



Review

The 2009 A (H1N1) influenza virus pandemic: A review[☆]Marc P. Girard^{a,*}, John S. Tam^b, Olga M. Assossou^c, Marie Paule Kieny^b^a University Paris-7 Denis Diderot, 39 rue Seignemartin, 69008, Lyon, France^b World Health Organization, 20 Av Appia, CH-1211 Geneva 27, Switzerland^c Unité de Recherche Clinique Lariboisière-St Louis, Hôpital Fernand Widal, 200 rue du Fbg Saint Denis, 75010, Paris, France

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ABSTRACT

In March and early April 2009 a new swine-origin influenza virus (S-OIV), A (H1N1), emerged in Mexico and the USA. The virus quickly spread worldwide through human-to-human transmission. In view of the number of countries and communities which were reporting human cases, the World Health Organization raised the influenza pandemic alert to the highest level (level 6) on June 11, 2009. The propensity of the virus to primarily affect children, young adults and pregnant women, especially those with an underlying lung or cardiac disease condition, and the substantial increase in rate of hospitalizations, prompted the efforts of the pharmaceutical industry, including new manufacturers from China, Thailand, India and South America, to develop pandemic H1N1 influenza vaccines. All currently registered vaccines were tested for safety and immunogenicity in clinical trials on human volunteers. All were found to be safe and to elicit potentially protective antibody responses after the administration of a single dose of vaccine, including split inactivated vaccines with or without adjuvant, whole-virion vaccines and live-attenuated vaccines. The need for an increased surveillance of influenza virus circulation in swine is outlined.

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1. Introduction

Since the global H1N1 influenza virus pandemic of 1918, influenza virus gene reassortment has been documented and observed to occur among human influenza viruses with different

subtypes, between human and avian viruses, and among animal (including avian and other animals) influenza viruses. Such reassortant viruses led to the global pandemics of 1957 (H2N2) and 1968 (H3N2) [1,2]. Although A/H1N1 viruses reappeared in 1977 and continued to circulate among humans, the seasonal epidemics of influenza A virus from 1968 to 2009 were dominated by A/H3N2 virus variants generated by antigenic drift [3,4]. Then, in early April 2009, a new influenza A (H1N1) virus emerged among humans in California and Mexico, quickly spreading worldwide through human-to-human transmission, and generating the first influenza pandemic of the 21st century [5,6]. The virus was found to be antigenically unrelated to human seasonal influenza viruses but genetically related to viruses known to circulate in pigs. In view

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of its likely swine origin, it is often referred to as 'swine-origin influenza virus' (S-OIV) A/H1N1, or pandemic influenza A (H1N1) 2009 virus.

Molecular studies of the new A (H1N1) 2009 pandemic virus genome showed that it was derived from several viruses which had been circulating in pigs for years, namely the North American H3N2 triple-reassortant (see details below), the classical swine H1N1 lineage, and the Eurasian 'avian-like' swine H1N1 virus [7,8]. The 'avian-like' virus lineage spread throughout Europe and Asia while also reassorting with other influenza virus strains. In Asian pig populations, for example, the classical swine H1N1 virus lineage still circulates together with the 'avian-like' swine H1N1, H1N2 reassortants and the North American H3N2 triple-reassortant [9]. Multiple lineages of influenza A viruses were found to co-circulate during any single season and to undergo frequent reassortment. This, in turn, has had a major impact on antigenic evolution [10].

Initial transmission of the pandemic A (H1N1) 2009 virus to humans is believed to have taken place at least several months before recognition of the first outbreak. Phylogenetic data even suggest that the reassortment of swine lineages may have occurred years before emergence in humans [11–14]. Surprisingly however, there has been no evidence so far that pigs have played any role in the epidemiology or in the worldwide spread of the virus in human populations [15].

On June 11, 2009, the World Health Organization raised the pandemic alert to level 6, in view of the number of countries and regions which officially reported A (H1N1) 2009 influenza cases in their communities. The virus was spreading rapidly around the world and appeared to affect primarily children and young adults as well as those with an underlying lung or cardiac disease condition [16]. The need for a specific vaccine was recognized in view of the continued outbreaks of severe human infections and the risk of a possible increase in pathogenicity and/or acquisition of antiviral resistance of the A (H1N1) 2009 virus through eventual reassortment. Vaccine development was promptly initiated in collaboration between the World Health Organization, Health Ministers and National Health Agencies, and the vaccine industry.

2. Epidemiology and disease burden

2.1. Epidemiology

The emergence of the pandemic H1N1 influenza virus in humans in early April 2009 in Mexico and California came as a total surprise. The virus first emerged in a small village in Vera Cruz, Mexico, but went unnoticed as no case of illness required hospitalization. The first two cases in California occurred in a 10-year-old boy and a 9-year-old girl who were hospitalized due to the infection. The H1N1 strain then quickly spread worldwide through human-to-human transmission. The number of countries, overseas territories and communities that reported laboratory-confirmed A (H1N1) 2009 cases in humans was 208 on December 30th, 2009 and more than 214 on April 18th, 2010. Most countries in the southern hemisphere reported more pandemic H1N1 in 2009 than any of the seasonal subtypes. In the temperate areas of the northern hemisphere, the spread of the pandemic was more gradual, initially spreading widely in the USA, Spain, Great Britain, Japan and Germany before invading other countries. In the tropics, infection rates appeared to be rapidly increasing in both Central and South America and Asia, especially in Thailand. However, very few epidemiological data are available regarding the spread of the virus in the African continent. It is not possible at this time to estimate what the future will look like and whether there will be, or not, a new wave of the pandemic in 2010. The most pessimistic estimates call

for 1 billion to 3 billion people (15–45% of the world's population) becoming infected.

On the basis of recorded clusters in the USA, the household secondary attack rate was initially estimated to be 27.3%. In a recent study of the A (H1N1) 2009 virus infection cases reported to the Centers for Disease Control and Prevention, an acute respiratory illness developed in none of the household contacts in 156 of 216 households (72%), in one contact in 46 households (21%), and in more than one contact in 14 households (6%). The transmissibility of the A (H1N1) 2009 influenza virus in households was therefore lower than that seen in past pandemics [17]. The mean time between the onset of symptoms in a patient case and the onset of symptoms in the household contact infected by that patient was 2.6 days (2.2–3.5).

In school outbreaks, a typical infected schoolchild spread the virus to 2.4 (range 1.8–3.2) other children on average within the school. The basic reproductive number, R_0 , thus ranged from 1.3 to 1.7 [18]. This is consistent with further pandemic spread causing illness in 25–39% of the world's population over a 1-year period, similar to the spread of the 1957–1958 Asian influenza A (H2N2) pandemic. In a study of an A (H1N1) 2009 outbreak in a New York City school, the estimated median generation time was 2.7 days (range 2.0–3.5) and the within-school reproductive number R_0 , 3.3 [19]. The natural history and rate of transmission of the A (H1N1) 2009 influenza virus appears to be similar to those of previously observed circulating pandemic and interpandemic influenza viruses.

The actual number of influenza A (H1N1) 2009 cases worldwide remains unknown, as most cases were diagnosed clinically and were not laboratory-confirmed [20]. In most countries, the capacity for laboratory diagnosis was so severely stressed that virological surveillance had to be restricted to patients attending hospitals [21]. However, it is likely that the total number of cases of pandemic H1N1 influenza worldwide was in the order of several tens of millions of cases. An early estimate of the extent of disease in the USA reported that ~1 of 6 Americans had experienced pandemic influenza as of early December 2009, accounting for ~50 million cases [22]. A recent estimate called for about 200 million pandemic H1N1 influenza cases worldwide, of which ~10 millions occurred in France [23].

A characteristic feature of the A (H1N1) 2009 pandemic is that it disproportionately affected children and young adults as compared to the older age groups [24]. One of the early studies in the USA showed that, although the age of the A/H1N1 patients in the study ranged from 3 months to 81 years, 60% of the patients were 18 years of age or younger [25]. In most countries, the majority of A (H1N1) 2009 cases have occurred in younger age groups, with the median age estimated to be 12–17 years in Canada, the USA, Chile, Japan and the UK. Of the 272 patients with A (H1N1) 2009 infection who were hospitalized in the USA from April to mid-June 2009, 45% were under the age of 18 years, whereas only 5% were 65 years of age or older [26]. Similarly, the mean age of the 426 persons infected with the A (H1N1) 2009 influenza virus who were quarantined in 61 hospitals in 20 provinces in China was 23.4 years [27].

This age distribution suggests partial immunity to the virus in the older population [28]. This hypothesis is supported by subsequent studies which showed that 33% of humans over 60 years of age had cross-reacting antibodies to A (H1N1) 2009 by hemagglutination-inhibition test and neutralization tests, although antibody titers to the pandemic virus did not significantly increase after vaccination with a seasonal vaccine, even when formulated with water-in-oil adjuvants [11,29]. In another study, no neutralizing antibodies against the pandemic A (H1N1) 2009 virus could be found in sera from people born after 1920 [30]. However, homology between the A (H1N1) 1918 and the A (H1N1) 2009 viruses could be demonstrated by cross-protection studies in mice [31].

Also, a strong conservation of more than 50% of T cell epitopes (whether T-helper or CTL epitopes) was described between the pandemic A (H1N1) 2009 virus and the seasonal H1N1 influenza virus strains used to prepare the 2007 and 2008 influenza vaccines, which would provide some level of cross-reactive cellular immunity to the pandemic virus in the vaccinated human population [32]. In addition, the possible role of the NA antigen in cross-protective immunity, which remains poorly explored, should be considered [33]. It should be noted that while the highest rate of severe disease leading to hospitalization has been in patients less than 5 years of age, the highest case fatality rate was recorded in the 50–60-year-old population.

2.2. Clinical presentation, severity and disease burden

Pandemic influenza A (H1N1) 2009 is mostly a mild, self-limiting upper respiratory tract illness with (or for some patient groups, without) fever, cough and sore throat, myalgia, malaise, chills, rhinorrhea, conjunctivitis, headache and shortness of breath. Up to 50% of patients present with gastrointestinal symptoms including diarrhea and vomiting. The spectrum of clinical presentation varies from asymptomatic cases to primary viral pneumonia resulting in respiratory failure, acute respiratory distress, multi-organ failure and death [34]. The A (H1N1) 2009 virus is able to bind to alpha 2,3-linked sialic acid receptors found on the surface of cells located deep in the lungs that seasonal influenza virus cannot bind (they only bind to alpha 2,6-linked sialic acid receptors found on cells of the upper respiratory tract), suggesting why people with the pandemic H1N1 influenza can experience more severe pulmonary symptoms as compared to seasonal influenza [35].

It was reported that 2–5% of confirmed cases in the USA and Canada and 6% of cases in Mexico required hospitalization. A fifth of them required clinical management in intensive care unit (ICU). Most of the hospitalized patients had underlying conditions such as cardiovascular disease, respiratory diseases including asthma and COAD, auto-immune disorders, obesity, diabetes or cancer [26]. Asthma appeared to be a significant risk factor for severe disease in children [36]. Among those severely affected were also previously healthy young people with no underlying health condition. These patients rapidly developed severe respiratory failure often associated with failure of other organs [37]. Due to the fact that testing and confirmation of pandemic A (H1N1) 2009 infection were more likely to occur in hospitalized patients and cases with severe or progressive disease, the reported hospitalization rates, especially at peak periods of infection, may not however reflect the actual situation.

Pregnant women, especially in their second and third trimester, are also at a higher risk for severe disease [16,38,39]. It was reported that more than one-third of pregnant women with confirmed A (H1N1) 2009 infection were hospitalized in the USA due to acute respiratory distress syndrome [40]. A California statewide survey showed that most (95%) of the pregnant women who were hospitalized from April 23 to August 11, 2009, due to pandemic H1N1 infection were in the second or third trimester, and only approximately one third had established risk factors other than pregnancy. Overall, 22% of these women required intensive care and 8% died [41].

Similar findings were reported from Australia and New Zealand, where the number of ICU admissions due to influenza A in 2009 was 15 times the number due to viral pneumonia in recent years: infants from 0 to 1 year of age and adults from 25 to 64 years of age were at particular risk, as well as pregnant women, adults with a body mass index greater than 35, and indigenous Australian and New Zealand populations. Mortality in hospitalized patients was 16% [42].

The overall A (H1N1) 2009 case fatality rate in Mexico was estimated to be 0.4% [43]. The average case fatality rate that can be deduced from laboratory-confirmed cases officially reported to

WHO as of 06 August 2009 was much lower (0.08%). Current estimates put the average case fatality rate at 0.15–0.25%. A (H1N1) 2009 deaths occurred mostly in middle-aged adults (median age around 40–50 years), contrary to seasonal Influenza where fatal disease occurs most often in the elderly (>65 years old). In a recent study of the first 16 weeks of the pandemic in California, which reported 1088 cases of hospitalization or death, the median age of hospitalized patients was 27 years of age, but the case fatality rate (11%) was definitely the highest in persons 50 years of age and older [44].

Most of the deaths due to pandemic H1N1 infection occurred in patients with an underlying medical condition. In South Africa, a majority of fatal cases occurred in HIV-infected people, including pregnant women. However, close to one third of the hospitalized influenza patients who died had no known underlying medical conditions that could have predisposed them for severe infection.

From data on medically attended and hospitalized A (H1N1) 2009 patients in Milwaukee and information from New York City hospitals on numbers of hospitalizations, use of intensive care units (ICUs) and deaths, it was estimated that about 1 in 2000 (between 1 in 4000 and 1 in 1000) people in the USA who presented with symptoms of pandemic influenza infection died; about 1 in 400 symptomatic cases required treatment in ICU; and 1 in 70 required hospital admission [22]. Among the medically attended cases in Milwaukee, 60% were in the 5–17 years age group, but severity of the cases was by far higher in the 18–50 years age group. Significantly higher figures have been reported elsewhere for the A (H1N1) 2009 case fatality rate [45,46], most likely reflecting a large uncertainty on the actual numbers of true pandemic influenza cases.

In Australia, where the rate of hospitalization was 23 per 100 000 population, the highest rate of hospitalization occurred among children under 5 years of age. The median age of the 190 patients who died of A (H1N1) 2009 influenza illness was 53 years, as compared with 83 years in previous seasons [47].

Rates of hospitalization of children with confirmed A (H1N1) 2009 influenza illness in Argentina were twice those for seasonal influenza the preceding year. Of the 251 children who were hospitalized with confirmed A (H1N1) 2009 influenza, 19% were admitted to an ICU, 17% required mechanical ventilation and 5% died, a 10 fold increase in the pediatric death rate as compared with seasonal influenza in previous years [48].

The official number of deaths from laboratory-confirmed pandemic influenza A (H1N1) 2009 infection worldwide reported to WHO as of 28 March 2010 was 17 483. This number appears to be very much lower than the estimated annual global mortality associated with seasonal influenza. However, the actual fatality of the A (H1N1) 2009 pandemic cannot be accurately ascertained at this time. Given the relatively high mortality rates for at-risk groups and hospitalized cases, as described above, the annual mortality due to A (H1N1) 2009 is expected to be higher. In addition, as the clinical presentation of pandemic influenza shared many common features with common respiratory diseases, patients may not have been tested for the virus. This is particularly important to consider in under-resourced countries, where deaths from respiratory diseases such as pneumonia are frequent.

The economic impact of the pandemic outbreak in Mexico was estimated as >\$3.2 billion (0.3% of gross national product) [49]. However, the global economic impact of the H1N1 pandemic is uncertain at the present time.

2.3. Modes of transmission

The modes of transmission of the pandemic A (H1N1) 2009 virus appear to be similar to those of seasonal influenza viruses and involve primarily close unprotected contact with respiratory droplets. The relative impact of close range exposure to large- and

small-particle droplets expelled when an infected person coughs is unknown but could be more prominent under special conditions such as aerosol-generating procedures. The virus is also likely transmitted through contacts with fomites that are contaminated with respiratory or possibly gastrointestinal fluids [50]. Many A (H1N1) 2009-infected patients experienced diarrhea, and viral RNA could readily be detected in the feces of these patients, making the potential for fecal-oral transmission a plausible risk [25]. However, viable infectious virus particles in feces have not been reported and the possibility of fecal transmission of the H1N1 virus remains to be proven.

The incubation period for A (H1N1) 2009 infection appears to range from 2 to 7 days, but most patients probably shed virus from day 1 before the onset of symptoms through 5–7 days after [51]. The median period during which the virus could be detected with the use of real-time PCR in quarantined patients was 6 days (range 1–17), whether or not fever was present [27]. Studies of transmission in animal models show that the pandemic H1N1 virus transmits just as efficiently as seasonal flu [52], contrary to earlier findings at the start of the pandemic [53].

3. Virology

The novel A (H1N1) 2009 virus can be grown in Madin Darby canine kidney (MDCK) cell cultures, primary human airway epithelial cell cultures, or in embryonated chicken eggs. Scanning electron microscopy revealed virions of mostly filamentous shape [30].

Sequence analyses showed the absence of markers associated with high pathogenicity in avian or mammalian species, such as a multibasic hemagglutinin cleavage site [54] or a lysine residue at position 627 in the PB2 protein [55]. The occurrence of a mutation at position 222 in the HA gene segment of H1N1 isolates from post-mortem specimens has been reported in various countries including Norway, the USA, China, Japan, Brazil and France. Although suspected to be associated with increased pathogenicity, this mutation did not change the antigenicity of the virus or its susceptibility to antiviral drugs, nor did it appear to provide the virus with increased transmissibility [56].

3.1. Molecular and antigenic characterization

Phylogenetic analyses of A (H1N1) virus isolates reveal a great homogeneity of genomic sequences. The virus is antigenically distinct from human seasonal influenza viruses but genetically related to three viruses that circulate in pigs [12,57], with the HA (H1), NP and NS gene segments coming from the classical swine H1N1 lineage. The H1 sequence can actually be traced back to the 1918 H1N1 pandemic virus (the “Spanish flu”), which has remained endemic in swine and continued to circulate among pigs in Asia, the Americas and, until the 1980s, in Europe [16,58,59].

The NA (N1) and M genes of the pandemic A (H1N1) 2009 virus come from the ‘avian-like’ Eurasian swine H1N1 lineage, which emerged in Europe in 1979 after reassortment between a classical swine and an avian H1N1 virus. The virus then spread through Europe and Asia [7,8,60], displacing the classical swine H1N1 virus from Europe and generating new reassortants in swine with different influenza A viruses of human origin [61].

Finally, the PA, PB1 and PB2 genes of the 2009 pandemic H1N1 virus are from the North American H3N2 ‘triple-reassortant’ lineage, which was first isolated from pigs in America in 1998 in which it showed unusual pathogenicity [62–64]. The name ‘triple-reassortant’ relates to the fact that the virus has genes from human, classical swine and North American avian influenza viruses.

The A (H1N1) 2009 virus has therefore inherited virus gene segments of all three sources: swine, human and avian origin.

However, no data are available to help evaluate when, where, or between which parent viruses the initial reassortment actually occurred [11,13,14].

Antigenically, all A (H1N1) 2009 virus isolates look similar to classical swine viruses and to the reassortant H1N1 viruses that have been circulating among pigs in the USA over the last decade, showing no antigenic cross-reactivity with contemporary human seasonal H1N1 viruses.

3.2. Experimental pathogenicity in animals

Experimental pathogenicity of the A (H1N1) 2009 virus was tested in mice, ferrets and nonhuman primates [30]. The virus replicated more efficiently in the lungs of infected mice, generating earlier bronchitis and alveolitis, than infection with a recent human H1N1 virus (A/Kawasaki/UTK-4). It also elicited markedly increased production of interleukin-10 (IL-10), interferon gamma (IFN- γ), IL-4 and IL-5. Similarly, the virus induced elevated fever, severe lung lesions with oedematous exudate and inflammatory infiltrates and high antigenic loads in pneumocytes in nonhuman primates, similar to what was reported for highly pathogenic avian H5N1 influenza viruses [65]. This may be related to the affinity of the virus for alpha 2,3-linked sialic acid receptors in the lower respiratory tract [35]. The virus was also more pathogenic in ferrets, replicating to higher titers in the trachea and lung and causing more severe bronchopneumonia with prominent viral antigen expression in the peribronchial glands and alveolar cells than human seasonal H1N1 viruses [66], while showing much less pathogenicity for the animal than the highly pathogenic avian influenza H5N1 virus [67,68]. In contrast, the A (H1N1) 2009 virus was devoid of overt pathogenicity for pathogen-free miniature pigs, although it did replicate efficiently in the respiratory tract of the animals [30].

The A (H1N1) 2009 influenza virus RNA was also detected in the intestinal tract of inoculated ferrets, consistent with the occurrence of gastrointestinal symptoms in many human A (H1N1) 2009 cases [53]. Transmission of the virus via aerosol or respiratory droplets was tested in ferrets, and found to be either as efficient as [66] or less efficient than [53] highly transmissible seasonal A (H1N1) virus. The latter observation is in agreement with the observation that the virus may not be that easily transmissible among humans [17,18,69].

3.3. Sensitivity to antiviral drugs

Genetic and phenotypic analyses indicate that the A (H1N1) 2009 pandemic influenza virus is susceptible to the neuraminidase inhibitors, oseltamivir and zanamivir, but resistant to the adamantanes [70]. Treatment with oseltamivir is efficacious if initiated within the first 36 h after infection [71]. Although a Cochrane review on oseltamivir cast doubt on the effectiveness and safety of the drug [72], the US FDA issued an emergency authorization approving the use of oseltamivir to treat influenza illness in infants under the age of 1 year and for chemoprophylaxis in infants older than 3 months of age.

Over 160 A (H1N1) 2009 viral isolates have been described that were resistant to oseltamivir, due to the same mutation in the neuraminidase (H275Y) as that described in oseltamivir-resistant seasonal H1N1 and avian H5N1 strains [73,74]. These cases have been sporadic and there was no evidence of further transmission of the resistance marker into the virus population. Most of the reported cases of resistance were associated with oseltamivir treatment, including prophylactic use of the drug against pandemic influenza infection.

New strategies to prevent and treat influenza virus infection may eventually be developed based on the use of broad-spectrum

neutralizing monoclonal antibodies such as CR6261, which has the potential to neutralize a spectrum of influenza virus subtypes and was shown to be highly protective against lethal H5N1 and H1N1 infections in mice [75].

4. Vaccines

Vaccines are considered to be one of the most effective tools, not only to prevent the spread of the influenza virus but also to mitigate the severity of illness and the impact of the disease [76]. In view of the rapid spread of the A (H1N1) 2009 influenza pandemic worldwide, the rapid implementation of a vaccine has been a global priority. The risk of the virus gaining additional virulence properties, such as enhanced pathogenicity and/or antiviral resistance through mutations and/or reassortment with other human or avian influenza viruses, further highlighted the urgency of rapid vaccine development. In addition, the lack of cross-protective immunity between the pandemic and seasonal influenza virus strains rendered the 2009 seasonal influenza vaccine ineffective in the fight against the A (H1N1) 2009 pandemic.

The development of a pandemic H1N1 influenza vaccine has raised complex challenges. In addition to the regulatory needs for assessment of immunogenicity and safety of the vaccine in human volunteers, other important issues have been raised. These include the need to ensure that sufficient seasonal influenza vaccine would still be available in time, accurate estimation of short- and medium-term production capacity of the different vaccine producers, and plans to reserve part of the foreseen production capacity for under-resourced countries [77,78].

As of June 2009, the total global annual capacity for trivalent seasonal influenza vaccine production stood at 876 million doses, with seven manufacturers responsible for 560 million doses (i.e. 64% of the capacity). In spite of the WHO global pandemic influenza action plan to increase the potential supply of pandemic influenza vaccine [79], the production of enough pandemic vaccine to immunize the world's population, if and when needed, would take several years! In addition, it was not clear early on whether one or two doses of pandemic vaccine would be required to induce full protection, or whether the use of water-in-oil adjuvants would have the same antigen dose-sparing effect as in the case of the H5N1 vaccines [80,81]. Finally, the yields of virus in eggs or cell cultures, which is an important determinant of the amount of vaccine doses that can be manufactured, were not quite up to expectation.

A total of 26 vaccine manufacturers from America, Europe, Russia, Australia and Asia have now developed or are developing pandemic A (H1N1) 2009 vaccines. These include inactivated whole-virion vaccines, split inactivated vaccines, subunit vaccines and live-attenuated vaccines, including a novel type of highly attenuated influenza virus strain that is deleted of the NS1 genomic RNA segment [82]. Of note is the participation of new vaccine manufacturers from China, India, Thailand and South America. All pandemic A (H1N1) 2009 vaccines currently registered were tested in clinical trials for safety and immunogenicity. A few clinical trials still are in progress for new vaccines and in certain at-risk patient subpopulations.

Preliminary reports indicated that a single 15- μ g dose of an inactivated split influenza A (H1N1) 2009 vaccine induced a hemagglutination-inhibition assay titer of 1:40 or more in nearly all 18–64-year-old volunteers [83], suggestive of possible cross-priming with previous exposure to the vaccine antigens and revealing that there was more similarity between the influenza A (H1N1) 2009 virus and recent seasonal virus strains than had been recognized previously [84]. The US NIAID Office of Communications also reported that among healthy volunteers who received a single 15- μ g dose of either the Sanofi-Pasteur or the CSL Limited

inactivated split A (H1N1) 2009 vaccine, a robust immune response was measured in 96% and 80%, respectively, of adults aged 18–64 years, and in 56% and 60%, respectively, of adults aged 65 and older [85,86].

In a recent Phase II trial on 410 children and 724 adults who received a single dose (15 μ g HA) of inactivated A (H1N1) vaccine in the USA, potentially protective serological titers of >1:40 were detected at 21 days after vaccination in 45–50% of 6–35-month-old, 69–75% of 3–9-year-old, 95–100% of 18–64-year-old, and 93–95% of elderly subjects [87]. No vaccine-related severe adverse event was reported, but approximately 50% of every age and vaccine group reported injection-site (pain, redness) and systemic (fever) reactions. Similarly, a multi-centered, double-blind, randomized trial on 12 691 subjects aged 3 years or older receiving a single dose (7.5 μ g HA) of a split virion A (H1N1) 2009 vaccine in China showed that potentially protective serological titers were detected on day 21 in 76.7% of 3–12-year-old children, 96.8% of 12–18-year-old adolescents, 89.5% of 18–60-year-old adults, and 80.3% of adults older than 60 years. In children, the administration of a second dose of the 7.5 μ g formulation increased the seroprotection rate to 97.7% [88].

The fact that it is possible to induce potentially protective antibody levels against A (H1N1) infection in adults within 2 weeks of administration of a single dose of vaccine has now been confirmed with every pandemic H1N1 vaccine tested [89]. This has been shown for split inactivated vaccines containing 15 μ g HA (Sanofi-Pasteur, CSL, Sinovac and others), split inactivated vaccines with a water-in-oil adjuvant containing either 7.5 μ g HA and MF59 (Novartis) [90] or 3.8 μ g HA and AS03 (GSK), and whole-virion vaccines containing 10 μ g HA (Baxter) or 6 μ g HA (Omnivest, Hungary) [91]. National authorities have recommended that young children should receive a two-dose schedule, as in the case of seasonal vaccines, but immunogenicity data from clinical trials indicate that with many vaccines a single dose induced appropriate levels of immune responses in children [92]. It seems prudent, however, to follow the current recommendations for two doses to infants and young children [93].

Vaccination against pandemic H1N1 influenza was first implemented in China [94], followed by a large number of other countries. The problems still remain, however, of vaccinating people living in under-resourced countries, which are dependent upon donations from governments of industrialized countries and the pharmaceutical industry and which are little able to afford the cost of mass vaccination. The deployment and application of these donations at targeted countries are challenging issues. The WHO is efficiently coordinating this effort.

Among the high priority groups for vaccination [95] are health care workers and pregnant women. The latter are at risk of severe illness and mortality. Their vaccination is a highly cost-effective strategy with substantial benefits to both the infants and the expectant mothers [96,97]. Other priority groups are young children and individuals with an underlying cardiovascular or respiratory medical condition including asthma, auto-immune disorders and diabetes.

The safety of the A (H1N1) 2009 vaccines has been thoroughly monitored during the various clinical trials. Current data show that the pandemic influenza vaccines are well tolerated and behave as the corresponding seasonal vaccines in terms of safety and lack of severe adverse events. A small number of cases of Guillain Barré syndrome were reported after pandemic H1N1 vaccine administration in large-scale campaigns, but they all recovered quickly [98]. Although oil-in-water adjuvanted vaccines have been approved by the European Association EMEA for use in all populations, including pregnant women, their use in the USA has raised regulatory problems, as no adjuvanted flu vaccine had ever been licensed in that country and no fast-track system was in place for their registration [99].

5. Discussion

The most damaging influenza pandemic in recent history was the 1918–1920 H1N1 pandemic that resulted in some 40–50 million deaths, mainly among the young adult population [100]. The new H1N1 virus that is causing the first influenza pandemic of the 21st century is clearly less virulent than the 1918 virus, although it partially shares the same H1 antigenicity.

As outlined by Nistal-Villan and Garcia-Sastre [101], the new pandemic has given the world sobering lessons on the experts' inability to predict the specific subtype that will start a new pandemic. Attention during the last several years was focussed on the avian H5N1 virus strain, due to its high intrinsic pathogenicity and, to a lesser extent, on H7, H9 and H2 viruses as potential new pandemic strains. The sudden emergence of the A (H1N1) 2009 virus was totally unexpected, and at the same time, provided additional evidence on the role of domestic pigs in the ecosystem of influenza A and the need for systematic swine surveillance in the future [14].

In spite of the rapid response of the WHO and National Authorities, it took several months to have a H1N1 vaccine available, thus no vaccination was possible during the 2009 winter season in the Southern Hemisphere where the new H1N1 virus was prevalent. This illustrates well the incapability of the vaccine industry to produce enough vaccine in a timely manner to protect vulnerable populations, especially in developing countries.

Such an incapability, together with the global impact of the A (H1N1) 2009 pandemic on public health, have further reinforced the idea of developing an 'universal' influenza vaccine that could provide efficacious cross-reactive immunity and induce broad protection against different influenza virus variants, clades, and even subtypes, making the need for yearly seasonal vaccination unnecessary. Recent research and developmental work have been most encouraging for this approach. It was demonstrated that the external region of the ion channel M2 viral protein (M2e), the sequence of which is relatively well conserved among influenza A subtypes, can elicit cross-protection through antibody-dependent cellular cytotoxicity [102–105]. The safety and efficacy of that approach remains, however, to be documented [106].

The recent finding that the human immune system can recognize a conserved neutralization epitope on the HA molecule that is shared across several influenza virus subtypes [107,108], combined with the fact that the well-conserved NP viral nucleoprotein could generate cross-protective cellular immunity [109] are also strong arguments in favor of the possibility of developing an 'universal' vaccine [110]. The evidence that influenza virus-specific cell-mediated immunity can be used as a basis for broad antiviral protection was recently demonstrated in the mouse model [111], but this approach needs to be studied further in other animals and humans in a more systematic way.

A most striking finding regarding the pandemic A (H1N1) 2009 virus was the recent demonstration of the homology between the H1 hemagglutinins of the 2009 and the 1918 pandemic A (H1N1) viruses [112,113]. The cocrystal structure of the 1918 HA with an antibody from a survivor of the 1918 "Spanish flu" that neutralized both 1918 and 2009 H1N1 viruses revealed an epitope that is conserved in both pandemic viruses [114], providing a likely explanation for the age-related immunity to the current influenza pandemic. The HA molecule from the 1918 H1N1 virus was therefore at least partially conserved for 91 years while circulating in pigs and finally reemerging in the A (H1N1) 2009 S-OIV [31].

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