Review Article

H1N1 update review

Faris Q. Alenzi, MSc, PhD.

ABSTRACT

هناك موجة قلق و رعب اجتاحت قارات العالم مع إنتشار موجات فيروس إنفلونزا الخنازير H1N1. مما أدى بمنظمة الصحة العالمية لرفع درجة خطورته الى الدرجه السادسة. وعليه فقد شدد قادة وعلماء العالم على تطبيق أنظمة و قواعد الرعاية الصحية والوبائية المعتمدة بمثل هذه الحالات للحد من الإصابات والوفيات الناتجة. يناقش هذا البحث بداية ظهور الفيروس، و تركيبه الجيني، وأعراضه، وطرق استكشافه، والنتائج الأولية الناتجة عن التجارب السريرية، والدور المناعي، والادوية المستخدمة، و اللقاحات ضد انفلونزا الخنازير H1N1، و سيطرة المريض، و المستشفى، و معالجة الأعراض. كما يصف بشرح مفصل مسؤوليات الممارسين الصحيين، و توضيح مناطق الصعوبة في تشخيص المختبر، طرق عزل المريض، و كيفية التحكم و التقليل من انتقال العدوى للعاملين. كلها عبارة عن طرق حية للمساعدة في تقليل خطورة الانتشار العالمي و المحلي لظهور داء انفلونزا الخنازير H1N1.

There is worldwide concern on the spreading pandemic wave of the new swine influenza virus (S-OIV). The WHO has placed the pandemic threat alert to level 6. World leaders and scientists importantly stress that regulations and pandemic preparedness may lower the morbidity and mortality. This review describes the background, origin, epidemiology, signs and symptoms, methods of detecting H1N1, the risk of H1N1 pandemic control plans, immunity to H1N1, vaccination against H1N1, hospital management, patient management, and treatment of symptoms. It also describes in considerable detail the responsibilities of health professionals in navigating the complex areas of laboratory diagnosis, patient isolation procedures, and how to minimize and manage any accompanying staff infections, all of which are vital processes to help mitigate and minimize the seriousness of local and national de-novo outbreaks of emerging H1N1 infection.

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From the Department of Clinical Laboratory Sciences, College of Applied Medial Sciences, Al-Kharj University, Al-Kharj, Kingdom of Saudi Arabia.

Address correspondence and reprint request to: Dr. Faris Q. Alenzi, Consultant, Associate Professor of Immunology, Department of Clinical Laboratory Sciences, College of Applied Medial Sciences, Al-Kharj University, PO Box 422, Al-Kharj 11942, Kingdom of Saudi Arabia. Tel. +966 (1) 5453817. Fax. +966 (1) 5454586. E-mail: fqalenzi@ksu.edu.sa

Worldwide concern accompanied the spreading of the pandemic wave of the new swine influenza virus (S-OIV). The World Health Organization (WHO) soon announced the pandemic threat alert to scale 6. World leaders and scientists importantly stress that regulations and pandemic preparedness may lower the morbidity and mortality. Affecting most countries, the 2009 H1N1 influenza virus or S-OIV is the predominant influenza virus in circulation worldwide. Late September 2009, the WHO had reported at least 318,925 laboratory-confirmed cases of 2009 H1N1 with more than 3,917 deaths. In the preceding 2 weeks, there had been an increase of at least 22,454 cases and more than 431 deaths.[1] Laboratory-confirmed cases represent a substantial underestimation of worldwide cases since many countries focus surveillance and laboratory testing only on those patients with severe illness. From April 19 to September 12, 2009, 60.6% of all influenza specimens reported to WHO were 2009 H1N1 viruses.[1] In temperate regions of the Southern Hemisphere, disease due to 2009 H1N1 is currently declining. Nevertheless, in the tropics there is still substantial disease attributable to 2009 H1N1. In temperate regions of the Northern Hemisphere, there is an increased influenza like illness (ILI) activity due to 2009 H1N1, and this includes most of the USA, Mexico and certain European countries. In late September, WHO reported more than 10,000, 2009 H1N1 influenza isolates had been tested worldwide and found to respond to the influenza antiviral, oseltamivir.[1] In March 2009, an outbreak of influenza infection in North America was found to be caused by a new strain of influenza virus, designated as "Influenza-A H1N1 2009", which is a reassessment of swine, avian, and human influenza viruses. Over a thousand cases were identified within the first month, mainly in the USA and Mexico, thereafter, thousands

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of cases were identified and reported throughout the world. The WHO declared Influenza-A H1N1 2009 as a pandemic, predicting that a third of the world's population will eventually be infected.[1] Appropriate actions concerning Influenza-A H1N1 2009 need to be made based on facts reflected by scientific prospects, however, not to be affected by political, legal, financial or any other interests.[2]

Origin. Influenza viruses are negative strand ribonucleic acid (RNA) viruses of the genus *Orthomyxoviridae*. They continually circulate in humans in yearly epidemics, mainly in the winter in temperate climates. Antigenically novel virus strains emerge sporadically as pandemic viruses.[3] The most successful influenza virus of the 20th century from the perspective of transmissibility among, and pathogenicity to, humans were the H1N1 virus that caused the Spanish flu pandemic of 1918. This virus is thought to have killed up to 100 million people.[4] The next most successful viruses were those that caused the Asian flu pandemic in 1957 (H2N2), which killed 70,000 people in the USA, and the Hong Kong flu pandemic in 1968 (H3N2) which killed 34,000 people in the USA. The reason for the high pathogenicity of the 1918 Spanish flu virus remains an enigma;[4] the available data suggest an avian virus origin, but the precursors are still unknown. It is possible that all gene segments were from mammalian-adapted avian influenza viruses. More is known about the 1957 and 1968 human pandemic strains. Each of these newly emerged H2N2 and H3N2 viruses possessed gene segments from avian and human influenza viruses.[5] Acquisition of novel surface glycoproteins (hemagglutinin [HA] and neuraminidase [NA]) allowed the viruses to circumvent the host's humoral immunity, and their possession of a novel PB1 gene implicates this gene in interspecies transmission. One recipe for success for a virus is therefore, reassortment, a process that results in the acquisition of novel surface antigens and accordingly, as here, the novel PB1 gene and the retention of segments of this gene, seems to be related to transmissibility among humans.[6]

Influenza as a disease of pigs was first recognized during the Spanish influenza pandemic of 1918-1919. The Veterinarian J. S. Koen first described the condition, observing frequent outbreaks of influenza in families followed immediately by illness in their swine herds, or vice versa.[7] Shope and Lewis in 1930 first isolated the influenza virus from pigs; the virus was isolated from humans several years later.[9] Swine influenza virus was first isolated in man in 1974, confirming that swine-origin influenza viruses (S-OIV) could infect humans. Pigs are known to have receptors both to avian and human influenza virus strains,[11] and this may have an important role in inter-species transmission. Consequently, they have been considered a possible "mixing vessel" in which genetic material can be exchanged, with the potential to result in novel progeny viruses, to which humans are immunologically naive and highly susceptible.[12,13] As the threat of a pandemic due to highly pathogenic H5N1 avian influenza virus strains looms, a better understanding of inter-species transmission of influenza is necessary. Myers et al (2007),[14] reviewed the literature to compile and summarize all reported cases of human infection with swine influenza virus. They extracted data regarding demographic characteristics, epidemiological investigations and the laboratory results finding were 50 cases of apparent zoonotic swine influenza virus infection, 37 of which involved civilians and 13 of which involved military personnel, with a case-fatality rate of 14% (7 of 50 persons). Most civilian subjects (61%) reported exposure to swine. Although sporadic clinical cases of swine influenza occur in humans, the true incidence of zoonotic swine influenza virus infection is unknown. People who work with pigs are known to be at increased risk of zoonotic influenza virus infection, so it is therefore, prudent to include them in pandemic planning efforts.[14]

The current S-OIV lineage carries 3 gene segments that share with the human seasonal virus, a common descent from the 1918 H1N1 virus. Whilst the 1918 influenza A (H1N1) virus probably appeared simultaneously from birds to humans and swine, the provenance of S-OIV is most likely from swine to humans. This was likely the result of a reassortment between 2 influenza A (H1N1) swine viruses. These 2 viruses were actually the products of at least 4 independent avian-to-mammalian cross-species transmission. During this process of evolution, there were at least 4 reassortments of gene segments among avian, human, and swine-adapted viruses.[15]

Epidemiology. As of 3 October 2009, 12,848 laboratory-confirmed cases of Pandemic (H1N1) 2009 were reported to the WHO by 21 out of 22 countries in the Mediterranean region. A total of 1,065 of these reported cases were locally transmitted. Djibouti became the latest country in the region to report cases of pandemic (H1N1). There are 78 related deaths from Pandemic (H1N1) reported from 12 countries in the region. These deaths were reported from Saudi Arabia [n=28], Oman [n=21], Kuwait [n=7], Islamic Republic of Iran [n=4], Yemen [n=4], Bahrain [n=4], Egypt [n=2], Lebanon [n=2], Syrian Arab Republic [n=2], Iraq [n=2], Palestine [n=1] and Qatar [n=1]. An additional 840 laboratory-confirmed cases of Pandemic (H1N1) 2009 were reported from 11 countries during the period from 26 September 2009 to 3 October 2009: Bahrain [n=102], Egypt [n=45], Jordan [n=61], Iraq [n=185], Islamic Republic of Iran [n=20], Morocco [n=13], Oman [n=248], Palestine [n=127], Syrian Arab

Republic [n=8], and Tunisia [n=31].[16] As of September 30, 2009, 24 countries in Africa have officially reported 12,382 laboratory-confirmed human cases of pandemic (H1N1) 2009 including 70 deaths. The deaths were reported from South Africa [n=59], Mauritius [n=8], Mozambique [n=2], and Namibia [n=1].[17] The greatest increase in confirmed cases and deaths was reported in North America during the past weeks. Mexico reported a large increase in activity of the pandemic (H1N1) 2009 virus during the month of September based on confirmed cases. In the United States, a total of 26 states reported widespread geographic activity of the influenza virus. In Canada, 2 outbreaks of influenza were reported, one in a school and one in a long term care facility. In Central America and some countries in the Caribbean, there has been an increase in the number of confirmed cases. The prevalence of S-OIV in America during the last week was reported in 8,869 new confirmed cases and 158 new confirmed deaths. By early October 2009, there had been 146,106 confirmed cases reported across all 35 countries in the Americas region. Twenty-five of these countries reported a combined total of 3,292 deaths amongst confirmed cases of the disease.[18] The WHO analysis of the current worldwide status of S-OIV is shown in **Table 1**.[19]

Signs and symptoms. Influenza viruses are spread from person to person primarily through a large-particle respiratory droplet transmission (when an infected person coughs or sneezes near a susceptible person). Transmission requires close contact between source and the recipient persons. Contact with respiratory-droplet contaminated surfaces is another possible cause. It is characterized by the abrupt onset of constitutional and respiratory signs including: fever, myalgia, headache, malaise, nonproductive cough, sore throat, and rhinitis. In children may include: otitis media, nausea, and vomiting. Clearly, identifying influenza illness in the absence of laboratory confirmation is challenging and suggests that the diagnosis of influenza should be considered in all patients presenting with respiratory symptoms or fever during the influenza season.[20]

Detection of novel pandemic influenza a (H1N1). The direct fluorescent antigen (DFA) influenza tests are also useful, but require technical expertise and a fluorescence microscope and take 1-4 hours to complete. One of the current detection methods was developed to use the DFA,[21] where they were comparing with real-time reverse transcriptase-polymerase chain reaction (rRT-PCR), and this showed a sensitivity of 93% (39/42) and a negative predictive value of 96%. These tests lack expansion to routine clinical laboratories and may require an expert eye often difficult to find in such laboratories. Providing that technical expertise is available, the DFA may requires 1-4 hours to complete.

The tests that are commonly used in clinical practice and routine hospital laboratory are the rapid influenza diagnostic tests. Early in the outbreaks that occurred in some parts of the world, a need for rapid screening test necessitated the presence of many commercial rapid tests. This test is based on visualization of colorimetric banding on a disposable single-use card used for one single use and then discarded, but the specimen type is important to achieve successful identification. Many commercial kits are available, and the selection of the required test is based on the user criteria. Influenza rapid point-of-care (POC) tests for the detection of the recently emerged swine lineage A(H1N1) virus can be used in the emergency room where it is the gatekeeper of all patients presenting and complaining of the flu symptoms. These popular PCO tests can provide results in less than 30 minutes. Several commercial kit suppliers make tests of differing quality, unfortunately, none of them are adequate for excluding the diagnosis of 2009 influenza A (H1N1) infection. For this reason, it is of extreme importance to supplement the initial result with an additional test before confirming the diagnosis. The sensitivity and specificity of the rapid screening test for influenza A is variable, and higher sensitivity should be sought, the user should be careful that a confirmatory validation test should follow if they felt appropriate. Earlier studies of the Centers for Disease Control and Prevention (CDC) in the USA, showed rapid test sensitivity of 40-69% in established cases; tests included in the comparison were the Binax NOW (Binax Inc.; Scarborough, Maine, USA), Directigen EZ (BD Medical; Sparks, Maryland, USA), and QuickVue (Quidel; San Diego, California, USA).[22-25]

Table 1 - Laboratory-confirmed cases of pandemic (H1N1) 2009 as officially reported to the World Health Organization (WHO) by States Parties as of 27 September 2009.[16]

Region	Cumulative total	
	Cases	Deaths
WHO Regional Office for Africa (AFRO)	8352	42
WHO Regional Office for the Americas (AMRO)	13747	3020
WHO Regional Office for the Eastern Mediterranean (EMRO)	12008	74
WHO Regional Office for Europe (EURO)	>56000	∝176
WHO Regional Office for South-East Asia (SEARO)	33594	413
WHO Regional Office for the Western Pacific (WPRO)	96197	383
Total	**>343298**	**∝4108**

During the pandemic stage, a rapid method for accurate detection and confirmation of 2009 H1N1 is of greatest importance. Improper interpretation of these tests is may lead to false-negatives and, when one considers that the lives of these patients lay in the hands of the technicians interpreting results of these tests, appropriate training of the technical staff is clearly vital. The rapid antigen test (RAT) or what it is known as QuickVue Influenza (A & B) was compared with detecting 2009 H1N1 versus seasonal H3N2 in concurrent epidemics in Australia. The testing of the studied cases (n=460) was 62% 2009 H1N1, 35% H3N2, and 3% other local Australian strains. The sensitivity of the RAT was 91/155 (59%) versus 69/95 (73%) (p=0.03); there were no false positives. The authors concluded that the RAT has decreased sensitivity for detecting 2009 H1N1.[26] The limited data at that time suggested that the currently available RAT detection kits have poor clinical sensitivity for diagnosis of human H5N1 disease. In a recent study, an analytic sensitivity for the detection of 2 contemporary H1N1, 2 H3N2, and 3 H5N1 viruses were investigated and determined using specialized virus culture as a gold standard, or what it is known as a reference method.[27] It was documented that the limits of detection of influenza viruses of all subtypes by antigen detection kits were >1000-fold lower than virus isolation. However, careful interpretation of these tests can have a major impact on their utility and may lead to proper employment of laboratory resources in achieving the correct diagnosis. Underestimating of the interpretation guidelines accompanying the kits may result in misleading clinical diagnosis. It is the duty of the laboratory management staff to explain this form of testing to avoid patient mismanagement (**Table 2**). In 1977, 3 circulating strains of influenza A(H3N2), A(H1N1), and B virus strains were detected by antibodies to the hemagglutinins of these virus strains in Moscow. The strain of influenza A circulated at that time have been detected in an elder group of people living in that community and showed that the community did not suffer from the strain of A(H1N1) since antibodies were found in the elderly.

Table 2 - Interpretation of rapid influenza detection tests.

Virus detected	Results (interpretation)
Positive for flu A	Seasonal H1N1
	H3N2
	Pandemic H1N1
Positive for flu B	Seasonal H1N1
	H3N2
	Pandemic H1N1
Negative for flu A and flu B	Still cannot rule out influenza infection

Interpretation of such findings concluded that the seasonal influenza did not include H1N1. Therefore, antibody detection can be a vital tool in detecting how many influenza strains are circulating in a community. The drawback of such detection may be that it can be used in a research investigation whereas a reference laboratory is monitoring seasonal influenza strains, and the availability of the antibodies required for this kind of investigation.[28] Also, in one earlier study, the detection by antibodies to A(H1N1) indicated that the elderly can be at high risk of this strain and therefore the antibodies may not prevent the infection even if it is present in this group and therefore outbreaks among the elderly can be anticipated within the institutionalized population.[29] The same procedure has been used in the form of enzyme-linked immunosorbent assay, also called (ELISA) (A subtype specific ELISA using purified hemagglutinin (HA) from influenza A[H1N1] and A[H3N2]) to detect the previous exposure to swine population to prove that multiple strains can be found in pigs using the anti-H1N1 and anti-H3N2.[30] The recent implementation of antibody assays in the novel pandemic strain of A(H1N1) has been shown to have low sensitivity in the veterinary use of the antibodies detection assay, and for this particular reason, an expert role should be implemented in order to use the assay in the proper way and therefore it should be excluded in the hospital setting. On the other hand, it is still a very valuable tool to investigate the relationship between flu strains and host infections, including humans.[31]

Since the beginning of the utilization of PCR, the assay has always revealed extremely important information on viruses and various infected species. One of the early uses of PCR for the investigation of the A(H1N1) PCR was used to amplify and sequence the complete haemagglutinin (HA1) region of the haemagglutinin (HA)-encoding genes of 10 clinical isolates of influenza virus of the H1N1 or H3N2 subtypes. The experimental work performed showed an expected change in the sequence of the HA from the egg passage in 90% of the original isolates. Referring to that experiment may illustrate that the isolates of influenza virus of the H1N1 or H3N2 subtypes can have amino acid substitutions in the egg-derived HA sequences which demonstrate the ability of the influenza virus to change, and hence relates to the changes that have occurred in the current novel pandemic A(H1N1) due to antigenic drift.[32] Powerful molecular biology tools have a major role in detection and tracking such drifting and shifting characteristic of influenza viruses. Sequencing, preceded by PCR, has been used to study many isolates of the A(H1N1); for instance, the phylogenetic analysis of sequencing data was performed to document that since 1995 there had been 3 different genetic lineages of

influenza A(H1N1) virus HA1 gene circulating in men in Shenzhen City, China.[33] A useful and simple molecular tool has also been utilized to reveal the rapid genotyping and monitoring of important sequence changes in the circulating influenza. Sequence information from recent H1N1, H3N2, and H5N1 human virus isolates was used to identify conserved regions within each internal gene, and gene-specific PCR primers capable of amplifying all 3 virus subtypes were designed. Virus subtyping was based on subtype-specific restriction fragment length polymorphism (RFLP) assay patterns within the amplified regions, which means circulating viruses can be distinguished from each new viruses.[34] The disadvantage of such molecular tools is the level of expertise needed that few trained people have found to assist and mostly found in the research laboratories, not in the hospital-based laboratories. In another approach using the PCR-based assay, an one-step multiplex reverse transcription (RT)-PCR assay that was aiming to targeting the HA1 segment of the human hemagglutinin gene was investigated as a rapid surveillance method where 3 major human influenza viruses in global circulation, H1N1, H3N2, and B were used in the investigation. The researchers were able to detect the 3 viruses using 112 randomly selected cultured-positive clinical samples collected in the study.[35] The samples were gathered during the 2000-2001 influenza season and used 3 subtype specific primers sets capable of producing PCR products and resulted in obtaining 3 base-pair lengths of 585 influenza H1, 402 influenza H1, and 290 (bands) corresponding to influenza B subtypes, and these were utilized together in a one step in one reaction tube. This assay of RT-PCR from the RNA samples of the viruses is called multiples RT-PCR and is aimed to subtype the circulating influenza from the positive cell cultures (100%), and to prove that the assay is a confirmatory molecular tool. Additionally, it can be used as a highly sensitive and timely surveillance tool for rapid detection and simultaneous subtyping of clinical influenza specimens isolated from different geographical locations. As such, it might contribute an accurate confirmation of local virus subtypes once it was utilized in public health laboratories in a designated area. These molecular tools can also be utilized to gather full information of the divergent genetic evolution of hemagglutinin in influenza viruses in general, including the A(H1N1) subtype.[36] It is very important to mention that the construction of a local database of the divergent genetic evolution of influenza viruses should be initiated, and utilized as part of a flu epidemic monitoring program. A Database of such endemic viruses, which may be pandemic, can be very useful in controlling pathogenic diseases and formulating vaccine batches. To view and compile the evidence of the concurrent circulation of H1N2, H1N1, and H3N2 influenza A viruses, also the RT-PCR with the immunoassays of HI which followed by partial cDNA sequencing can be invaluable as a molecular tool to prove the nature of the circulating swine flu viruses where herds of pigs are domesticated.[37] The molecular tools, however, depend on 2 critical factors, the sequence that is usually carefully selected to represent the viruses under surveillance,[38,39] and the primers representing the required virus sequence that needs to be amplified by RT-PCR.[22] In some scenarios, the sequence selected for RT-PCR by some commercially available kits has to be validated in tedious work to verify the presence of the novel pandemic H1N1[40] that the kits makers initially declared. Moreover, probes and primers should be selected within the recommended regions such as CDC M-gene reagents and accompanied by in-house preparations of the same regions of the virus genome. This will avoid cross-reactivity with seasonal influenza viruses and prevent wrong conclusions. Nevertheless, it is only the systematic preference of the expert molecular biologists that can easily identify the proper molecular tool to document the presence of the novel pandemic H1N1 in the laboratory. Cell culture detection has been discussed,[24,41,42] but this can be determined by a very specialized virology laboratory in which biosafety level 3 (BLS3) is facilitated. Routine hospital laboratories might not be able to provide this level of sophistication and therefore should not be an option unless a reference laboratory is available. Since the clinical specimens are of extreme importance in achieving accurate results, the following section provides guidance in the management of specimens.

Specimens. Specimens are preferably nasopharyngeal swabs, nasal aspirates, or a combination of nasopharyngeal swab and oral pharyngeal swab. For intubated patients, the preferred specimens are endotracheal aspirate, sputum, or bronchial alveolar lavage.

Transport. For transportation to the laboratory, specimens should be placed in a sterile viral transport medium and cooled by ice packs at 4°C. Polyester or Dacron synthetic tips attached to an aluminum or plastic shaft should be used to obtain cultures. Wooden-stick swabs and calcium alginate swabs are inappropriate to use.

Risk of pandemic H1N1 control plans. The WHO raised its pandemic preparedness alert level to phase-6 on a 6-phase scale due to the outbreak and rapid spread of the virus. Epidemiological figures show that a pandemic is underway with sustained community-level outbreaks in numerous countries all over the globe. All authorities have started to organize, communicate, and implement plans of disease mitigation. Despite the mild pattern of the illness currently, the impact of the pandemic during

the second wave could worsen as larger numbers of people become infected. The impact of large numbers of severely ill patients creating an urgent burden on health services to the point of being capable of overwhelming intensive care units may possibly also impinge on the management of other disease. However, H1N1 cannot be described as severe pandemic yet, although the outbreak has some social and economic impacts that have already prompted governments, corporate leaders, health care facilities, educational institutions, and other organizations take action.

During the early stages of the outbreak, governments in various countries (such as the USA) declared a "public health emergency" and released stockpiles of antiviral medications. Other countries, such as Mexico, closed schools, gyms, pools, and banned most large gatherings. Accordingly, the WHO re-evaluated its plans and capacity to respond to a pandemic. Due to lack of experience of managing and controlling H1N1, a higher severity of pandemic is likely to exceed the projections of what many corporate and governmental leaders have envisaged. Therefore, health authorities are well advised to review their ability to respond to public panic and disruptions to their operations. Control plans for H1N1 pandemic need strong international alliance where all preventive and control measures must be considered. Control plans should outline the steps that each government will take to reduce the risk to the health and safety of its population. Control plans should be updated in accordance with evolving epidemiological figures of current illness patterns.

Strategies that address appropriate caution should be undertaken before any pandemic begins.[43] These include the following: H1N1 should be treated as a truly catastrophic pandemic; Pandemic planning committees, supported by real budgets should promptly established; Services and critical products should be prioritized; Probable post pandemic changes must be estimated and appropriate plans should be considered; Pandemic risk management plans should be assessed; and All critical issues that have direct concerns to public health should be considered. These issues include travel, hygiene, antiviral medications, and health care support to ensure that they are consistent with the guidance from the CDC.

Health professional responsibilities. Health authorities should consider the following measures in their control plans:[44] 1) An emergency management team should be established to manage response to the H1N1 pandemic. 2) Health authorities should prioritize their control plans to target the most vulnerable groups within the population. 3) Control plans should be validated, monitored, and evaluated according to constantly updated epidemiological figures. 4) Each organization should establish its own educational, preventive, and control plans for its staff in accordance with the international control standards. 5) Health authorities should establish appropriate social networking systems to communicate information during the H1N1 pandemic. 6) Control measures should be in place to deal with defaults in social activities including transportation system, public school, and their effects on local economics. 7) Educational authorities should develop plans for online instructional continuity during the H1N1 pandemic. 8) When prioritizing plans, schools should be placed as highest priority on protecting human life and the well-being of the surrounding community. Other factors should consider the protection of property and the environment and maintaining the integrity of related research programs. 9) Plans to ensure the successful delivery of services during the H1N1 pandemic should be established by the authorities concerned so that adequate resources are in place to handle public demand for health services. 10) During the H1N1 pandemic, hospitals and other health facilities should remain operational. However, in the event that adequate resources are not available to ensure health care services can be delivered safely, clinics may triage requests for service. 11) Travel control policies should be established in accordance to the CDC travel advisory plans during an H1N1 pandemic. 12) The H1N1 vaccine should be provided to staff and students in accordance with Government distributions guidelines. Because initial supplies may be limited, the Director of Student and Employee Health should develop an internal prioritization list. 13) Each organization should develop their control plans in accordance with the CDC recommendations on infection control and prevention practices during an H1N1 pandemic. 14) Health authorities should order and maintain an inventory of antiviral medications for H1N1. 15) Health authorities should be prepared to implement multiple measures to protect workers and ensure business continuity. 16) Governments should review human resource policies to make sure these practices are consistent with public health requirements. 17) Governments should engage all local authorities in the control plans to confirm channels of communication are effective for the dissemination of local outbreak information. 18) Governments should be prepared to close schools and temporarily dismiss students in order to protect public health, and be alerted of the decision effect on business's functioning, especially affecting absenteeism.

In the absence of widespread effective vaccination, the H1N1 virus spreads more readily than the seasonal influenza viruses. During the seasonal flu period everyone will be more susceptible to the H1N1

infection. Older children and young adults appear to be at greater risk of H1N1 virus infection. Therefore, health care professionals must deliver accurately the important responsibilities thrust upon them by this pandemic in order to reduce the risk of disease and sustain effective control measures. In the event of influenza pandemic, the workload of healthcare workers (HCWs) would raise dramatically. In fact, healthcare workers are the first line of response to influenza pandemic. Moreover, due to the nature of their occupation, their own risk of infection is also increased. Pandemic preparedness plans should consider both the proportion of ill healthcare employees, as well as the proportion of employees who may be absent due to personal fears or private responsibilities. Appropriate protective measures should be clarified and communicated during the pre-pandemic phase. Initiatives to strengthen workers' confidence in the fact that everything would be carried out to protect them against becoming ill in the event of a pandemic also need to be implemented.[45]

Limitation of healthcare personnel entering the isolation room. Healthcare staff should only be in the room of a patient in isolation if they are directly involved in the care of that patient.

Isolation precautions. All healthcare personnel who enter the patient's isolation room should take standard and contact precautions. Eye protection should also be used for all healthcare activities when patients are being evaluated, or are in the isolation, for novel H1N1. There should be adherence to hand hygiene by washing with soap and water or using alcohol-based hand sanitizer immediately after removing gloves and other equipment, and after any contact with respiratory secretions. Non-sterile gloves and gowns along with eye protection should be put on when entering a patient's room.

Respiratory protection. The appropriate respiratory protective equipment necessary to protect health care workers from the novel swine-origin influenza A (H1N1) virus is not known.[46] However, CDC recommends that all healthcare personnel who enter the rooms of patients in isolation with confirmed, suspected, or probable novel H1N1 influenza should wear a fit-tested disposable N95 respirator. Healthcare workers should therefore always ensure that adequate respiratory protection is in place when entering a patient's room.[47]

Management of visitors. Hospitals should limit visitors for patients who are in isolation for the novel H1N1 infection to just those people who are absolutely necessary for the patient's emotional well-being and care. Visitors who have been in contact with the patient before and during hospitalization are a possible source of novel H1N1. Therefore, visitors should be scheduled and controlled to allow for appropriate screening for acute respiratory illness before entering the hospital and given appropriate instructions on the use of personal protective equipment and other precautions (such as hand hygiene, limiting surfaces touched) whilst in the patient's room. Visitors should be instructed to limit their movement within the facility.[47] Visitors must be offered a gown, gloves, eye protection, and respiratory protection (namely N95 respirator) and, before entering the patient's room, should be instructed by healthcare personnel on their use.[47]

Surveillance of healthcare personnel. In communities where novel H1N1 virus transmission is occurring, healthcare personnel should be monitored daily for signs and symptoms of febrile respiratory illness. In communities without novel H1N1 virus transmission, healthcare personnel working in areas of a facility where there are patients being assessed or isolated for novel H1N1 infection should be monitored daily for signs and symptoms of febrile respiratory infection. This would include healthcare personnel exposed to patients in an outpatient setting or in the emergency department. Healthcare personnel who develop these symptoms should be instructed not to report to work, or if at work, should cease patient care activities and notify their supervisor and infection control personnel. Healthcare personnel who do not have a febrile respiratory illness may continue to work. Asymptomatic healthcare personnel who have been exposed to novel H1N1 should be put on antiviral prophylaxis before they are allowed to continue working.

Management of ill healthcare personnel. Healthcare personnel should not report to work if they have a febrile respiratory illness. In communities where novel H1N1 transmission is occurring, healthcare personnel who develop a febrile respiratory illness should be excluded from work for 7 days or until symptoms have resolved, whichever is longer. In communities without novel H1N1 transmission, healthcare personnel who develop a febrile respiratory illness and have been working in areas of the hospital where swine influenza patients are present, should be excluded from work for 7 days or until symptoms have resolved, whichever is longer. In communities where novel H1N1 transmission is not occurring, healthcare personnel who develop febrile respiratory illness and have not been in areas of the facility where swine influenza patients are present should follow facility guidelines on returning to work.[47]

Health care professionals can contribute effectively to obtaining successful control measures through the following strategies: 1) Fair vaccine distributions for the highly susceptible groups of the population. This reduces the chance of infection among cases, contact groups, young, elderly or immuno-suppressed patients. 2) Coordination with school-based clinics to offer non-

mandatory vaccination to prevent future pandemics. 3) Setting up isolation procedures for sick patients by encouraging them to stay at home and reduce their household contacts. 4) Ensure early identification of all H1N1 cases and provide appropriate care, treatment and isolation procedures. 5) Issue weekly reports of the number of laboratory-confirmed influenza cases (Laboratory confirmation includes rapid influenza tests, RT-PCR, DFA, IFA, or culture. Include all cases with a positive influenza test, whether or not typing was carried out). 6) Arrange hospitalization for pneumonia and influenza syndrome and issue weekly reports for the number of pneumonia and influenza hospitalizations. 7) Perform surveillance for influenza-associated mortality with weekly reports for the laboratory-confirmed influenza deaths. 8) Plan for a surge of patients and increased demands for hospital/laboratory services for people who seek medical care. 9) Consider extending hospital hours of operation to care for patients with novel H1N1 flu. 10) Take steps to protect the health of workforce during the H1N1 pandemic because healthcare workplaces are classified as very high or high exposure risk for pandemic influenza. 11) Provide immunization against H1N1 at no cost to the public. 12) Ensure wide understanding of the pandemic planning and response activities of the hospitals, outpatient facilities and local public health in the nearby community. 13) Be prepared for a range of unexpected situations. The true impact of novel H1N1 flu outbreaks in the coming months will not be known until it happens. 14) Be prepared for the distinct possibility that there will be a very significant increased demand for services.

Immunity to H1N1. It is believed that the current H1N1 virus will have a similar effect to the Spanish flu virus, infecting lung cells, leading to over-stimulation of the immune system through the release of many cytokines into the tissue of the lungs. This then leads to extensive white blood cell migration towards the lungs, causing destruction of lung tissue and excessive secretions, causing patients to have difficulty in breathing. In contrast to other pandemics, which have mostly killed the old and the very young, the 1918 pandemic killed unusual numbers of young adults, which may have been due to their healthy immune systems mounting a too-strong and damaging response to the infection. A similar situation appears to be the case with the current H1N1 influenza pandemic. Flu viruses change every year, and therefore older people (who have previously been infected with other H1N1 flu viruses) probably have a partial immunity to this new currently circulating H1N1 virus. Partial immunity means that they may still get infected with the flu virus, but they would be less likely to be hospitalized or have severe consequences. It is recommended that patients who fall into high-risk categories receive vaccinations, including those with chronic illness, heart disease, lung disease, diabetes or suppressed immune systems, and those who are pregnant, are between 20 and 40 years old, or are infants. Of the cases reported to the CDC,[47] 64% were in the age range 5-24 years with only 1% being in individuals >65 years; this represents a very different pattern to that seen with seasonal influenza. Perhaps older individuals enjoy some degree of pre-existing immunity from previous immunizations with seasonal flu vaccines.

Vaccination against Influenza-A H1N1 2009. Generally speaking, to protect individuals from a particular disease, immunization is essential. Immunization can be active (vaccination) or passive, natural or artificial. An unintentional immunization can also happen when an individual is inadvertently exposed to an infectious agent. During vaccination, individuals receive a modified antigen that may consist of attenuated, inactivated or dead organisms, subcellular components, or detoxified toxins that trigger an immune response. The outcome of vaccination is that subsequent exposure to the unmodified antigen will lead to rapid activation of the immune system to eliminate that pathogen before it can cause disease. Unfortunately, no vaccination is without risk. This certainly became evident with the swine flu epidemic that occurred in 1976, when there was a rush to protect individuals who were at risk (especially infants and the elderly), but significant neurological complications such as Guillain Barre syndrome and others were observed.[48] Consequently, this has raised serious concerns on the safety and efficacy of receiving the vaccine against the current Influenza-A H1N1 2009 pandemic. Media and broadcasts confirm that many people worldwide expressed their concerned on the safety and efficacy of the newly developed H1N1 vaccine. Many questions of concern were raised such as do we have enough knowledge on the side-effects of the newly developed vaccine (such as Guillain Barre Syndrome), which can lead to paralysis and even death? Are there any other side effects that we don't yet know about? Is it worth the risk to receive the vaccine even when it becomes available? Influenza virus is known to be one of the most complex and confusing of all viruses due to its continuous genetic mutations. Unlike other viruses, such as measles (which stay unchanged year upon year), every 1-3 years influenza viruses mutate and therefore at risk people have to get vaccinated yearly. Early each year, health bodies worldwide routinely decide which flu strains will be included in the subsequent seasonal flu vaccine. However, one of the current dilemmas is

the possibility that, by the time the vaccine becomes available later on in the year, usually in September or October, the viruses may have undergone further genetic mutation and therefore have already changed. This therefore poses the question of whether it is of practical use to expose people to the vaccine and its possible side effects?

The concerns on the new vaccine are mainly due to what happened at Fort Dix in 1976, also linked to a swine flu-like outbreak.[48] In 1976, over 40 million people received the H1N1 vaccination over the period of a few months.[48] The incidence of Guillain-Barre syndrome amongst them was approximately one in 50,000. Guillain-Barre syndrome is a rare clinical disorder wherein the body's immune system attacks the nerves, causing weakness, and numbness to the arms and legs and sometimes even paralysis. This compares to approximately one in a million people who develop the syndrome from the seasonal flu vaccine. Moreover, Guillain-Barre syndrome occurs naturally following upper respiratory illnesses, digestive illnesses and is rarely associated with drugs and vaccines. The new H1N1 vaccine is expected to be vigorously tested before it can be available to the public, making sure that no incidence with Guillain-Barre outbreak can occur this time around. However, what, if any, of these rare side effects occur, and whether they do, will not be known until hundreds of thousands or even millions people have received the new vaccine. In addition to the above, the contents of the newly developed vaccine, is important to be understood, and similarly, what the regular flu vaccine normally contains[49] (**Table 3**). There is also another concern of whether the newly developed vaccine will even work at all.

It may be understandable that individuals who are in good health refuse to take the vaccine because they may feel that if they become infected with H1N1 they will develop natural immunity against the virus. They may also feel that, in the worst scenario, if they do develop any clinical complications, they will still have access to the antiviral treatment that is currently available. However, it would be risky for individuals who have other health problems and children not to take the vaccine when it becomes available. The risk groups have already been identified by the WHO and included in the CDC recommendations.[47]

Vaccines are the most powerful public health tool for the control of influenza infection. In the past 30 years, many hundreds of millions of doses of trivalent H1, H3 and B influenza vaccines have been administered without significant clinical complications, while clearly saving countless lives. Currently, the CDC in the USA, has already isolated the new Influenza-A H1N1 virus and has modified the virus so that it can be used to make hundreds of millions of doses of the vaccine.[50,51] Vaccine manufacturers are now using these materials to begin vaccine production.[52-54] Making vaccine is a multi-step process which can take several months to be completed. Candidate vaccines must be tested through clinical trials over the next few months following production. The 2009 newly developed Influenza-A H1N1 vaccine is expected to be available in the fall semester. Despite all the concerns stated earlier, many people who were around during the swine flu outbreak in 1976 have been found to have some immunity to H1N1.[54-58] Also, people over the age of 50 who have been getting annual flu vaccines for most of their adult lives, also appear to have partial immunity (all flu vaccines contain some form of the H1N1 virus).[54,56] Individuals are strongly advised to get the seasonal flu vaccine because while a resurgence of the H1N1 flu virus may be on the horizon, there may be other strains of flu making their rounds, and people should not leave themselves susceptible to them.[54,56] The 1976 swine flu virus and the Influenza-A 2009 H1N1 virus are different enough to the extent that its unlikely that a person vaccinated in 1976 will have full protection from the 2009 H1N1. People vaccinated in 1976 should still be given the 2009 H1N1 vaccine.[54]

It is anticipated that both seasonal flu and Influenza-A 2009 H1N1 vaccines may be administered at the same time. However, it is expected that the seasonal vaccine will be available earlier than the H1N1 vaccine. The usual seasonal influenza viruses are still expected to cause illness during fall and winter. However, at risk individuals are encouraged to get their seasonal flu vaccine as soon as it is available. The CDC's Advisory Committee on Immunization Practices (ACIP) has recommended that only certain at risk groups among the whole population are highly encouraged to receive the 2009 H1N1 vaccine as soon as it becomes available. These target groups include pregnant women, people who live with or taking care of children younger than 6 months of age, healthcare and emergency medical services personnel, persons between the ages of 6 months and 24 years old, and people of 25 through 64 years of age who are at higher risk for 2009 H1N1

Table 3 - Possible materials contained in the influenza H1N1 vaccine.

Possible materials	Reactions
Gelatin & Tween80™ & Resin	Can cause allergic reactions
Formaldehyde	Carcinogen
Triton X100	Detergent
Gentamycin	Antibiotic
Thimerosal	Mercury is still in multi-dose flu shot vials

because of chronic health disorders or compromised immune systems.[50,51] It is expected that the 2009 H1N1 influenza-A vaccine will have a similar safety profile as that of seasonal flu vaccines, which have a very good safety track record. Over the years, hundreds of millions of people have received seasonal flu vaccines. The most common side effects following flu vaccinations are unremarkable, typically soreness, redness, tenderness or swelling at the site of injection. Accordingly, the CDC expects any side effects following vaccination with the 2009 H1N1 influenza-A vaccine would be similarly unusual. If side effects occur, they will likely be similar to those experienced following seasonal influenza vaccine. Mild problems that may be experienced include fainting (mainly adolescents), headache, muscle aches, fever, and nausea. If these problems occur, they usually begin soon after the vaccination is given and last 1-2 days. Life-threatening allergic reactions to vaccines are very rare.[50,51] If they do occur, they are usually within a few minutes to a few hours after the vaccination is given. Additionally, the CDC is planning to work actively with numerous partners including other federal agencies, state and local health departments, professional organizations, and academic institutions to follow individuals after vaccination, and to monitor for any potential adverse events.[7,8] Seasonal influenza vaccines are known to be highly effective in preventing influenza disease. The expectation is that a vaccine against 2009 H1N1 influenza-A would probably work in a similar fashion to the seasonal influenza vaccines. The CDC believes that the benefits which will be gained from vaccination with the 2009 H1N1 influenza-A vaccine will far outweigh the risks.[50,51]

Pregnant women, children, and teens are the extreme risk groups among the population to be most susceptible to the clinical complications due to infection with H1N1. Such complications include death, and therefore these groups should be vaccinated no matter what.[50] Others, however, may have some leeway on the decision to vaccinate. Individuals who had a documented case of H1N1 during the 2008-2009 flu season; probably have already acquired partial immunity. But if the current strains have no update yet, people could be highly susceptible to contracting the new virus without any immunity. Overall, the development of antiviral vaccination was recognized as the most cost efficient use of public money in the entire health field, both in saving lives and for economic impact.

Treatment. The first 642 cases identified in 41 American states were characteristically described. The median age of these patients was 20 years (range: 3 months to 81 years). Forty percent of patients were 10-18 years, and 35% were 19-50 years. Presenting symptoms were fever (94%), cough (92%), sore throat (66%), diarrhea (25%), and vomiting (25%). A few percent of patients were hospitalized, but many of these had pre-existing chronic medical conditions. They reported the virus as sensitive to both oseltamivir and zanamivir.[59]

According to the CDC, the novel H1N1 influenza virus is susceptible to oseltamivir and zanamivir, these are neuraminidase-inhibitor antiviral medications, which target the early phase of the infection. However, this strain is resistant to adamantanes, such as amantadine and rimantadine.[60] Currently, the CDC recommends antiviral treatment and chemoprophylaxis with either oseltamivir or zanamivir against this virus for people at high risk.[61-63] Both drugs were found to be safe with breastfeeding.[64] Expanding the use of a 7-valent pneumococcal conjugate vaccine (PCV7) to people aged ≥5 years is not indicated because circulation of the 7 serotypes included in the vaccine has declined substantially and disease caused by these serotypes is now uncommon.[65]

In conclusion, there is a currently considerable risk to large numbers of the population to the consequences of novel H1N1 infection. Efforts must be made by central healthcare planners and local healthcare providers to ensure that appropriate subgroups of the population receive preventative vaccination, that adequate diagnostic capacity is available, and that adequate and effective preparation for isolation and treatment strategies for potentially large numbers of infected patients is in place prior to the anticipated peaks of H1N1 infection outbreaks.

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References

1. World Health Organization. Influenza A (H1N1). (Updated 2009 December 23; Accessed 2009 October 4). Available from URL: http://www.who.int/csr/disease/swineflu/en/index.html
2. Gallaher WR. Towards a sane and rational approach to management of Influenza H1N1 2009. ***Virol J*** 2009; 6: 51.
3. Cox NJ, Subbarao K. Global epidemiology of influenza: past and present. ***Annu Rev Med*** 2000; 51: 407-421.
4. Taubenberger JK, Reid AH, Janczewski TA, Fanning TG. Integrating historical, clinical and molecular genetic data in order to explain the origin and virulence of the 1918 Spanish influenza virus. ***Philos Trans R Soc Lond B Biol Sci*** 2001; 356: 1829-1839.
5. Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. ***J Virol*** 1989; 63: 4603-4608.

6. Lipatov AS, Govorkova EA, Webby RJ, Ozaki H, Peiris M, Guan Y, et al. Influenza: emergence and control. ***J Virol*** 2004; 78: 8951-8959.
7. Easterday B. Swine influenza: historical perspectives. Proceedings of the 4th International Symposium on Emerging and Re-emerging Pig Diseases (Rome); 200 June 14-14; Parma, Italy. Italy: Department of Animal Health, Faculty of Veterinary Medicine, University of Parma 2003. p. 241-244
8. Shope R, Lewis P. Swine influenza: experimental transmission and pathology. ***J Exp Med*** 1931; 54: 349-359.
9. Smith W, Andrews CH, Laidlaw PP. A virus obtained from influenza patients. ***Lancet*** 1933; 225: 66-68.
10. Smith TF, Burgert EO Jr, Dowdle WR, Noble GR, Campbell RJ, Van Scoy RE. Isolation of swine influenza virus from autopsy lung tissue of man. ***N Engl J Med*** 1976; 294: 708-710.
11. Ito T, Couceiro JN, Kelm S, Baum LG, Krauss S, Castrucci MR, et al. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. ***J Virol*** 1998; 72: 7367-7373.
12. Scholtissek C, Hinshaw VS, Olsen CW. Influenza in pigs and their role as the intermediate host. In: Nicholson K, Webster RG, Hay AJ, editors. Textbook of influenza. 1st ed. Malden (MA): Blackwell Science; 1998. p. 137-145.
13. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. ***Microbiol Rev*** 1992; 56: 152-179.
14. Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. ***Clin Infect Dis*** 2007; 44: 1084-1088.
15. Zimmer SM, Burke DS. Historical perspective--Emergence of influenza A (H1N1) viruses. ***N Engl J Med*** 2009; 361: 279-285.
16. World Health Organization. Influenza A (H1N1). (Update 15 January 2010; Accessed 2009 October 4). Available from URL: www.emro.who.int/csr/h1n1/.
17. World Health Organization. Pandemic Influenza (H1N1) 2009. (2009 December 20, Accessed 2009 October 4). Available from URL: www.afro.who.int/ddc/influenzaa/index.html.
18. World Health Organization. Regional Update. Pandemic (H1N1) 2009. (2010 January 14, Accessed 2009 October 4). Available from URL: www.new.paho.org/hq/index.php?option=com_content&task=view&id=1866&Itemid=1167
19. World Health Organization. Pandemic (H1N1) 2009 - update 68. (Update 15 January 2010; Accessed 2009 October 5). Available from URL: www.who.int/csr/don/2009_10_02/en/index.html.
20. Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. ***MMWR Recomm Rep*** 2008; 57: 1-60.
21. Pollock NR, Duong S, Cheng A, Han LL, Smole S, Kirby JE. Ruling out novel H1N1 influenza virus infection with direct fluorescent antigen testing. ***Clin Infect Dis*** 2009; 49: e66-e68.
22. Leblanc JJ, Li Y, Bastien N, Forward KR, Davidson RJ, Hatchette TF. Switching gears for an influenza pandemic: validation of a duplex RT-PCR for simultaneous detection and confirmation of pandemic (H1N1) 2009. ***Journal of Clinical Microbiology*** 2009; 47; 3805-3813.
23. Vasoo S, Stevens J, Singh K. Rapid antigen tests for diagnosis of pandemic (Swine) influenza A/H1N1. ***Clin Infect Dis*** 2009; 49: 1090-1093.
24. Ginocchio CC, Zhang F, Manji R, Arora S, Bornfreund M, Falk L, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. ***J Clin Virol*** 2009; 45: 191-195.
25. Hurt AC, Baas C, Deng YM, Roberts S, Kelso A, Barr IG. Performance of influenza rapid point-of-care tests in the detection of swine lineage A(H1N1) influenza viruses. ***Influenza Other Respi Viruses*** 2009; 3: 171-176.
26. Foo H KJ, Blyth CC, Iredell J, Dwyer DE. An evaluation of a rapid antigen test and two influenza PCRs during co-circulation of pandemic influenza A/H1N1 09 and seasonal influenza. Program and abstracts of the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). 2009 September 12-15. San Francisco, California, USA. Washington, (DC): ASM Press; 2009.
27. Chan KH, Lam SY, Puthavathana P, Nguyen TD, Long HT, Pang CM et al. Comparative analytical sensitivities of six rapid influenza A antigen detection test kits for detection of influenza A subtypes H1N1, H3N2 and H5N1. ***J Clin Virol*** 2007; 38: 169-171.
28. Slepushkin AN, Obrosova-Serova NP, Schastny EI, Pugaeva VP, Chernetsov IuV. [Immune structure of the population of Moscow to the strains of the influenza viruses A(H3N2), A(H1N1) and B circulating in 1977]. ***Vopr Virusol*** 1979; 2: 175-180.
29. Mathur U, Bentley DW, Hall CB, Roth FK, Douglas RG Jr. Influenza A/Brazil/78(H1N1) infection in the elderly. ***Am Rev Respir Dis*** 1981; 123: 633-635.
30. Lee BW, Bey RF, Baarsch MJ, Emery DA. Subtype specific ELISA for the detection of antibodies against influenza A H1N1 and H3N2 in swine. ***J Virol Methods*** 1993; 45: 121-136.
31. Barbé F, Labarque G, Pensaert M, Van Reeth K. Performance of a commercial Swine influenza virus H1N1 and H3N2 antibody enzyme-linked immunosorbent assay in pigs experimentally infected with European influenza viruses. ***J Vet Diagn Invest*** 2009; 21: 88-96.
32. Rocha EP, Xu X, Hall HE, Allen JR, Regnery HL, Cox NJ. Comparison of 10 influenza A (H1N1 and H3N2) haemagglutinin sequences obtained directly from clinical specimens to those of MDCK cell- and egg-grown viruses. ***J Gen Virol*** 1993; 74 (Pt 11): 2513-2518.
33. Cheng X, Li L, He J. [Characterization of HA1 genes of influenza A (H1N1) viruses isolated in Shenzhen City]. ***Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*** 1999; 13: 340-344.
34. Cooper LA, Subbarao K. A simple restriction fragment length polymorphism-based strategy that can distinguish the internal genes of human H1N1, H3N2, and H5N1 influenza A viruses. ***J Clin Microbiol*** 2000; 38: 2579-2583.
35. Daum LT, Canas LC, Schadler CA, Ujimori VA, Huff WB, Barnes WJ, et al. A rapid, single-step multiplex reverse transcription-PCR assay for the detection of human H1N1, H3N2, and B influenza viruses. ***J Clin Virol*** 2002; 25: 345-350.
36. Al-Faress S, Cartet G, Ferraris O, Norder H, Valette M, Lina B. Divergent genetic evolution of hemagglutinin in influenza A H1N1 and A H1N2 subtypes isolated in the south-France since the winter of 2001-2002. ***J Clin Virol*** 2005; 33: 230-236.
37. Maldonado J, Van Reeth K, Riera P, Sitja M, Saubi N, Espuna E, et al. Evidence of the concurrent circulation of H1N2, H1N1 and H3N2 influenza A viruses in densely populated pig areas in Spain. ***Vet J*** 2006; 172: 377-381.
38. Chutinimitkul S, Chieochansin T, Payungporn S, Samransamruajkit R, Hiranras T, Theamboonlers A, et al. Molecular characterization and phylogenetic analysis of H1N1 and H3N2 human influenza A viruses among infants and children in Thailand. ***Virus Res*** 2008; 132: 122-131.
39. Sinha NK, Roy A, Das B, Das S, Basak S. Evolutionary complexities of swine flu H1N1 gene sequences of 2009. ***Biochem Biophys Res Commun*** 2009; 390: 349-351.

40. Centers for Disease Control and Prevention (CDC). Update: novel influenza A (H1N1) virus infections - worldwide, May 6, 2009. ***MMWR Morb Mortal Wkly Rep*** 2009; 58: 453-458.
41. He J, Bose ME, Beck ET, Fan J, Tiwari S, Metallo J et al. Rapid multiplex reverse transcription-PCR typing of influenza A and B virus, and subtyping of influenza A virus into H1, 2, 3, 5, 7, 9, N1 (human), N1 (animal), N2, and N7, including typing of novel swine origin influenza A (H1N1) virus, during the 2009 outbreak in Milwaukee, Wisconsin. ***J Clin Microbiol*** 2009; 47: 2772-2778.
42. Steain MC, Dwyer DE, Hurt AC, Kol C, Saksena NK, Cunningham AL, et al. Detection of influenza A H1N1 and H3N2 mutations conferring resistance to oseltamivir using rolling circle amplification. ***Antiviral Res*** 2009; 84: 242-248.
43. Hawker J, Begg N, Blair I, Reintjes R, Weinberg J. Communicable disease control handbook. United Kingdom: Blackwell Science; 2005 p. 241-286
44. Webber R. Communicable disease epidemiology and control. United Kingdom: CABI Publishing; 2005. p. 166-180
45. Wicker S, Rabenau HF, Gottschalk R. Influenza pandemic: Would healthcare workers come to work? An analysis of the ability and willingness to report to duty. ***Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*** 2009; 52: 862-869.
46. Radonovich LJ Jr, Perl TM, Davey V, Cohen H. Preventing the soldiers of health care from becoming victims on the pandemic battlefield: respirators or surgical masks as the armor of choice. ***Disaster Med Public Health Prep*** 2009; 3 Suppl 2: S203-S210.
47. Centers for Disease Control and Prevention. H1N1 Flu (Swine Flu). (15 January 2010; Accessed 2009 October 5). Available from URL: [http://www.cdc.gov/swineflu]
48. Lessler J, Cummings DA, Fishman S, Vora A, Burke DS. Transmissibility of swine flu at Fort Dix, 1976. ***J R Soc Interface*** 2007; 4: 755-762.
49. Tenpenny S. The Truth About the Flu Shot. (2009 November 28; Accessed 2009 October 5. Available from URL: [http://drtenpenny.com/the_truth_about_the_flu_Shot.aspx]
50. Centers for Disease Control and Prevention. H1N1 Flu (Swine Flu) (Update 2010 January 15; Accessed 2009 October 5) Available from URL: http://www.cdc.gov/H1N1FLU/
51. Centers for Disease Control and Prevention. Influenza: The Disease. (Update 2009 October 14; Accessed 2009 October 4). Available from URL: http://www.cdc.gov/flu/about/disease/index.htm
52. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. ***N Engl J Med*** 2009; 361: 2424-2435.
53. De Groot AS, Ardito M, McClaine EM, Moise L, Martin WD. Immunoinformatic comparison of T-cell epitopes contained in novel swine-origin influenza A (H1N1) virus with epitopes in 2008-2009 conventional influenza vaccine. ***Vaccine*** 2009; 27: 5740-5747.
54. Cohen J. Swine flu outbreak. Past pandemics provide mixed clues to H1N1's next moves. ***Science*** 2009; 324: 996-997.
55. Michaelis M, Doerr HW, Cinatl J Jr. An Influenza A H1N1 Virus Revival - Pandemic H1N1/09 Virus. ***Infection*** 2009; 18. [Epub ahead of print]
56. Butler D. Swine flu goes global. ***Nature*** 2009; 458: 1082–1083
57. Sylte MJ, Suarez DL. Influenza neuraminidase as a vaccine antigen. ***Curr Top Microbiol Immunol*** 2009; 333: 227-224.
58. Centers for Disease Control and Prevention (CDC). Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. ***MMWR*** 2009; 58: 521-524.
59. Dawood FS, Jain S. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. ***N Engl J Med*** 2009; 360: 2605-2615.
60. Centers for Disease Control and Prevention. Update: drug susceptibility of swine origin influenza A (H1N1) viruses, April 2009. ***MMWR Morb Mortal Wkly Rep*** 2009; 58: 433-435.
61. Centers for Disease Control and Prevention. Pregnant Women and Novel Influenza A (H1N1) Virus: Considerations for Clinicians (Update 2009 June 30; Accessed 2009 September 30). Available from URL: http://www.cdc.gov/H1N1flu/clinician_pregnant.htm
62. Centers for Disease Control and Prevention. What Should Pregnant Women Know About 2009 H1N1 Flu (Swine Flu)? (Update 2009 October 19, Accessed 2009 September 30). Available from URL: http://www.cdc.gov/h1n1flu/guidance/pregnant.htm
63. Centers for Disease Control and Prevention. Interim Guidance on Infection Control Measures for 2009 H1N1 Influenza in Healthcare Settings, Including Protection of Healthcare Personnel (Update 2009 October 14, Accessed 2009 September 30). Available from URL: http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm
64. Tanaka T, Nakajima K, Murashima A, Garcia-Bournissen F, Koren G, Ito S. Safety of neuraminidase inhibitors against novel influenza A (H1N1) in pregnant and breastfeeding women (review). ***CMAJ*** 2009; 18: 55-58.
65. Centers for Disease Control and Prevention. Interim guidance for use of 23-valent pneumococcal polysaccharide vaccine during novel influenza A (H1N1) outbreak (Update 2009 October 26, Accessed 2009 September 2009). Available from URL: http://www.cdc.gov/h1n1flu/guidance/ppsv_h1n1.htm