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The A/2009 H1N1 influenza virus pandemic

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Introduction

Since the global H1N1 influenza virus pandemic of 1918, influenza virus gene reassortment has been well documented and observed to occur frequently between human virus subtypes, between human and avian and among avian influenza viruses. Such reassortments led to the global pandemics of 1957 (H2N2) and 1968 (H3N2) [407] [408] . Although A/H1N1 viruses continued to circulate among humans, seasonal epidemics of influenza A virus from 1968 to 2009 were dominated by A/H3N2 virus variants generated by antigenic drift [409] [410] , until, In early April 2009, a new influenza A (H1N1) virus brutally emerged among humans in California and in Mexico, quickly spreading worldwide through human-to-human transmission, and generating the first influenza pandemic of the twenty-first century [411] [412] . 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 of its likely swine origin, it is often referred to as 'swine-origin influenza virus' (S-OIV) A/H1N1, or 2009 A (H1N1) influenza virus.

Swine H1N1 influenza viruses had been circulating in pig populations for at least 80 years but too often lacked surveillance and molecular characterization. In 1998, a new triple-reassortant H3N2 virus emerged in the North American pig population, comprising genes from classical swine H1N1, North American avian and human H3N2 influenza viruses. Co-circulation and mixing of this North American triple-reassortant with viruses of swine lineage generated further H1N1 and H1N2 reassortant swine viruses. In Europe, an avian H1N1 virus ('avian-like' swine H1N1) was first isolated from pigs in Belgium in 1979 [413] , and gradually replaced classical swine H1N1 viruses [414] . The 'avian-like' virus lineage spread all over Europe and Asia while also reassorting with other influenza virus strains. In Asian pig populations, the classical swine H1N1 virus lineage still circulated together with the 'avian-like' swine H1N1, H1N2 reassortants and the North American H3N2 triple-reassortant [415] . Multiple lineages of influenza A viruses have been found to co-circulate during any single season and to undergo frequent reassortment which may in turn have a major impact on antigenic evolution [416] .

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Molecular studies of the new A/H1N1 pandemic virus genome showed that it was derived from several viruses which had been circulating in pigs for a long time, namely the North American H3N2 triple-reassortant, the classical swine H1N1 lineage, and the Eurasian 'avian-like' swine H1N1 virus (see details under Virology). Initial transmission to humans is believed to have taken place at least several months before recognition of the first outbreak and phylogenetic data even suggest that the reassortment of swine lineages may have occurred years before emergence in humans [417] [418] [419] [420] . Surprisingly however, there has been no evidence so far that swine are playing any role in the epidemiology or in the worldwide spread of the virus in human populations [421] .

On June 11, 2009, the World Health Organization raised the pandemic alert to level 6, in view of the number of regions which officially reported A/H1N1 influenza cases in their communities. In view of the rapid spread of the H1N1 virus, its propensity to primarily affect children and young adults, as well as those with an underlying lung or cardiac disease condition [422] , and the risk of a possible increase in pathogenicity through further reassortment with avian or human virus strains, the development of a specific vaccine was promptly engaged in collaboration between the World Health Organization, Health Ministers and National Health Agencies and the vaccine industry.

Disease Burden and Epidemiology

Epidemiology

The emergence of the pandemic HINI influenza virus in humans in early April 2009 in Mexico and California came as a total surprise. The virus first emerged in a little 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 both necessitated hospitalization. The H1N1 strain then quickly spread worldwide through human-to-human transmission. The number of countries, overseas territories or communities that reported laboratory-confirmed A/H1N1 cases in humans was more than 207 on November 22nd, 2009. 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 as well in the Fall. In the tropics, rates appeared to be quickly increasing in countries in both Central and South America and Asia, especially in Thailand, but very few data exist regarding the African continent. It is not possible at this time to guess what will the future look like. The most pessimistic estimates call for 1 billion to 3 billion people (15% to 45% of the world's population) getting infected.

On the basis of recorded clusters in the USA, the household secondary attack rate was estimated to be 27.3%. In school outbreaks, a typical schoolchild infected on average 2.4 (range 1.8-3.2) other children within the school. The basic reproductive number, R0, thus ranged from 1.3 to 1.7 [423] . This is consistent with further pandemic spread causing illness in 25% to 39% of the world's population over a 1-year period, similar to the spread of the 1957-1958 Asian influenza A (H3N2) pandemic.

The actual number of influenza A (H1N1) cases worldwide remains

unknown, as most cases are diagnosed clinically and are not laboratory-confirmed [424] but it most probably is in the order of several millions cases. In most countries, the capacity for laboratory diagnosis has been so severely stressed that virological surveillance had to be restricted to patients attending hospitals [425] . The number of influenza-like illnesses during the 2009 spring outbreak in New York City has been estimated at 750 000- 1 million cases.

Age distribution

A characteristic feature of the H1N1 pandemic is that it disproportionately affected so far children and young adults [426] . One of the early American studies showed that, although the age of H1N1 patients in the study ranged from 3 months to 81 years, 60% of patients were 18 years of age or younger [427] . In most countries, the majority of H1N1 cases have been occurring in young people, with the median age estimated to be 12 to 17 years in Canada, the USA, Chile, Japan and the UK. Of the 272 patients with 2009 H1N1 influenza who were hospitalized in the USA from April to mid-June 2009, 45% were under the age of 18 years, whereas 5% only were 65 years of age or older [428] . This age distribution speaks in favor of at least partial immunity to the virus in the older population.

Of note, however, is the fact that if the highest rate of severe disease leading to hospitalization has been in the less than 5 years of age, the highest case fatality rate was recorded in the 50-60 years-old population. Subsequent studies have shown that 33% of humans over 60 years of age had cross-reacting antibodies to S-OIV A(H1N1) by hemagglutination inhibition test and neutralization tests, but antibody titers to A/H1N1 did not substantially increase after vaccination with a seasonal vaccine, even when formulated with water-in-oil adjuvants [417] [429] . In another study, no neutralizing antibodies against the pandemic A/H1N1 (2009) virus could be found in sera from people born after 1920 [430] . However, a strong conservation of more than 50% of T cell epitopes (whether T-helper or CTL epitopes) was described between the 2009 A(H1N1) S-OIV and the seasonal influenza virus strains used to prepare the 2007 and 2008 influenza vaccines, which would provide a definite level of cross-reactive cellular immunity to the pandemic virus in the vaccinated human population [431] . In addition, the possible role of the NA antigen in cross-protective immunity, which remains poorly explored, should not be forgotten [432].

Clinical presentation and severity of the disease

H1N1 is most of the times a rather mild, self-limiting upper respiratory tract illness with (or at times without) fever, cough and sore throat, body aches, fatigue, 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 [433] . The H1N1 virus can bind alpha2-3 sialic acid receptors found on the surface of cells located deep in the lungs that seasonal influenza virus cannot bind, suggesting why people with the pandemic flu can experience more severe pulmonary symptoms [434].

Thus, 2% to 5% of confirmed cases in the USA and Canada and 6% of cases in Mexico had to be hospitalized, a fifth of them requiring management in intensive care unit (ICU). The rate of hospitalization could

actually be as high as 10% in some cities. Most, but not all of the hospitalized patients had underlying conditions such as cardiovascular disease, respiratory disease including asthma, auto-immune disorders, obesity, diabetes or cancer [428].

Pregnant women, especially in their second and third trimester, also are at a high risk for severe disease [422] [435] . Thus, more than one-third of the pregnant women with confirmed H1N1 infection had to be hospitalized during the current pandemic in the USA due to acute respiratory distress syndrome [436] . 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 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. In-hospital mortality was 16% [437].

The overall H1N1 case fatality rate in Mexico was estimated to be around 0.4% [438] . The average case fatality rate that can be deduced from the 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% to 0.25%. H1N1 deaths mostly occur 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). Most of the deaths occurred in patients with an underlying medical condition, but close to one third of the hospitalized patients who died had no known underlying medical condition. In South Africa, a majority of fatal cases occurred in HIV-infected people, including pregnant women. In a recent study of the first 16 weeks of the pandemic in California, which saw 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 [439].

From data on medically attended and hospitalized H1N1 patients in Milwaukee and informations from New-York City hospitals on numbers of hospitalizations, use of intensive care units (ICU) 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 H1N1 influenza infection died; about 1 in 400 symptomatic cases required treatment in ICU; and 1 in 70 required hospital admission [440] . 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. Quite higher figures have been reported elsewhere for the H1N1 case fatality rate [441] [442] , most likely reflecting a large incertitude on the true numbers.

The official number of deaths from H1N1 infection worldwide that had been reported to WHO on November 22, 2009 was 7820.

During the pandemic outbreak in Mexico, an estimated 150 000 cases of influenza-like illness with 3312 hospitalizations occurred in metropolitan Mexico City. The economic impact of the pandemic was estimated as >$3.2 billion (0.3% of gross national product) [443].

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 large respiratory droplets. The contribution of close range exposure to 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 [444] . Many S-OIV-infected patients experienced diarrhea, and viral RNA can readily be detected in the feces of these patients, making the potential for fecal-oral transmission a risk to take seriously into account [427].

The incubation period for S-OIV 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 to 7 days after. Studies of transmission in animal models show that the H1N1 virus transmits just as efficiently as seasonal flu [445] , contrary to earlier findings at the start of the pandemic [446].

Virology

The novel S-OIV A/H1N1 2009 can be grown in canine kidney (MDCK) cell cultures or primary human airway epithelial cell cultures, or in embryonated chicken eggs. Scanning electron microscopy revealed virions of remarkably filamentous shape [430].

Sequence analyses showed the absence of markers associated with high pathogenicity in avian or mammalian species, such as a multibasic hemagglutinin cleavage site [447] or a lysine residue at position 627 in the PB2 protein [448] . The occurrence of a mutation at position 222 in the HA gene segment of H1N1 isolates from post mortem specimens has been recently 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 nor its susceptibility to anti-viral drugs, nor did it appear to provide the virus with increased transmissibility.

Molecular and antigenic characterization

Phylogenetic analyses of A (H1N1) virus isolates reveal a great homogeneity of genomic sequences. The virus is antigenically distant from human seasonal influenza viruses but genetically related to three viruses that circulate in pigs [418] [449] , with the HA (H1), NP and NS gene segments coming from the classical swine H1N1 lineage. The H1 sequence could 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 America's and until the 1980s also in Europe [422] [450] [451].

The NA (N1) and M genes of the 2009 H1N1 pandemic 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, then spread through Europe and Asia [452] , displacing the classical swine H1N1 virus from Europe and generating new reassortants in swine with different human origin influenza A viruses [453].

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 American pigs in 1998 in which it showed unusual pathogenicity [454] [455] [456] . The name 'triple-reassortant' relates to the

fact that the virus has genes of human influenza virus origin, of classical swine influenza virus origin and of North American avian virus origin.

The 2009 S-OIV H1N1 therefore has inherited virus gene segments of all three swine, human and avian origin: its HA, NP and NS gene segments have been inherited from swine classical virus, its NA and M segment from the avian-like Eurasian reassortant lineage, its PB1 segment from human H3N2 virus, and its PA and PB2 segments from North American avian lineage. Indeed, all gene segments of the pandemic A (H1N1) S-OIV were already established in the North-American 'triple-reassortant' (H3N2) swine virus and in the 'avian-like' Eurasian (H1N1) swine virus but no data are available to help evaluate when, where, nor between which parent viruses did the initial reassortment actually occur [417] [419] [420].

Antigenically, all S-OIV isolates look similar to classical swine viruses and to 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. Surprisingly, there is no evidence that pigs play any role in the epidemiology or in the worldwide spread of the pandemic A (H1N1) virus in the human population [421].

Pathogenicity in animals

Experimental pathogenicity of the 2009 A/H1N1 S-OIV was tested in mice, ferrets and nonhuman primates [430] . S-OIV replicated more efficiently in the lungs of infected mice, generating earlier bronchitis and alveolitis, and eliciting more markedly increased production of interleukin-10 (IL-10), interferon gamma (IFN-Y), IL-4 and IL-5 than infection with a recent human H1N1 virus (A/Kawasaki/UTK-4). Similarly, it induced in nonhuman primates more elevated fever, more severe lung lesions with oedematous exudate and inflammatory infiltrates and higher antigenic loads in pneumocytes, similar to what was reported for highly pathogenic avian H5N1 influenza viruses [457] . This may have to do with affinity of the virus for alpha2-3 sialic acid receptors in the lower respiratory tract [434] . The pandemic virus also was more pathogenic in ferrets, replicating to higher titers in trachea and lungs than human seasonal H1N1 virus and caused more severe bronchopneumonia with prominent viral antigen expression in the peribronchial glands and alveolar cells. In contrast, it was devoid of overt pathogenicity for pathogen-free miniature pigs, although the virus did replicate efficiently in the respiratory tract of the animals.

The 2009 A(H1N1) S-OIV was also found to be more pathogenic than a seasonal 2007 A (H1N1) virus in ferrets and mice, with extensive virus replication occurring in the trachea, bronchi and bronchioles of the animals, while replication of the seasonal virus was limited to the upper respiratory tract [458] . The 2009 A(H1N1) influenza virus also replicated in the intestinal tract of inoculated ferrets, consistent with gastrointestinal involvement in many human A(H1N1) cases [446]. Transmission of the virus via aerosol or respiratory droplets was also tested in ferrets, and found to be either as efficient as [458] or less efficient than [446] 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 as only 10% of patients' household contacts become infected [459].

Sensitivity to antiviral drugs

Genetic and phenotypic analyses indicate that S-OIV is susceptible to neuraminidase inhibitors oseltamivir and zanamivir, but resistant to the adamantanes [460] . Treatment with oseltamivir is efficacious if initiated within the first 36 hrs after infection. The FDA issued an emergency use 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.

Close to one hundred A(H1N1) S-OIV isolates have been described that were resistant to oseltamivir. These cases have been sporadic and there was no evidence of further transmission of the resistance marker into the virus population.

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 [461] . Today, the implementation of a pandemic A(H1N1) influenza vaccine in the fastest time is a global priority. This stems both from the rapid spread of the pandemic worldwide, from the fear that the A(H1N1) virus might accidentally gain added virulence through mutations and/or reassortment with other human or avian influenza virus, and from the total lack of cross-immune reactivity observed between the pandemic and seasonal influenza virus strains, making the 2009 seasonal vaccine useless in the fight against the A(H1N1) pandemic.

The development of a pandemic influenza vaccine however raises complex challenges, such as ensuring that sufficient seasonal influenza vaccine will still be available in due time, estimating with accuracy short- and medium-term production capacity of the different producers, reserving part of the foreseen production capacity for under-resourced countries with no or little access to the vaccine, etc [462] [463].

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 [464] , the supply of enough pandemic vaccine to immunize the world's population -should this be needed- would therefore take at least four years! Added to which, it was not clear at that early time whether one or two doses of pandemic vaccine would be required to induce full protection, nor whether the use of water-in-oil adjuvants would have the same antigen dose-sparing effect as in the case of the H5N1 vaccines [465] [466] . Finally, the yields of virus production 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 presently developing pandemic A(H1N1) vaccines, whether inactivated whole-virus vaccines, split inactivated vaccines, subunit vaccines, live-attenuated vaccines or other formulations. Of note is the participation of new vaccine manufacturers in China, India, Thailand and South America. All H1N1 vaccines were tested in clinical trials for safety and immunogenicity. Clinical trials still are in progress in certain at-risk subpopulations.

Preliminary reports indicated that a single 15-µg dose of an inactivated split influenza A (H1N1) 2009 vaccine induced a hemagglutination-inhibition assay titer of 1:40 or more in 96.7% of 18-64 years-old subjects [467] . The robust immune response observed in the 18-49 years-old volunteers cohort was unanticipated, suggesting that there is more similarity between the influenza A (H1N1) 2009 virus and recent seasonal virus strains than had been recognized so far [468] . The NIAID Office of Communications also reported that among healthy volunteers who received a single 15-µg 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 to 64, and in 56% and 60%, respectively, of adults aged 65 and older [469].

In a recent Phase II trial on 410 children and 724 adults who received a single - dose (15 µg HA) of inactivated A(H1N1) vaccine in the USA, protective serological titers of >1:40 were detected at 21 days after vaccination in 45%-50% of 6-35 month-old infants, 69%-75% of 3-9 year-old children, 95-100% of 18-64 year-old adults, and 93%-95% of elderly adults [470] . No vaccine-related severe adverse event occurred, but about 50% of every age and vaccine group reported injection-site and systemic reactions. Similarly, a multi-center, double-blind, randomized trial on 12691 3 years of age or older persons receiving a single-dose (7.5 µg HA) of a split virion A/H1N1 vaccine in China showed that 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 µg formulation increased the seroprotection rate to 97.7% [471].

The fact that it is possible to induce in adults protective antibody levels against A (H1N1) infection within two weeks of administration of a single dose of vaccine has now been confirmed with every pandemic H1N1 vaccine tested [472] , whether split inactivated vaccines containing 15 µg HA (SanofiPasteur, CSL, Sinovac and others), split inactivated vaccines with a water-in-oil adjuvant containing either 7.5 µg HA and MF59 (Novartis) [473] or 3.8 µg HA and AS03 (GSK), or whole-virion vaccines containing 10 µg HA (Baxter) or 6 µg HA (Omnivest, Hungary) [474] . The same one-dose schedule applies to intranasal live attenuated influenza virus (LAIV) vaccines (MedImmune in the USA and Microgen in Russia). National authorities have recommended that young children should receive a two-dose schedule, as is the case for seasonal vaccines, but immunogenicity data from clinical trials indicate that with many vaccines a single dose induces appropriate levels of immune responses.

Vaccination against pandemic H1N1 influenza was first implemented in China [475] , followed by a large number of countries. The problem still remains, however, of vaccinating the populations living in under-resourced countries, which cannot afford to buy the vaccine, and which depend on donations from governments of industrialized countries and from the pharmaceutical industry. The WHO is efficiently coordinating this effort.

[476] are health care workers and pregnant women, whose vaccination is a highly cost-effective strategy with substantial benefits to both the infants and the expectant mothers [477] [478] . Other priority groups are individuals with an underlying cardiovascular or respiratory medical condition including asthma, auto-immune disorders and diabetes, as well as young children.

The safety of the pandemic H1N1 vaccines has been thoroughly monitored during the various clinical trials. Current data show that the 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 Guillain Barré syndromes have been reported after H1N1 vaccine administration in large-scale vaccination campaigns, but they all reverted quickly. Although oil-in-water adjuvanted vaccines have been approved for use in all populations by the European Association EMEA, including pregnant women, their use in the USA raises regulatory problems, as no adjuvanted flu vaccine has ever been licensed in the country and as no fast-track system is in place for their registration [479].

The emergence of the 2009 H1N1 pandemic and its global impact on Public Health have revived the dream of a 'universal' influenza vaccine that could provide solid, broad subtypic protection against influenza viruses and skip the need for yearly seasonal vaccinations. 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 [480] [481] , combined with the fact that the well-conserved NP viral nucleoprotein could generate cross-protective cellular immunity [482] are strong arguments in favor of the possibility of developing such a vaccine [483] . It also has been shown that the conserved external region of the ion channel M2 viral protein (M2e) can elicit cross-protection through antibody-dependent cellular cytotoxicity (ADCC) [484] [485] [486] [487] . However, pigs which were vaccinated with a human influenza M2e vaccine [487] showed more severe clinical signs than non-vaccinated control animals when challenged with a swine H1N1 influenza virus. Three out of six vaccinated pigs died after challenge, suggesting that antibodies to human M2e, especially in combination with cell-mediated immune responses, exacerbated swine influenza disease [488] . These results cast doubt on the feasibility of using safely M2e as an immunogen in humans.