.

In conclusion, the SSN, which was established for the early detection of outbreaks, proved useful in the early detection and monitoring of the 2009 influenza pandemic in Madagascar. Nevertheless, our system proved to be inefficient to detect an increase in mortality and to estimate the disease burden of the pandemic. Thus, it is important to implement surveillance at the hospital level in the future, to better estimate mortality due to new variants.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Dr Yolande Raelina Rajaona (Direction des Urgences et de la Lutte contre les Maladies Négligées), Dr Armand Randrianarivo-Solofoniaina (Direction de la Veille Sanitaire et de la Surveillance Epidémiologique), and all staff working at the Ministry of Health for supporting the sentinel surveillance network. We are deeply indebted to all general practitioners and nurses involved every day in the sentinel surveillance in Madagascar. We also recognize the tremendous work of all technicians from the virology unit, all physicians and nurses who worked at the private and public clinics during the pandemic, and particularly Joël Lust, Lydia Ramandimbisoa, and Isabelle Rasoarahona (Lycée Français de Tananarive), Dr Jean-Marie Berthezène (Centre médico-social de l' Ambassade de France), and Dr Marc Ramanantseheno. We also thank John McCauley and his team from the National Institute for Medical Research for their help in antigenic assays and genetic analysis, as well as Mark Katz and Adam Cohen for helpful discussions during all process of writing of this manuscript.

Disclaimer. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Ministry of Health.

Financial support. This work was supported by the Office of the Assistant Secretary for Preparedness and Response, US Department of Health and Human Services (GRANT No. IDSEP060001-01-01), the Centers for Disease Control and Prevention (cooperative agreement U51/IP000327-01), and the WHO office in Madagascar.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

- Hsieh YC, Wu TZ, Liu DP, et al. Influenza pandemics: past, present and future. *J Formos Med Assoc* **2006**; 105:1–6.
- Nguyen-Van-Tam JS, Hampson AW. The epidemiology and clinical impact of pandemic influenza. *Vaccine* **2003**; 21:1762–8.
- Update: novel influenza A (H1N1) virus infection—Mexico, March–May, 2009. *MMWR Morb Mortal Wkly Rep* **2009**; 58:585–9.
- Update: swine influenza A (H1N1) infections—California and Texas, April 2009. *MMWR Morb Mortal Wkly Rep* **2009**; 58:435–7.
- Chan M. World now at the start of 2009 influenza pandemic—statement to the press by WHO Director-General. http://www.who.int/mediacentre/news/statements/2009/h1n1_pandemic_phase6_20090611/en/index.html. Accessed 26 December 2011.
- WHO. Current WHO phase of pandemic alert. http://www.who.int/csr/disease/avian_influenza/phase/en/. Accessed 20 December 2011.
- Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* **2009**; 360:2605–15.
- Randrianasolo L, Raelina Y, Ratsitorahina M, et al. Sentinel surveillance system for early outbreak detection in Madagascar. *BMC Public Health* **2010**; 10:31.
- WHO. Recommended surveillance standards WHO/CDS/CSR/ISR/99.2. <http://www.who.int/csr/resources/publications/surveillance/whocdscsr992.pdf>. Accessed 20 December 2011.
- Razanajatovo NH, Richard V, Hoffmann J, et al. Viral etiology of influenza-like illnesses in Antananarivo, Madagascar, July 2008 to June 2009. *PLoS One* **2011**; 6:e17579.
- Shu B, Wu KH, Emery S, et al. Design and performance of the CDC real-time reverse transcriptase PCR swine flu panel for detection of 2009 A (H1N1) pandemic influenza virus. *J Clin Microbiol* **2011**; 49:2614–9.
- Tamura K, Dudley J, Nei M, et al. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **2007**; 24:1596–99.
- Echavarría M, Querci M, Marcone D, et al. Pandemic (H1N1) 2009 cases, Buenos Aires, Argentina. *Emerg Infect Dis* **2010**; 16:311–3.
- Sigmundsdóttir G, Gudnason T, Ólafsson O, et al. Surveillance of influenza in Iceland during the 2009 pandemic. *Euro Surveill* **2010**; 15:19742.
- Zimmer SM, Crevar CJ, Carter DM, et al. Seroprevalence following the second wave of pandemic 2009 H1N1 influenza in Pittsburgh, PA, USA. *PLoS One* **2010**; 5:e11601.
- Lopez L, Huang S. New Zealand Public Health Surveillance, Influenza Weekly Update 2009/45. http://www.surv.esr.cri.nz/PDF_surveillance/Virology/FluWeekRpt/2009/FluWeekRpt200945.pdf. Accessed 20 December 2011.
- Devaux I, Kreidl P, Penttinen P, Salminen M, Zucs P, Ammon A. Initial surveillance of 2009 influenza A(H1N1) pandemic in the European union and European economic area, April–September 2009. *Euro Surveill* **2010**; 15:19740.
- Baguelin M, Hoschler K, Stanford E, et al. Age-specific incidence of A/H1N1 2009 influenza infection in England from sequential antibody prevalence data using likelihood-based estimation. *PLoS One* **2011**; 6:e17074.
- Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. *Lancet* **2010**; 375:1100–8.
- Van Kerkhove MD, Vandemaële KA, Shinde V, et al. Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. *PLoS Med* **2011**; 8:e1001053.
- Liang M, Lye DC, Chen MI, et al. New influenza A (H1N1) 2009 in Singapore: the first ten adult imported cases. *Singapore Med J* **2009**; 50:581–3.
- Rajatonirina S, Heraud JM, Randrianasolo L, et al. Pandemic influenza A(H1N1) 2009 virus outbreak among boarding school pupils in Madagascar: compliance and adverse effects of prophylactic oseltamivir treatment. *J Infect Dev Ctries* **2011**; 5:156–62.

23. Kasper MR, Wierzba TF, Sovann L, Blair PJ, Putnam SD. Evaluation of an influenza-like illness case definition in the diagnosis of influenza among patients with acute febrile illness in Cambodia. *BMC Infect Dis* **2010**; 10:320.
24. Navarro-Mari JM, Perez-Ruiz M, Cantudo-Munoz P, Petit-Gancedo C, Jimenez-Valera M, Rosa-Fraile M. Influenza-like illness criteria were poorly related to laboratory-confirmed influenza in a sentinel surveillance study. *J Clin Epidemiol* **2005**; 58:275–9.
25. Call SA, Vollenweider MA, Hornung CA, Simel DL, McKinney WP. Does this patient have influenza? *JAMA* **2005**; 293:987–97.
26. Gessner BD, Shindo N, Briand S. Seasonal influenza epidemiology in sub-Saharan Africa: a systematic review. *Lancet Infect Dis* **2011**; 11:223–35.
27. Rabarijaona LP, Rakotondrarija NT, Rousset D, Soares JL, Mauclere P. [Influenza epidemiologic and virologic surveillance in Antananarivo from 1995 to 2002]. *Arch Inst Pasteur Madagascar* **2003**; 69:20–6.