CMR
Journals.ASM.org

Two Years after Pandemic Influenza A/2009/H1N1: What Have We Learned?

Vincent C. C. Cheng,[a,b] Kelvin K. W. To,[a,c] Herman Tse,[a,c] Ivan F. N. Hung,[c,d] and Kwok-Yung Yuen[a,c]

Department of Microbiology, Queen Mary Hospital, Hong Kong Special Administrative Region, China[a]; Infection Control Team, Queen Mary Hospital, Hong Kong Special Administrative Region, China[b]; State Key Laboratory for Emerging Infectious Diseases, Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong Special Administrative Region, China[c]; and Department of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China[d]

INTRODUCTION 223
TAXONOMY, NOMENCLATURE, AND GENERAL VIROLOGY 224
VIRAL LIFE CYCLE 227
CELL ENTRY AND VIRULENCE FACTORS 227
EPIDEMIOLOGY 229
  Animal Surveillance 229
  Mathematical Modeling 229
  Seroepidemiology 229
  Risk Factors for Severe Disease 231
HISTOPATHOLOGY AND PATHOGENESIS 232
  Histopathological Changes in the Respiratory Tract 233
  Histopathological Changes in Extrapulmonary Sites 233
  Host Cytokine Profile 233
  Host Immune Response 233
CLINICAL MANIFESTATIONS 238
CLINICAL MANAGEMENT AND LABORATORY DIAGNOSIS 238
  Specimen Collection 239
  Nucleic Acid Amplification 240
  Antigen Detection 240
  Viral Culture 240
  Antibody Testing 243
  Antiviral Susceptibility Testing 243
ANTIVIRAL AND IMMUNOMODULATORY THERAPIES 244
  Antiviral Treatment 244
  Antiviral Resistance 244
  Safety of Antivirals 245
  Passive Immunotherapy 245
  Controversial Use of Steroids and Other Immunomodulators 246
EXTRACORPOREAL MEMBRANE OXYGENATION 247
VACCINES 248
INFECTION CONTROL IN THE COMMUNITY AND HOSPITALS 249
CONCLUSION 250
ACKNOWLEDGMENTS 250
REFERENCES 250

INTRODUCTION

Previous pandemic influenza viruses involved an antigenic shift to a different subtype. However, the antigenic shift demonstrated by the pandemic influenza A H1N1 2009 virus (A/2009/H1N1) was an antigenic change from a human H1N1 subtype to a swine H1N1 subtype. Pig surveillance programs have not been able to detect the immediate precursor of this virus in pigs in South China and other parts of the world (51). Although South China was predicted to be the starting point of influenza pandemics, the first human case and the initial epidemic were detected in North America and Mexico, respectively (140, 184). Unlike the poor predictability of pig surveillance, human seroepidemiology correctly predicted an impending pandemic due to the lack of immunity in the general population with relative protection in the elderly (387). However, seroepidemiology and laboratory studies were unable to make an accurate assessment of the disease severity in order to recommend a commensurate pandemic alert level. Regarding the risk factors for severe disease, obesity was an important predisposing factor, in addition to extremes of age, pregnancy, and underlying medical illness (364, 458, 522). In patients with severe disease, viral clearance was delayed, with a persistent elevation of proinflammatory cytokines and associated multiorgan damage despite antiviral therapy (510). Additionally, a lower serum IgG2 level appeared to be associated with disease severity, especially in pregnant patients (80, 207). Severe disease and lung pathology were associated with immune complex deposition. In terms of laboratory diagnosis, a comparative laboratory test eval-

Address correspondence to Kwok Yung Yuen, kyyuen@hkucc.hku.hk.
Copyright © 2012, American Society for Microbiology. All Rights Reserved.
doi:10.1128/CMR.05012-11

uation showed that a rapid diagnosis was best achieved by reverse transcription-PCR (RT-PCR), which was markedly superior to antigen detection by enzyme immunoassays (EIAs) (296). None of the viral genomic signatures, such as PB2-K627, full-length PB1-F2, and the PDZ motif of NS1, which were previously speculated to be useful in predicting the virulence of the influenza A virus were present in this pandemic virus (293). Only the hemagglutinin D222G (H1 numbering) mutation with a predilection for α-2,3-linked sialic acid was associated with clinically severe disease and the involvement of the lower respiratory tract (96). In addition, the similar crystal structures, antigenic makeups, and patterns of glycosylation for the hemagglutinin of this virus and that of the 1918 virus explained the relative protection of the elderly through the induction of cross-reactive humoral and cellular immune responses against the surface and internal proteins, respectively. As for treatment options, the intrinsic resistance to adamantanes leaves the early initiation of neuraminidase inhibitors as the only option available in most countries. Moreover, further mutations may affect the usefulness of these antivirals. In severe cases, intravenous peramivir or zanamivir, convalescent-phase plasma, and hyperimmune intravenous immunoglobulin can be considered in clinical trial settings. Despite the technological advances in using cell-based inactivated whole-virus vaccines and improved adjuvants, vaccine production failed to prevent the first peak in tropical areas and the Southern Hemisphere. The bottlenecks for the rapid mass production of vaccines must be overcome before the next pandemic. Social distancing methods, such as canceling entertainment and sporting events, closing stores, office buildings, and public transportation systems, border screening, the isolation and quarantine of febrile patients and contacts, school closures, and hospital infection control measures may achieve only a few more weeks of preparedness by slowing down the introduction and spread of the pandemic virus if instituted early enough. In this article, we review the biology of the virus in relation to the clinical manifestations, pathogenesis, laboratory diagnosis, host susceptibility, immune response, and options for treatment, immunization, public health, and infection control. Because there have been a large number of publications on this topic, we can select only those publications related to the understanding and practice of clinical microbiology and infectious diseases.

TAXONOMY, NOMENCLATURE, AND GENERAL VIROLOGY

The influenza virus is a negative-sense, single-stranded RNA virus with a lipid-containing envelope and an eight-segment genome encoding 11 or 12 proteins (545). The pleomorphic viral particles observed in cell culture or clinical specimens may vary from spherical to filamentous in shape, with a diameter of 120 nm. The A/2009/H1N1 virus produces filamentous viral particles when grown in cell lines examined by electron microscopy (274). This virus belongs to the family *Orthomyxoviridae*, which includes the genera of influenza virus, Thogoto virus, and infectious salmon anemia virus (155, 424). The genus of influenza virus is divided into types A, B, and C as defined by the antigenicity of the nucleocapsid and matrix proteins in the viral core (424). These proteins are the antigens used in the complement fixation test for type-specific antibodies (250). Among the three genera, the influenza A virus is associated with more severe disease and pandemics in humans (234, 569). The influenza A virus is further subtyped by two surface proteins, hemagglutinin (H), which attaches the virion to the host cell for cell entry, and neuraminidase (N), which facilitates the spread of the progeny virus by cleaving the host sialic acid receptors attached to the progeny virus (137). There are 16 H subtypes and 9 N subtypes, which make up all of the subtypes of the influenza A virus by various combinations of H and N (180). The infidelity of RNA polymerase (one mutation per genome per replication) and the selective pressure of host immunity lead to the accumulation of mutations and a change in the surface antigenicity of these surface proteins, which are the targets of neutralizing and hemagglutination-inhibiting antibodies (2). This antigenic change is called antigenic drift (424). Thus, every year in February, the World Health Organization (WHO) selects the strains of virus that are to be used for the annual influenza vaccination in humans according to this antigenic drift. The nomenclature of the viral strain is written in the following order: type/place of isolation/strain number/year of isolation(subtype). Using the year 2005 as an example, the WHO recommended changing the virus strain from influenza A/Fujian/411/2003(H3N2) to A/California/7/2004(H3N2) for vaccination purposes. These changes are necessary because the hemagglutination inhibition antibody titers in the general population that would be induced by the older vaccine strain would not be sufficient to provide good protection against a newly drifted or, in the case of a pandemic, newly shifted strain (429). All of the combinations of the 16 H and 9 N viral subtypes are found in water fowl, whereas the H1 to H3 and N1 and N2 viral subtypes are commonly found in humans infected with influenza (4). Because of its segmented genome, the shuffling of gene segments can occur if two different subtypes of the influenza A virus infect the same cell. This genetic reassortment of the gene segments from different animals can lead to a major surface antigenic change, resulting in a new reassorted virus to which the global population has no effective neutralizing antibodies. A pandemic may ensue if this new virus has preserved replicative efficiency in human cells and transmissibility between humans.

A phylogenetic analysis of the A/2009/H1N1 virus suggested that the ancestors of the 8 gene segments are different and can be traced to avian, human, and swine origins (481). In previous pandemic viruses, the H subtypes shifted from H1 to H2 in 1957 and H2 to H3 in 1968, whereas the 2009 pandemic virus had North American swine H1 and Eurasian swine N1, replacing the circulating human seasonal H1N1 virus subtype (472). Using all of the available H1N1 virus sequences in GenBank, phylogenetic analysis suggested that the A/2009/H1N1 virus strains, and the 1976 New Jersey swine strains, and the 1918 pandemic H1N1 strains are distinct and diverge from the human seasonal H1N1 strains circulating since 1918 (Fig. 1). This form of genetic shift is different from those of the 1957 pandemic H2N2 virus, which evolved from the circulating H1N1 virus by acquiring the H2, N2, and PB1 genes from avian species (301), and the 1968 pandemic H3N2 virus, which is likely to have evolved from the circulating H2N2 virus by acquiring the H3 and PB1 genes from avian species (173, 301). Thus, animal surveillance programs for influenza virus will continue to play an important role in the detection of the immediate precursor virus in animals before it becomes a pandemic virus in humans. However, the vast geographic distribution of pig and poultry farming, especially in developing countries, presents an enormous challenge for surveillance programs to detect such a precursor virus before a pandemic begins.

The 8 gene segments of the influenza A virus genome, encoding 11 to 12 viral proteins with various functions in the life cycle, often

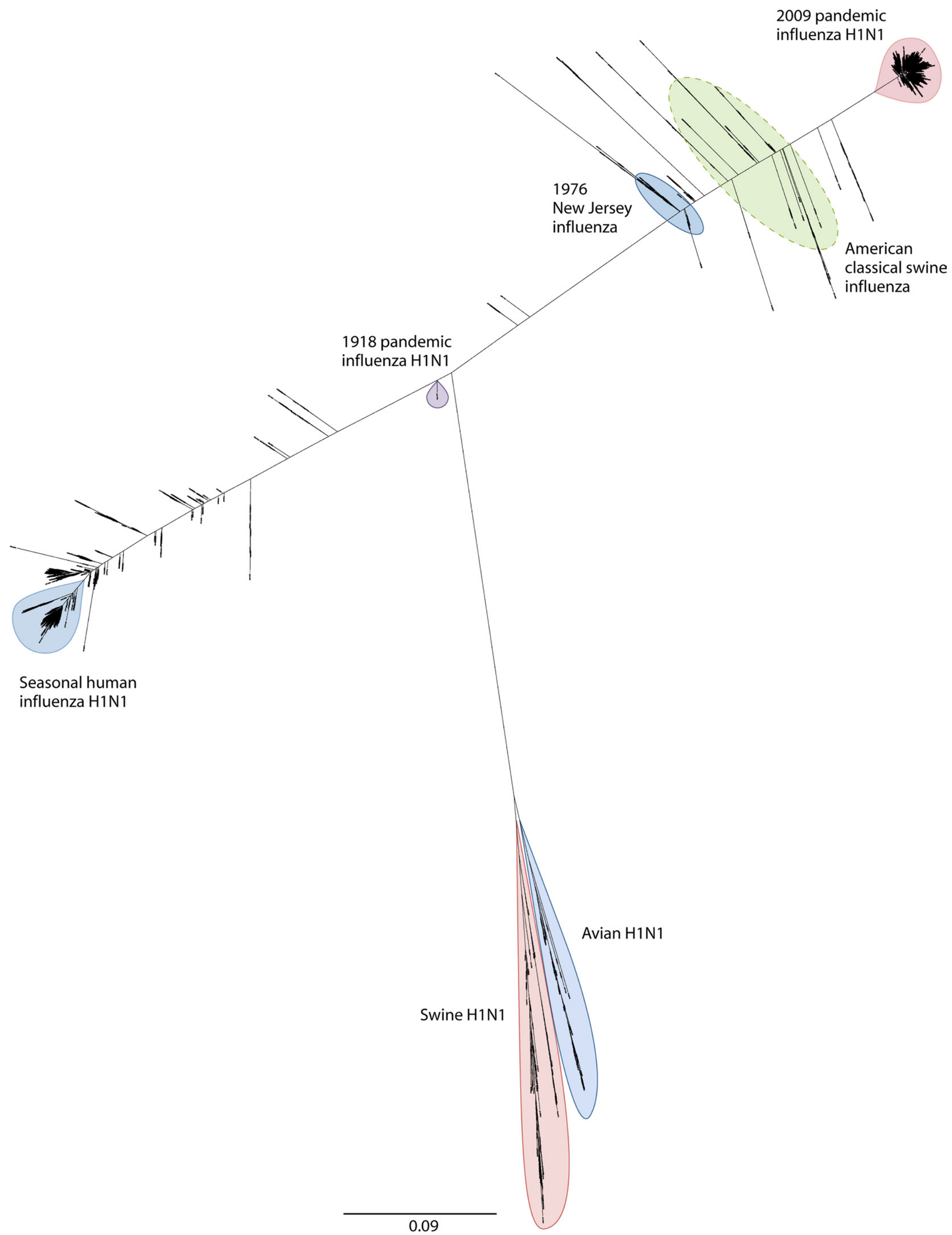
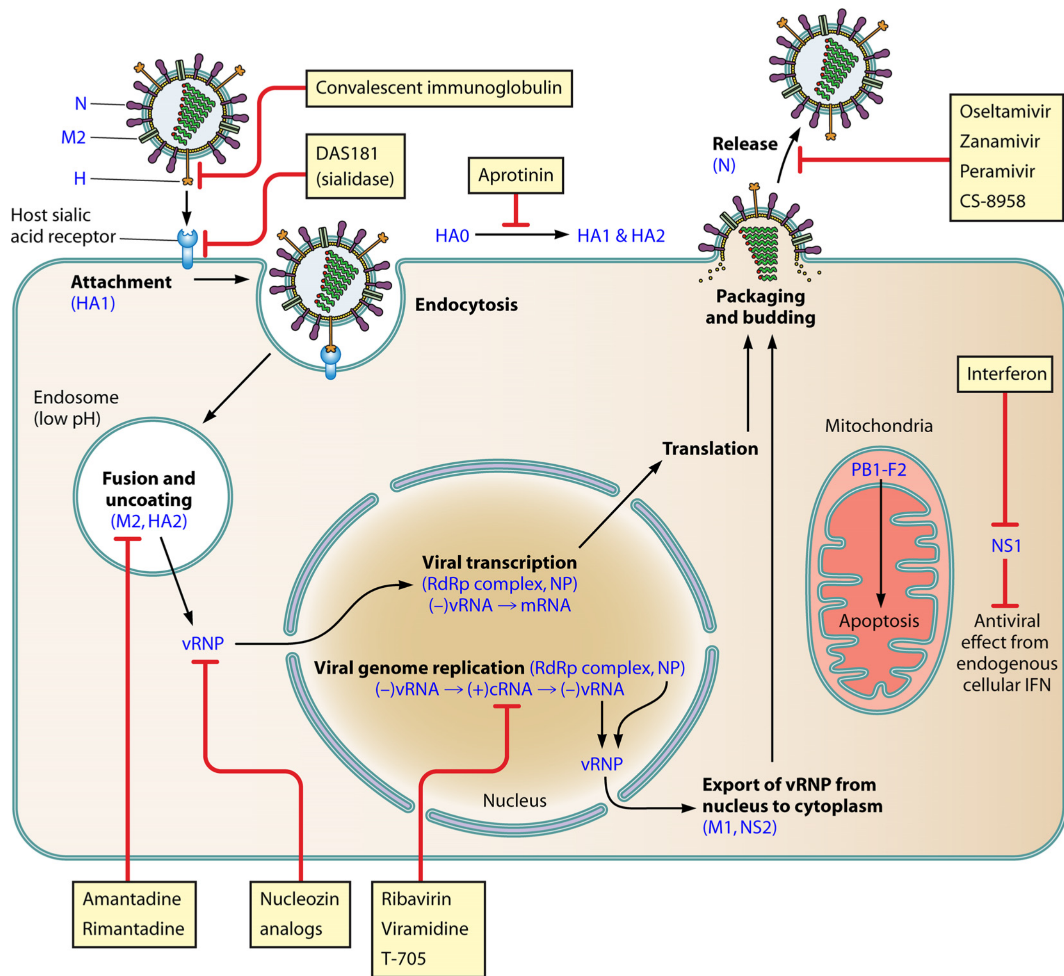


FIG 1 Maximum-likelihood phylogenetic tree of 3,764 full-length hemagglutinin nucleotide sequences of A/2009/H1N1 strains from the NCBI Influenza Virus Resource. Phylogenetic reconstruction was performed using RAxML version 7.2.6. Positions of 1918 pandemic, 1976 New Jersey swine influenza, seasonal, and 2009 pandemic human influenza A virus strains are highlighted.

serve as antiviral and diagnostic targets (Fig. 2). Three gene segments encode the polymerase proteins PB1, PB2, and PA and two smaller proteins, PB1-F2 and N40. The polymerase proteins are antiviral targets of ribavirin, viramide, and T-705 (141, 312). PB2 is necessary for cap binding and endonuclease activity but can also inhibit the induction of type I interferon (276). PA functions as an RNA polymerase subunit and in proteolysis (441). PB1-F2 is a mitochondrial toxin that causes cellular apoptosis and inhibits the induction of type I interferon (524). Additionally, a newly identified protein of unknown function, N40, is expressed from the PB1 fragment (545).

Nucleoprotein (NP) can be an antiviral target of nucleozin and its analogs (295). NP is the structural component of ribonucleoprotein (RNP). H is the target for neutralizing antibodies and has



Diagnostic test (include antiviral susceptibility testing)

H – Hemagglutination inhibition and viral neutralizing antibody assay for strain- or subtype-specific antibody; D222G (marker of higher virulence); precursor polypeptide HA0 is proteolytically cleaved into HA1 and HA2 for cell entry.
N – Oseltamivir resistance (H275Y); zanamivir resistance (S247N, I223R, I223K)
M2 – Amantadine resistance (S31N)
M1, NP – RT-PCR and type specific antibody testing by complement fixation test for type specific antibody

Inhibitory action

FIG 2 Potential diagnostic and antiviral targets in the viral life cycle of influenza virus.

a precursor form called HA0 that undergoes proteolytic cleavage into two components, HA1 and HA2. HA1 functions in the attachment of the virus onto the sialic acid receptor, and HA2 is responsible for membrane fusion. H has 4 major antigenic domains, with frequent mutations in 5 hypervariable regions at HA1. N is the antiviral target of oseltamivir, zanamivir, and peramivir (243). N serves as a sialidase that is required for the release of progeny virions (425). N binds plasminogen for the activation of H by proteolytic cleavage (208). The matrix protein M1 is a structural protein for the nuclear export of viral RNA (vRNA) and viral budding, whereas M2 functions as an ion channel for the acidification of the viral core during the uncoating of virus in endosomes. M2 is the antiviral target of the adamantanes, which include amantadine and rimantadine. In addition, M2 induces heterotypic antibodies associated with protection. The nonstructural protein NS1 is an interferon antagonist that may also stimulate proinflammatory cytokines in infected cells, whereas NS2 is a nuclear export factor.

In addition to the spikes in the H and N proteins that cover the surface of the virion (402), the M2 protein is embedded in the lipid envelope with its ectodomain exposed on the surface (444). Below the lipid envelope is a layer of the M1 protein that surrounds the RNP core (573). The core consists of 8 RNA segments that are associated with one or more copies of the viral polymerase complex and covered by NP molecules (16). Recent studies suggested that the genetically highly conserved M2 ectodomain and the stalk region of HA2 could be the antigenic targets of neutralizing antibodies for the production of a universal influenza A vaccine (37, 483). Glycosylation has been associated with the masking of epitopes on H, and the A/2009/H1N1 virus has significantly reduced glycosylation compared to the previous seasonal influenza H1N1 virus (563). This finding may partly explain why a single dose of the A/2009/H1N1 vaccine was sufficient enough to induce immunity in immunologically naïve individuals.

The pandemic influenza virus behaves like other influenza viruses, which are infectious for more than 24 h under low (<25%)

and high (>80%) relative humidity but less stable at intermediate humidity (50%) and higher temperatures (366, 490). Thus, the virus should be most stable in cold winters. Unexpectedly, the A/2009/H1N1 virus emerged in the spring in North America and spread during the summer in tropical areas and the Southern Hemisphere. This virus produced a larger second wave during the early autumn in the Northern Hemisphere. Thus, the pressure of seasonality on transmission was overwhelmed by the lack of herd immunological control. The virus can be readily inactivated by detergents, disinfectants, ionizing radiation, or temperatures greater than 50°C (287).

VIRAL LIFE CYCLE

The virus initiates its life cycle when H attaches to the receptors on a cell surface that contains sialic acid, such as glycolipids or membrane glycoproteins. Human influenza viruses, including the A/2009/H1N1 virus, preferentially bind to sialic acid with an α-2,6-linkage to galactose, which contains oligosaccharides that are abundantly present in the upper respiratory tract (473). In contrast to the other human seasonal influenza A/H1N1 virus, the A/2009/H1N1 virus also binds to the α-2,3-linked sialic acid receptors that are present in the lower respiratory tract (113). The internalization of the virus into endosomes occurs by endocytosis, which is mediated by epsin-1, a cargo-specific adaptor for virus entry through the clathrin-mediated pathway or by the clathrin-independent pathway (91). The precursor polypeptide HA0 must be activated by proteolytic cleavage into HA1 and HA2 by trypsin or a trypsin-like endogenous host cell protease before it can undergo an acid pH-triggered conformational change into a fusogenic form (97). Then, the viral and endosomal membranes can be fused. The ion channel M2 in the viral membrane is then activated by endosomal acid pH, resulting in an influx of protons into the virion and the dismantling of M1 from the RNP core (465). The dismantling process will release the RNP into the cytoplasm. PB2 and NP both have a nuclear localization signal (NLS) and can bind to importin α_1 (a cellular nuclear import factor), the most abundant importin in human cells (189). Next, the RNP enters through the nuclear pore into the nucleus, where the transcription and replication of the viral genome occur (254). This nuclear localization of influenza virus NP results in the egg yolk appearance of an infected cell that is stained by an immunofluorescent antibody against the NP in clinical specimens, such as nasopharyngeal aspirate. Moreover, blocking the trafficking of the viral NP from the cytoplasm into the nucleus by nucleozin analogs was shown to be a viable antiviral mechanism (295). The nuclear viral RNP then becomes the template for the production of mRNA that snatches its 5′ cap of 9 to 15 nucleotides from host mRNA (320, 460). In addition, this viral RNP is the template for the full-length complementary copy that becomes a template for the amplification of viral RNA genomes. With an NLS, viral PB1, PB2, PA, and NP are transported into the nucleus, where they associate to form RNP (290, 397, 406, 413). Both vRNA and cRNA complexed with RNP have a panhandle/fork/corkscrew structure (178). The export of viral RNP back to the cytoplasm depends on M1, which binds to the RNP and interacts with NS2, which has a nuclear export signal (471). Under the cytoplasmic membrane, the RNP-M complex assembles under patches of H and N to form virions (499). The packaging of vRNA in viral particles depends on the 3′ and 5′ noncoding regions with *cis*-acting signals (268). Additionally, the coding sequences of the viral RNA contribute to the efficient packaging, but the exact mechanism is unknown (401). The budding of infectious progeny virions is followed by their release from their aggregation by their viral H bound to sialic acid, which is mediated by the sialidase activity of N. Because the mRNAs of H, M, NP, and NS are more abundantly expressed than the PA, PB1, PB2, and N genes, RT-PCR diagnostic tests are often targeted against the M gene, which is abundant and genetically conserved, and the H gene, which is abundant and subtype specific (153, 331, 447). The most abundant protein that is expressed in infected cells and constitutes the structure of a free virion is the NP (448). Thus, most EIAs for the influenza virus antigen are targeted toward the NP, which is type specific, but these tests cannot differentiate between subtypes.

CELL ENTRY AND VIRULENCE FACTORS

Virus-containing droplets may settle on nasopharyngeal, tracheobronchial, conjunctival, or other respiratory mucosal epithelia. The virus moves randomly through the mucus layer of the mucosa with the aid of neuraminidase to find the appropriate cell receptor for binding by viral H. The α-2,6-linked sialic acid receptors are present in both the upper and lower respiratory tracts of humans, the latter of which are lined by tracheobronchial pseudocolumnar epithelium and type 1 pneumocytes. The α-2,3-linked sialic acid receptors are present in the distal bronchiole, type 2 pneumocytes, and alveolar macrophages of humans (473). The H of the A/2009/H1N1 virus adheres to the α-2,6-linked sialic acid receptors and to the α-2,3-linked sialic acid receptors, which are more highly expressed in the human lower respiratory tree. The latter binding affinity is similar to that of the H subtypes of the avian influenza A virus, such as the H5N1, H9N2, or H7N7 subtype, which also preferentially attach to the α-2,3-linked sialic acid receptors that are present on the respiratory and alimentary epithelia of birds, human conjunctiva, and the ciliated portion of human respiratory pseudostratified columnar epithelium; therefore, this binding affinity allows for the sporadic jumping of avian influenza viruses into humans (Fig. 3). Moreover, this binding affinity allows the A/2009/H1N1 virus to infect and replicate to high titers in the lungs of ferrets, mice, and monkeys, with a greater proinflammatory response than for seasonal influenza virus. Nevertheless, the damage caused by the A/2009/H1N1 virus is less than that of the H5N1 virus (274, 369, 400, 520). This finding may partially explain the predilection of this new virus to cause pneumonia in healthy individuals (113). However, this *in vitro* glycan binding phenomenon has not been replicated in other studies (41, 99, 354, 369, 564, 567).

In addition to its pathogenetic significance, the differential binding affinity of H for different receptors affects the evolution of influenza virus. The respiratory epithelium of pigs contains both α-2,6- and α-2,3-linked sialic acid receptors; therefore, pigs can be infected by human, swine, and avian influenza viruses. Because pigs live in close proximity to humans and poultry in residential backyards in Southeast Asia, they can theoretically serve as "mixing vessels" for gene reassortment between avian, swine, and human influenza viruses, which may create future pandemics.

The conserved amino acid residues within the influenza virus H receptor binding site that are implicated in receptor specificity include amino acid positions 98, 136, 153, 183, 190, 194, 222, 225, 226, 227, and 228. Remarkably, the only difference between the 1918 H1 of the A/New York/1918/H1N1 virus and an avian H consensus receptor binding signature is a single E190D mutation

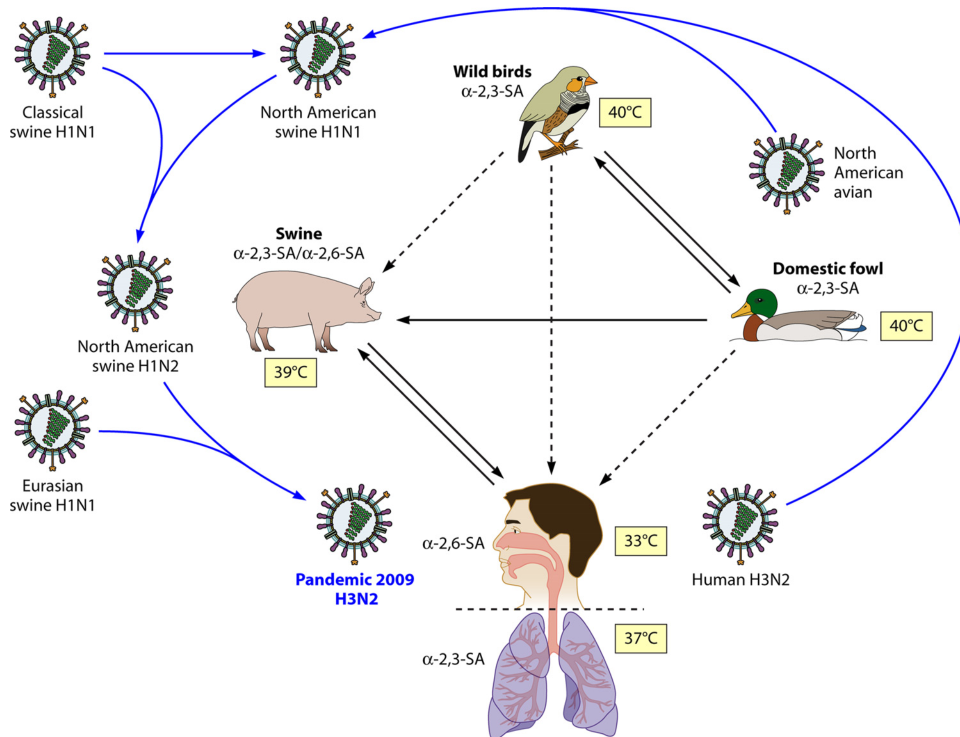


FIG 3 Emergence of A/2009/H1N1 virus from other human and animal influenza viruses. Influenza viruses are usually limited to infecting specific hosts, with tissue tropism and receptor specificity being important restriction factors. A change in tropism sometimes occurs, with pigs being an important "mixing vessel" due to their tracheae containing receptors with both α-2,3-linked and α-2,6-linked sialic acid moieties. In the case of A/2009/H1N1 virus, sequential reassortment of genes from human, avian, and swine influenza viruses culminates in a virus with replication competence comparable to those of other human influenza viruses.

(H3 numbering) (476). The H crystal structure of the A/2009/H1N1 virus reveals a close resemblance to the 1918 H1N1 virus (563). The H1 of the A/South Carolina/1918/H1N1 virus has an additional G225D (H3 numbering) substitution, which is sufficient to switch the receptor preference from α-2,3 to α-2,6 in cell-based assays (501). The A/2009/H1N1 virus possesses a "human virus"-type amino acid at positions 190 and 225 (222 by H1 numbering), which most likely supports the efficient transmissibility of these viruses in humans. In addition to these two positions, a difference in only two other amino acids, at positions 200 and 227, differentiated the receptor binding specificities of the swine H1 virus and the pandemic H1 virus (149). When the A/2009/H1N1 virus was compared with the classical swine virus, the most discriminative positions were all located in the receptor binding pocket, suggesting that these positions may be important in the human adaptation of the virus (386). Interestingly, some isolates from patients with severe disease, especially in lower respiratory tract specimens, possess an amino acid substitution at position D222G (H1 numbering) (96), and D222G is associated with viremia (516). The D222G mutant has been readily generated in mouse adaptation experiments that demonstrated a significant increase of virulence in mice (580). Additionally, the D222G mutant has been shown to have stronger binding to turkey erythrocytes than wild-type virus (85). Homology modeling and molecular docking showed that the receptor binding pocket of the A/2009/H1N1 virus is smaller than those of other influenza A viruses, which allows for a tighter binding of the virus with the receptor. The amino acid at position 222 of H may affect the positioning of the conserved Q223 residue, hence modulating the flexibility of the binding pocket and steric hindrance during receptor binding. In addition, the molecular property of residue 222 can directly influence the "lysine fence" via the polarity of the amino acid residue, where the D222G substitution enhances the electrostatic interactions between the receptor and the protein (515). Furthermore, the lysine fence at the H of the A/2009/H1N1 virus, which is a positively charged structure composed of K145, K133, and K222, favors the binding of the virus to both α-2,3- and α-2,6-glycans (487).

Viruses that possess PB2-K627 but not viruses that possess PB2-E627 were previously observed to grow efficiently in the upper respiratory tracts of mammals (241), which suggests that PB2-K627 confers efficient replication at 33°C (the temperature of the upper airway in humans), whereas PB2-E627 does not. In contrast, both variants mediate efficient replication at 37°C. Collectively, these findings suggest that PB2-K627 allows for efficient replication in the lower and upper respiratory tracts of mammals, a feature that may facilitate transmission. Notably, the replacement of PB2-K627 with glutamic acid reduced the transmissibility of human influenza viruses in a guinea pig model (489). In addition, the amino acid substitution at position 701 of PB2 has emerged as a determinant of virulence, a role probably related to its facilitation of the binding of PB2 to importin in mammalian cells. The recently emerged A/2009/H1N1 virus possesses "low-pathogenic"-type amino acids at positions 627 and 701, i.e., glutamic acid and aspartic acid, respectively (426). However, these low-virulence pathotypes may be overcome by PB2-R591, which has been shown to confer efficient replication of the virus (566).

Other virulence-associated genomic signatures, such as the

full-length PB1-F2, the cleavage and polyadenylation specificity factor 30-kDa subunit (CPSF30), the E92 and PDZ domains of NS1, and the multibasic H cleavage sequence, which were previously speculated to be useful in predicting the severity of disease, are absent in the pandemic A/2009/H1N1 virus (193). Most virus strains have a truncated version of PB1-F2 that consists of 11 amino acids. Three isolates of the A/2009/H1N1 virus were reported to have PB1-F2, which consisted of 57 amino acids and was associated with an improvement in *in vitro* replication (421). In cell culture and in an animal model, the introduction of various virulence-associated mutations into PB2 generally did not enhance the viral titer or virulence of the A/2009/H1N1 virus (252). The only consistent viral signature associated with clinical severity, higher viral titer, and virulence in animal models is the D222G mutation (Table 1), which is likely due to its high affinity for binding to α-2,3-linked sialic acid receptors in the lower respiratory tract. However, these mutants may have diminished transmissibility between the upper respiratory tracts from host to host, which may have selected against their prevalence.

A comparison of the influenza viruses from different origins found that the residues E14 and F55 in the M2 proteins were predominantly in the human influenza virus but not in the swine or avian influenza viruses, suggesting that these residues may be important in human-to-human transmission (427). The M1 protein has been shown to be essential in the high transmissibility of the A/2009/H1N1 virus in a guinea pig model (118). Furthermore, a genomic analysis of the A/2009/H1N1 strains that were collected during the early prepandemic period showed that there have been multiple changes in the viral genome (382), and several mutations in NP (V100I), NS1 (I123V), N (V91I and N233D), and H (S206T) have emerged as the predominant type, from fewer than 11% of the strains collected from April 2009 to more than 75% of the strains collected from June to December 2009 (426). Because most of these mutant residues were located in the functional domain, it was proposed that these changes may enhance human adaptation of the virus or its virulence.

EPIDEMIOLOGY

When the chronology of the 2009 pandemic was examined (Table 2), it was found that there were many similarities with the severe acute respiratory syndrome (SARS) epidemic (105, 106, 109, 437, 438), except that the 2009 pandemic was considered to be mild in the first wave (449). The outbreak started in a developing country, Mexico, but the virus was first isolated in California, where the public health and laboratory infrastructures are well established (140). Although a phylogenetic analysis strongly suggested that swine would be the immediate animal host from which the virus jumped into humans, the immediate virus precursor could not be detected by animal surveillance programs. However, in analogy to the bat SARS coronavirus (332), a more distant precursor virus was found in swine. Fortunately, for the SARS epidemic, the immediate precursor virus of the human SARS coronavirus was found in civets, which were later banned in wet markets to prevent the relapse of the SARS epidemic (216). Paradoxically, the retrograde passage of the A/2009/H1N1 virus back into pigs was well documented with genetic reassortment (530). Though oseltamivir resistance or D222G viral mutation associated with severe disease was reported within 9 months, the proportions were very low (372). In contrast to the SARS coronavirus, which could be controlled by infection control measures and social distancing, the A/2009/H1N1 virus rapidly infected over 40% of the susceptible population and became endemic by replacing the previously circulating human seasonal H1N1 virus.

Animal Surveillance

The scientific community anticipates that a future influenza pandemic is likely to originate from animals; however, many scientists were surprised that the 2009 pandemic may have originated from swine (481) and not from avian populations, which had been severely affected by the H5N1 and H9N2 viruses since 1997, with occasional poultry-to-human transmission (25, 52, 103, 104, 214, 215, 439, 440, 576). In the prepandemic period, intensive screening was dedicated to poultry surveillance, especially in East Asia, where the lethal H5N1 first emerged (94, 214, 215, 351, 576) and where antigenically drifted H3N2 strains often emerged and spread throughout the world (462). There were several warning signals for the 2009 pandemic. First, a triple-reassortant swine influenza H1N1 virus that represented a major antigenic shift event, composed of genomes from the H1N1, H3N2, and H1N2 viruses, emerged in the swine population in the late 1990s (417). Second, this reassortant virus repeatedly jumped the species barrier and caused sporadic cases in humans, with 20% of these cases requiring invasive mechanical ventilation (408, 472). Increased surveillance of pigs was proposed immediately before the pandemic (212). However, the vast geographic extent of pig farming, especially in developing areas, makes pig surveillance difficult.

Mathematical Modeling

Mathematical modeling has been widely used to predict the spread of influenza virus and the effectiveness of containment strategies (342). Although it is useful as a conceptual model, prepandemic modeling assumed that the pandemic began in Southeast Asia (120, 174). During the current pandemic, real-time predictions of the spread of the pandemic have been performed but with only modest accuracy (19, 176). Mathematical modeling can estimate the worst- and best-case scenarios to gauge the magnitude of the pandemic only when a sufficient caseload is available to calculate the basic parameters for the modeling. However, the interval for data accumulation has diminished the predictive value of mathematical modeling and its impact on epidemiological control. There have been few examples of policy changes or epidemiological controls that were based directly on the findings of mathematical modeling.

Epidemiological studies of laboratory-confirmed cases of 2009 pandemic influenza (Table 3) showed that for community outbreaks, the clinical attack rate ranged from 5.4% to 20.6%, the reproductive number ranged from 1.1 to 3.1, the generation time ranged from 0.8 to 3.9 days, and the incubation period ranged from 2 to 5 days (20, 36, 65, 134, 154, 184, 205, 226, 262, 359, 416, 506, 543, 556, 558, 571). These figures did not differ from those in the epidemiological settings of households, schools, hospitals, and tours. No clear differences were observed in the studies conducted across different continents.

Seroepidemiology

Seroepidemiological studies have demonstrated that children and young adults were seronegative before 2009 and were more commonly infected than the elderly after the first wave (387). Compared with patients with seasonal influenza, those with the A/2009/H1N1 virus were younger and less likely to have underly-

TABLE 1 Viral mutations and characteristics associated with virulence of A/2009/H1N1 influenza virus[a]

Viral protein	Study design	Finding(s)	Reference
PB2	Ferret model; introduction of E627K and D701N into A/2009/H1N1	No enhancement of virulence	252
	Cell culture (A549) and mice; introduction of E627K and D701N into A/2009/H1N1	Both mutants grew to 1-2-log-lower peak titer than parental strain in A549 and had attenuated viral replication in mouse lung; E627K mutant displayed only rare focal inflammation, compared with focal bronchiolitis caused by parental strain	280
	Human (293T) and porcine cells; to study the mechanism for human adaptation for A/2009/H1N1 which carries avian type E627 in the PB2 subunit	Serine at position 590 and arginine at position 591 found important in the regulation of polymerase activity in human cells	381
	Mouse and ferret model; to study the effect of varying the amino acids at positions 591, 627, and 701 of PB2 in A/2009/H1N1	R591Q reduced polymerase activity, while E627K and D701N had only mild effects; in competition studies between wild-type virus and R591Q mutant, relative amounts of wild-type virus increased over time in infected ferrets	566
	Cell culture (MDCK, 293T, DF-1, A549)	271A responsible for high polymerase activity of the virus	50
	Mouse model; analysis of the effect of amino acid substitution	E158G/A enhanced transcription and replication activity	271
	Mouse and ferret model; to study the importance of position 591 of PB2	A basic amino acid at position 591 of PB2 can compensate for the lack of lysine at position 627 in pH1N1 for efficient viral replication in mammals	566
	Mouse model; selection of virulent mouse strain and comparison of the PB2 gene	Position 158 is a pathogenic determinant	584
	Mouse model	Substitution of 627K in PB2 gene does not confer higher virulence	589
H	Compare patients with fatal and nonfatal cases (worldwide)	Prevalence of D222G in fatal vs nonfatal cases (7.1% vs $<$1.8%); D222G enhanced binding to α-2,3-linked sialic acid cell receptors	553
	Analyze 189 respiratory specimens from influenza surveillance network in Spain	D222G found in 2 fatal cases and 1 patient with severe illness who recovered; D222E found in 61 samples; no mutations found at position 187	12
	Analyze 117 clinical specimens in Hong Kong	D222G found in 12.5% of patients with severe disease but not in patients with mild disease; D222G identified mainly in endotracheal samples; D222N found in 2 severe cases and in 1 mild case	96
	Analyze 11 specimens from 5 deceased patients and 9 specimens from 5 ICU patients in Canada	D222G found in 3 ICU patients but none in deceased patients; D222G was more frequent among lower respiratory tract specimens than nasopharyngeal specimens (45% vs 14%; $P = 0.18$)	156
	Analyze 61 severe cases and 205 mild cases in Norway	D222G found in 11/61 severe cases but not found in any mild cases ($P <$ 0.001); D222N was found in 3 severe cases and 1 mild case ($P = 0.039$); no difference in the frequency of D222E between severe and mild cases ($P = 0.072$)	304
	Analyze 219 severe and 239 nonsevere cases in Hong Kong	D222G found in 4.1% of severe cases, but not found in nonsevere cases ($P = 0.002$)	371
	Analyze 8 fatal cases, 23 severe pneumonia cases, and 13 mild cases in Greece	D222G found in 5/31 (16.1%) severe/fatal cases and 2/13 (15.4%) mild cases; S162R found in 1 patient with severe illness	382
	Analyze 23 fatal cases, 9 seriously ill cases, and 26 community cases in UK	D222G found in 8.7% of fatal cases but not found in seriously ill or community cases; D222N found in 22% of seriously ill patients but not found in fatal or community cases; no difference in frequency of D222E between severe and mild cases	388
	Analyze 130 patients (23 with severe disease) in Italy for D222G and describe the transmission of A/2009/H1N1 with D222G	Only 1 patient with severe disease infected with D222G virus; this virus was transmitted to the father of the index patient, who was "moderately ill" without hospitalization	451
	Mouse and ferret model	D222G caused ocular disease in mice but no enhanced virulence in mice or ferrets; D222G attached to a higher proportion of alveolar macrophages and type II pneumocytes	119
	Analyze 63 patients with A/2009/H1N1 infection	D222G in 17.4% of severe pneumonia and in 26.7% of ICU patients	89
	Analyze the H of A/2009/H1N1 from 273 severe cases and 533 nonsevere cases in Spain	D222G present in 5.12% of severe cases; D222E present in 17.21% of severe cases and 9.75% of nonsevere cases; D222N found in 3 severe cases	337
	Mouse model; to study the effect of D222G substitution on virulence	D222G was more lethal in chicken embryo and produced higher viral load; the mutant had a much lower 50% lethal dose than wild-type virus (1.5×10^2 PFU vs 2×10^6 PFU); mortality of mice due to mutant virus higher than that due to wild type ($P < 0.0001$)	580
	Mouse model; to compare the D222G mutant with wild-type virus	D222G caused more severe disease in both nonpregnant and pregnant mice	85
	Analyze the receptor binding characteristics by carbohydrate microarray analyses (neoglycolipid technology)	A/2009/H1N1 (A/California/4/2009 and A/Hamburg/5/2009) bound to both α-2,6- and α-2,3-linked sialyl sequences	113
	Infection of *in vitro* culture of human airway epithelium (HTBE) with D222G mutant virus; examine receptor binding characteristics by carbohydrate microarray analyses	D222G virus infected a higher proportion of ciliated cells; also bound to a broader range of α-2,3-linked sialyl sequences	360
	Analyze the receptor binding characteristics by use of recombinant H	A/2009/H1N1 binds only α-2,6-linked and not α-2,3-linked sialyl sequences	567
	Analyze the receptor binding characteristics by use of recombinant H	A/2009/H1N1 binds α-2,6-linked but only minimally binds α-2,3-linked sialyl sequences	369
	Analyze the receptor binding characteristics by use of recombinant H	Swine H1 binds α-2,6 residue better than H1 of A/2009/H1N1 due to difference in positions 200 and 227	149
	Analyze the receptor binding characteristics by use of recombinant H	Compared to 1930 H1N1 and PR8, A/2009/H1N1 has stronger binding to α-2,6 sialic acid	188
	Analyze the change of amino acid on H	A single point mutation (I219K) in the glycan receptor binding site quantitatively increases its human receptor binding affinity	285

(Continued on following page)

TABLE 1 (Continued)

Viral protein	Study design	Finding(s)	Reference
	Mouse model; evaluate the binding of H from different seasonal H1N1 strains, 1918 H1N1, and A/2009/H1N1, using chimeric viruses	Viruses expressing A/2009/H1N1 H were associated with significant pathology in the lower respiratory tract and showed low binding activity for surfactant protein D	452
	Analyze the change of amino acid on H	Q223R enhanced infectivity of H pseudotypes in 293T cells	535
	Evaluate the *in vitro* and *in vivo* effects of D222G	D222G increased virus replication in MDCK cells and pathogenicity in mice	562
	Use random peptide library of H from A/2009/H1N1 to identify antigenically important regions	Most antigenic sites are located in the conserved H stem region responsible for membrane fusion	565
	Mouse model: analyze amino acid changes in mouse-adapted H1N1	D131E, S186P, and A198E contribute to virulence in mice	572
N	Use of homology modeling and molecular dynamic stimulation to study the N from different influenza viruses	Amino acid residues unique to A/2009/H1N1 are responsible for its affinity for α-2,6 sialic acid	291
NS1	Chickens; evaluate the role of NS1 protein in overcoming innate host defenses and pathogenicity in chickens using recombinant Newcastle disease virus with NS1 gene of A/2009/H1N1	Recombinant virus expressing the A/2009/H1N1 NS1 grew to higher titers than that expressing H5N1 NS1, antagonized IFN-β synthesis more efficiently in HeLa cells than in chicken embryo fibroblast cells, and inhibited PKR activation in infected HeLa cells	307
	Mouse and ferret model; compare NS1 protein of A/2009/H1N1 to NS1 of other human-adapted H1N1 viruses	Recombinant A/2009/H1N1 expressing NS1 of human-adapted seasonal strains induced less morbidity in mice and reduced titers in upper respiratory tracts of ferrets	229
	Cell culture and mouse model	XSEV PDZ ligand motif in NS1 contributes to efficient replication of A/2009/H1N1 in cell culture; RSEV, RSKV, and ESEV PDZ motifs in combination with 220W increase mouse pathogenicity	421
	Effect of NS1 on cellular pre-mRNA polyadenylation and mRNA translation	NS1 does not contribute to translation of mRNA and does not inhibit cellular pre-mRNA polyadenylation in A549 cells	55
	Vaccine based on NS1	Live vaccine based on A/2009/H1N1 NS1 can provide protection against infection in mice and ferrets	583
PB1-F2	Mouse and ferret model; determine the effect of PB1-F2 in A/2009/H1N1 by creating recombinant A/2009/H1N1 expressing PB1-F2	When comparing wild-type A/2009/H1N1 with recombinant A/2009/H1N1 expressing PB1-F2, there were no significant differences in morbidity and mortality	227
	Cell culture and mouse model; determine the effect of PB1-F2 mutation	F2-stop12L mutants (a stop-to-leucine substitution at position 12 in the PB1-F2) replicated more efficiently in MDCK cells but did not increase pathogenicity in mice	421
PA	Mouse model; analysis of the effect of amino acid substitution	L295P responsible for transcription and replication activity	271
	Mouse model	PA appears crucial in maintaining viral gene functions (substitution of PA gene impaired activity of all polymerase complexes)	484
	In vitro study	S186 is necessary for the protein to function optimally	537
	Mouse model; analyze amino acid changes in mouse-adapted A/2009/H1N1	E298K contributes to virulence	572
NP	Mouse model; analyze amino acid changes in mouse-adapted A/2009/H1N1	D101G contributes to virulence	572

[a] H1 numbering is used in denoting polymorphism position. H, hemagglutinin; HTBE, human tracheo-bronchial epithelial cells; ICU, intensive care unit; MDCK, Madin-Darby canine kidney; PKR, protein kinase R.

ing diseases (511). This finding was not unexpected because the A/2009/H1N1 virus is antigenically and structurally similar to the A/1918/H1N1 pandemic virus at antigenic sites Sa, Sb, Ca1, Ca2, and Cb over the globular head of H in crystallization and computer modeling but is different from other H1 viruses (356, 563). However, the preexisting seropositivity rate alone cannot explain some of the observations. For example, a study in Hong Kong showed an absence of preexisting seropositivity in the age group of 61 to 70 years, but the incidence of the A/2009/H1N1 virus was low in this population (579).

Risk Factors for Severe Disease

In addition to extremes of age and chronic underlying medical illness, other risk factors for severe disease or complications emerged from the A/2009/H1N1 pandemic. Immunosuppressive therapy was reported in a greater percentage of fatal cases in North America than of fatal cases in Asia and the Far East (15.7% versus 6.1%; $P < 0.001$) (127, 152, 170, 181, 187, 305, 313, 365, 435, 574) (Table 4). Additionally, underlying neurological conditions appeared to be more common in fatal cases in North America (37.7% versus 6.7%; $P < 0.001$) (127, 152, 181, 281, 305, 365, 435, 493, 523, 574). Unexpectedly, obesity emerged as a new risk factor for severe influenza, which can predict mortality (364, 458, 522). Although obesity adversely affects pulmonary function, it has recently been associated with immunodysregulation involving adipokines (269, 298, 407). Pregnancy has been confirmed as a risk factor for severe influenza (363). Pregnant women who were infected during the third trimester were at a higher risk for complications. Notably, no deaths were reported in pregnant women in Asia and the Far East (305). In a mouse model, pregnancy was associated with severe pulmonary damage and cytokine dysregulation, but there was no involvement of the placenta or fetus (85). In addition, smoking and allergies have been proposed as risk factors, but no systematic control studies have been performed (233, 570). In children, neurodevelopmental disorders emerged as a predominant risk factor (8). Additionally, an IgG2 subclass deficiency has been linked to severe pandemic influenza, especially in pregnant women (80, 206), and lower IgG2 levels in severe disease may be associated with cytokine dysregulation (80). However, the usefulness of intravenous immunoglobulin replacement therapy still needs to be confirmed in randomized controlled trials (207).

Primary viral pneumonia is typically seen in patients with underlying comorbidities, such as chronic cardiopulmonary diseases, but 12.5% and 6.3% of cases occur in healthy adults and

TABLE 2 Sequence of important events related to A/2009/H1N1 influenza

Date	Important event[a]
Mid-February 2009	Outbreak of respiratory illness in La Gloria, Veracruz, Mexico
12 April 2009	Mexican public health authorities reported outbreak to PAHO
15 April 2009	CDC identified A/2009/H1N1 in a boy from San Diego, CA
17 April 2009	CDC identified A/2009/H1N1 in a girl from Imperial, CA
19 April 2009	Mexico declared a national alert
21 April 2009	CDC alerted doctors to a new strain of H1N1 influenza virus
24 April 2009	WHO issued Disease Outbreak Alert
27 April 2009	WHO raised the pandemic alert from phase 3 to 4
29 April 2009	WHO raised the pandemic alert from phase 4 to 5
11 June 2009	WHO raised the pandemic alert from phase 5 to 6
8 July 2009	Virus strains resistant to oseltamivir identified
13 July 2009	WHO issued recommendations on pandemic H1N1 2009 vaccines
5 November 2009	Detection of infection of farmed swine by the pandemic virus
20 November 2009	Virus mutation detected in fatal and severe cases in Norway
2 December 2009	Oseltamivir-resistant virus identified in hospitalized and immunosuppressed patients
18 February 2010	WHO issued recommendations for the composition of influenza virus vaccines for the upcoming Northern Hemisphere influenza season
10 August 2010	WHO issued recommendations for the postpandemic period

[a] CDC, Centers for Disease Control and Prevention; PAHO, Pan American Health Organization; WHO, World Health Organization.

pregnant women, respectively (455). Genetic susceptibility may predispose healthy individuals to severe disease. In a case-control study, single nucleotide polymorphisms (SNPs) in the genes encoding the FcγRIIA protein, the replication protein A-interacting protein, and the complement component 1q subcomponent binding protein and another gene in chromosome 3 were associated with severe pneumonia (591). Interestingly, the SNP in the FCGR2A gene was found more frequently in patients with severe A/2009/H1N1 infection in China; however, this finding was not statistically significant (80). Recently, a genome-wide knockdown study with a short hairpin RNA (shRNA) library identified several important host genes that are involved in the viral life cycle, including fusion, uncoating, transport of the viral RNP complex into and out of the nucleus, replication, transcription and translation of the genome, assembly, and budding (297, 319, 468). The relationship between these host genes and disease severity should be examined further.

HISTOPATHOLOGY AND PATHOGENESIS

A total of 250 autopsies that examined the lungs, livers, spleens, and bone marrow specimens from 2009 pandemic H1N1 cases were reported in the literature (66, 200, 236, 378, 398, 403, 442, 470, 486, 500). Among 190 fatal cases for whom clinical data were reported, the male-to-female ratio was 101:89. The ages ranged from 2 months to 83 years, with a median age of 35 years (66, 200, 236, 378, 398, 403, 470, 486, 500). The interval between symptom onset and death ranged from 1 to 44 days, with a median of 8 days. The histopathological changes in fatal cases depended on the date of death after symptom onset. The changes in patients who died within the first 10 days after symptom onset were dominated by virus-induced cytolysis and acute inflammation, which involved the upper to lower respiratory tracts. The changes in patients who

TABLE 3 Epidemiological characteristics of patients in different continents with laboratory-confirmed A/2009/H1N1 infection

Epidemiological setting of transmission	Patient characteristics[a] (reference[s])				
	Asia	Oceania	Europe	North America	South America
Community[b]	AR, 5.4–20.6; R_o, 1.22–2.3; T_g, 0.8–1.9 (134, 359, 416, 556, 558)	AR, NM; R_o, 1.16–1.29; T_g, NM (262)	AR, 6–20; R_o, 0.5; T_g, 2.7 (20, 154, 226)	AR, —[c]; R_o, 1.7–1.8; T_g, 2.2–3.3 (65, 543)	AR, 7.7–61; R_o, 1.2–3.1; T_g, 1.9–3.2 (36, 184, 205, 262, 506, 571)
Household	AR, 8–26.1; R_o, NM; T_g, NM (124, 318)	AR, 14.5; R_o, NM; T_g, NM (59)	NA	AR, 4–45; R_o, NM; T_g, 3.9 (64, 182, 284, 393, 428, 571)	AR, 35; R_o, 1.8; T_g, 3.61 (436)
Nosocomial	AR, 23.5; R_o, NM; T_g, NM (10)	NA	AR, 35; R_o, NM; T_g, NM (114)	AR, 5–40; R_o, NM; T_g, NM (74, 172)	NA
School	AR, 21.3; R_o, NM; T_g, NM (326)	NA	AR, 2–60; R_o, NM; T_g, NM (53, 221, 248, 480)	AR, 2.8–21; R_o, NM; T_g, NM (72, 133, 275, 347, 514)	NA
Other	AR, 21–41[d]; R_o, NM; T_g, NM (231)	AR, 3.5[e]; R_o, NM; T_g, NM (18)	NA	AR, 8–22[f]; R_o, NM; T_g, NM (73, 130, 547)	NA

[a] AR, attack rate (percent); R_o, basic reproduction number, defined as the number of secondary infections generated by a primary infection in a susceptible population and which thus measures the intrinsic transmissibility of an infectious agent; T_g, disease generation time, defined as the mean time interval (days) between infection of one person and infection of the people whom individual infects; NA, not available; NM, not mentioned.
[b] In a retrospective analysis of the national surveillance data involving over 2,000,000 cases of A/2009/H1N1 influenza in Japan, it was noted that males $<$20 years of age may be more likely to suffer from A/2009/H1N1 than females in the same age categories (167).
[c] —, the attack rate in one study was highest among children aged 5 to 14 years (147 per 100,000 population), followed by children aged 0 to 4 years (113 per 100,000). The attack rate for children aged 5 to 14 years was 14 times higher than that for adults aged $\geq$60 years.
[d] Outbreak among tour group members in China.
[e] Outbreak on a passenger aircraft; their seating was within two rows of infected passengers, implying a risk of infection of about 3.5% for the 57 passengers in those rows.
[f] Including an outbreak on a docked Navy ship in the United States, an outbreak at the U.S. Air Force Academy, and an outbreak on a Peruvian navy ship in the United States.

TABLE 4 Underlying comorbidities of fatal hospitalized cases with laboratory-confirmed A/2009/H1N1 infection

Underlying condition	No. of patients with condition/no. of deaths (% of patients with condition)[a]				
	Asia ($n = 952$)	Africa ($n = 104$)	Europe ($n = 928$)	North America ($n = 1{,}200$)	South America ($n = 156$)
Chronic respiratory condition	105/908 (11.6)	11/85 (12.9)	181/788 (23)	345/1,200 (28.8)	37 (23.7)
Chronic cardiac condition	46/169 (27.2)	9/71 (12.7)	161/928 (17.3)	166/751 (22.1)	18 (11.5)
Diabetes mellitus	33/141 (23.4)	15/85 (17.6)	82/620 (13.2)	203/1177 (17.2)	18 (11.5)
Renal failure	63/908 (6.9)	0	35/499 (7.0)	71/751 (9.5)	8 (5.1)
Liver cirrhosis	42/898 (4.7)	0	28/499 (5.6)	14/324 (4.3)	6 (3.8)
Rheumatological condition	0	0	9/138 (6.5)	0	0
Neurological condition	61/916 (6.7)	0	127/499 (25.5)	292/774 (37.7)	0
Malignancy	9/44 (20.5)	0	48/499 (9.6)	45/324 (13.9)	0
HIV/AIDS	0	19/45 (42.2)	3/228 (1.3)	4/110 (3.6)	0
Immunosuppressive therapy	55/898 (6.1)	2/13 (15.4)	89/483 (18.4)	118/751 (15.7)	29 (18.6)
Obesity	103/916 (11.2)	19/86 (22.1)	116/928 (12.5)	49/517 (9.5)	41 (26.8)
Pregnancy	0	29/101 (28.7)	19/928 (2)	25/447 (5.6)	10 (6.4)
No risk factor	46/169 (27.2)	16/76 (21.1)	85/259 (29.4)	6/19 (31.6)	51 (32.7)

[a] The information should be interpreted with care because the denominators of the underlying conditions are different. References are as follows: for patients in Asia, references 131, 302, 305, 493, and 574); for patients in Africa, references 15 and 313); for patients in Europe, references 67, 152, 187, 435, 523, and 544); for patients in North America, references 68, 75, 127, 181, 281, and 365); and for patients in South America, reference 170.

died much later with intensive care support were dominated by both damage and reparative processes.

Histopathological Changes in the Respiratory Tract

Upper and middle airway infections caused by the A/2009/H1N1 virus were characterized by the multifocal destruction and desquamation of the pseudocolumnar and columnar epithelia, with significant submucosal edema and hyperemia to the extent of thrombus formation at the bronchiolar level. The acute inflammation could be severe, as evidenced by hemorrhagic tracheobronchitis and desquamative bronchiolitis with necrosis of the bronchiolar wall. Once necrosis occurred, there was infiltration by polymorphs and mononuclear cells. The histological changes in influenza pneumonia included interstitial edema with inflammatory infiltrate, alveolar proteinaceous exudation with membrane formation, capillary thrombosis, necrosis of the alveolar septa, intra-alveolar hemorrhage, desquamated pneumocytes with pyknotic nuclei into the alveolar spaces, and diffuse alveolar damage with dominant mononuclear interstitial infiltration by lymphocytes and histiocytes (Fig. 4A and B) (200, 378, 470). During the late stage, organizing diffuse alveolar damage, fibrosis, type II pneumocyte hyperplasia, epithelial regeneration, and squamous metaplasia were found, which are compatible with the fibroproliferative phase of acute respiratory distress syndrome (ARDS) and diffuse alveolar damage (Fig. 4C). Histochemical staining for the viral NP showed that type II pneumocytes were the predominantly affected cell type (470), and the staining was positive in patients who died within 3 days after symptom onset. Bacterial coinfections were documented in 26% to 33.3% of the case series that were analyzed (Fig. 4D) (66, 200, 236, 378, 470). The most common bacteria found in the autopsy series included *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, community-associated methicillin-resistant *S. aureus*, *Streptococcus mitis*, *Haemophilus influenzae*, and *Acinetobacter baumannii* (66, 107, 236). Mouse studies suggested that during A/2009/H1N1 infection, the innate defense against secondary pneumococcal infection was impaired due to the increased gamma interferon (IFN-γ) response during the recovery stage of infection (496).

Histopathological Changes in Extrapulmonary Sites

In addition to the pulmonary pathologies, the autopsy studies revealed pathological changes in other sites. Myocarditis with myofibril degeneration and interstitial lymphocytic infiltrate were found in several fatal cases of 2009 pandemic H1N1 infection (Fig. 4E) (195, 510). Hemophagocytosis was found in several severe cases, as in cases of influenza A H5N1 virus infection (236, 470, 510). Pulmonary thromboembolism was evident in 32 cases (Fig. 4F and 4G) (200, 236, 378, 470, 510). Splenic infarction associated with thrombosed arterial supply was observed in the postmortem examinations (Fig. 4H).

Host Cytokine Profile

The majority of the studies on the blood cytokine and chemokine levels showed that interleukin-6 (IL-6), IL-8, IL-10, IL-15, IFN-γ, and tumor necrosis factor alpha (TNF-α) were consistently elevated in severe disease (Table 5). Although the blood IL-17 level was found to be elevated in one study (31), this finding was not consistent and could be related to the sampling times at the different stages of the disease process (13, 225, 308, 510). IL-17 has recently been shown to be important in the recruitment of B cells (536). *In vitro* studies of the mRNA expression profiles and cytokine activation levels in virus-infected peripheral blood mononuclear cells showed no significant differences between the A/2009/H1N1 and seasonal H1N1 viruses except for the downregulation of zinc finger proteins and small nucleolar RNAs by seasonal H1N1 virus (341). The cytokine activation findings in animal challenge experiments with mice, ferrets, and macaque monkeys were heterogeneous, as expected with species variations (28, 85, 274, 294, 461).

Host Immune Response

Upon infection, influenza virus will trigger a series of host immune responses. The initial innate immune response includes the following: the expression of pattern recognition receptors (PRRs), such as Toll-like receptors (150), the retinoic acid-inducible gene I (RIG-I) protein (443), and NOD-like receptors; the complement

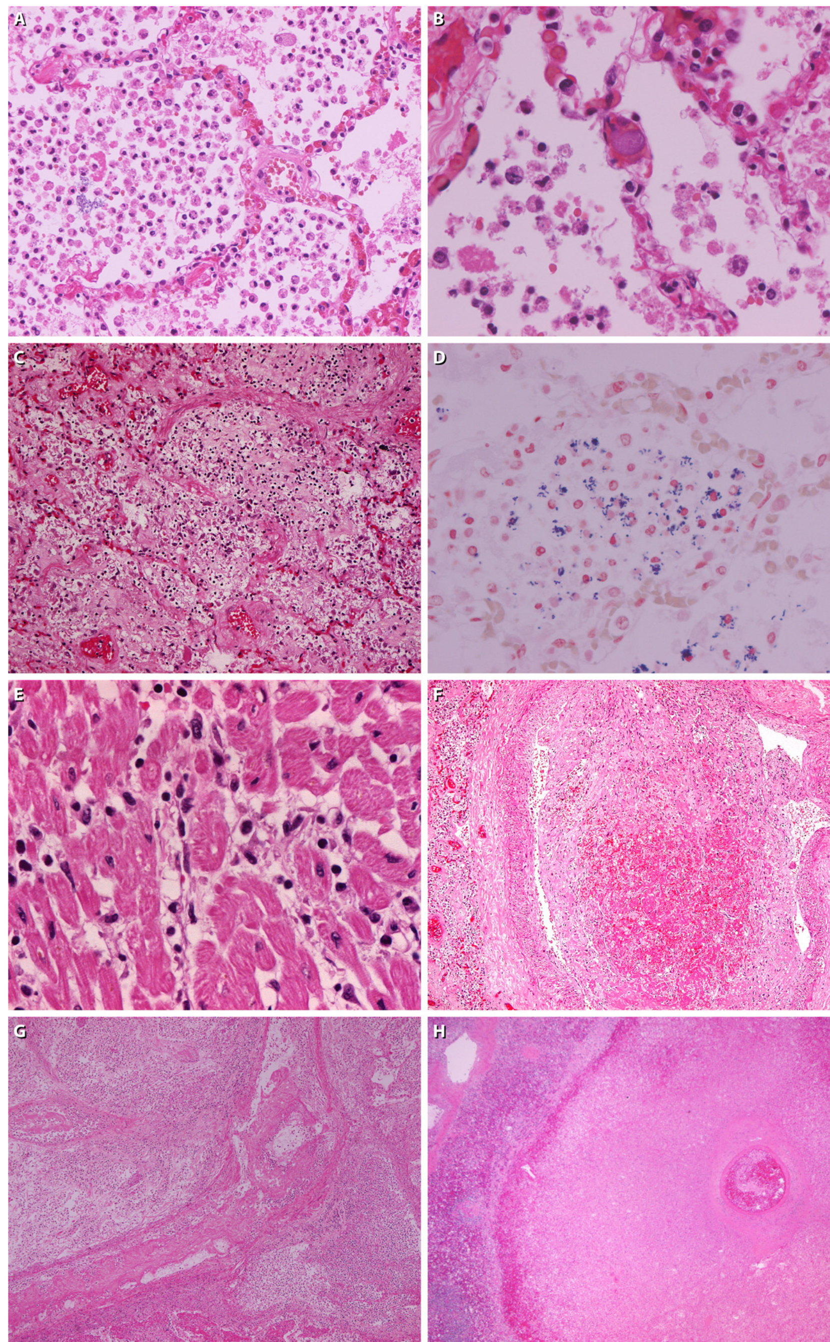


FIG 4 Histopathological examination in fatal cases of A/2009/H1N1. Hematoxylin and eosin (H&E) staining was used for panels A to C and E to H; Gram staining was used for panel D. (A) Lung parenchyma showing the acute phase of viral pneumonia and ARDS, with numerous macrophages within alveolar space. The aveolar septa are congested. Magnification, ×200. (B) A pneumocyte displaying cytopathic change with an enlarged nucleus during the acute phase of ARDS due to viral pneumonia. Magnification, ×400. (C) Lung parenchyma showing the chronic fibroproliferative phase of diffuse alveolar damage. The alveolar septa are thickened, and the alveolar spaces are replaced by fibrogranulation tissue. Magnification, ×200. (D) The alveolar spaces contain many Gram-positive cocci, with some being ingested by macrophages in a patient with secondary bacterial pneumonia. Magnification, ×200. (E) A case with myocarditis, showing lymphoid infiltrate in the myocardium. Magnification, ×200. (F) Organized thrombus of a branch of pulmonary artery. Magnification, ×100. (G) A branch of pulmonary artery with recent thrombus formation. The surrounding lung parenchyma shows heavy acute inflammatory infiltration. Magnification, ×40. (H) Splenic infarct associated with a thrombosed arteriole. Magnification, ×40. (All photos courtesy of Chung-Ying Leung, reproduced with permission.)

cascade; and antimicrobial peptides. The subsequent adaptive immune response consists of B and T lymphocytes, which are triggered by antigen-presenting cells, such as dendritic cells and macrophages.

Studies with prototype strains of the influenza A H1N1 virus showed that the virus triggers an immediate response from the innate immune system through nonspecific PRRs, including TLR3, -7, and -9 (150, 220), at the plasma membrane or within

TABLE 5 Changes in blood levels of cytokines and chemokines in severe and mild cases of A/2009/H1N1 infection

	Reference(s) in which level was found to be:		
Cytokine[a]	Higher in severe cases	Lower in severe cases	Not different in severe and mild cases
G-CSF	510		
GM-CSF	30		510
VEGF	30		
IFN-α2	510		
IFN-α	3		
IFN-γ	30		225, 308, 510, 541
IL-1α	510		13
IL-1RA	13, 30		
IL-2	13		510
IL-5	498[b]		510
IL-6	13, 30, 225, 308, 340, 510		3, 541
IL-8 (CXCL-8)	30, 225, 340, 510		3, 13, 541
IL-10	13, 30, 510		3
IL-12p70	30		13, 510
IL-15	225, 510		
IL-17	31	510[c]	13, 225, 308
IP-10	30, 308, 510		31, 225
MCP-1 (CCL2)	30, 340, 510	3	31
MIP-1α (CCL3)	13		510
MIP-1β (CCL4)	13		31, 510
TNF-α	31, 225, 510		13
sTNFR1	340		
TGF-β1	541		

[a] G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor; IL-1RA, IL-1 receptor antagonist; IP-10, IFN-γ-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP-1β, macrophage inflammatory protein 1β; sTNFR1, soluble TNF receptor 1; TGF-β1, transforming growth factor β1.
[b] Compared children with or without pneumonia.
[c] Lower in severe cases only if $\leq$3 days after onset of symptoms.

endosomes. TLR3 recognizes double-stranded RNA (dsRNA); however, it is unclear whether the influenza virus produces dsRNA in infected cells. TLR7 and TLR8 recognize uridine-rich sequences of single-stranded RNA (ssRNA). The triggering of these TLRs will lead to the recruitment of MyD88 and the activation of transcription factor NF-κB and mitogen-activated protein kinase (MAPK) inflammatory cascades. After virus entry and uncoating, viral RNA is recognized by the cytoplasmic RIG-I-like receptors, and the melanoma differentiation-associated gene 5 (MDA5), which are regulated by LPG2 and ubiquitin ligase (299). A dsRNA binding protein, called PACT, is a potent binder and activator of RIG-I and boosts the antiviral interferon response (316, 352). The activation of RIG-I results in a type I interferon response in epithelial cells. However, one key virulence factor of influenza virus, NS1, can inhibit this RIG-I-dependent interferon production in two ways. First, NS1 can interfere with the activation of RIG-1 by viral ssRNA harboring free 5′-triphosphate groups (443). Alternatively, NS1 reduces RIG-I signal transduction by inhibiting the TRIM25-mediated RIG-I–CARD ubiquitination (190). The significance of these molecular interactions between the viral and host factors is shown in cell culture and mouse challenge models infected with the prototype influenza A H1N1 virus (PR8). Compared with the PR8 virus with NS1 deleted, the cells and mice infected by the wild-type virus do not mount an innate immune response for almost 2 days postinfection as indicated by cytokine and chemokine assays and histopathology, during which the viral load starts to peak. This quiet stealth phase was followed by a sudden burst of lung inflammation in mice, with the subsequent initiation of adaptive immunity by migratory lung dendritic cells (390). These findings are compatible with the clinical findings that the nasopharyngeal viral load in infected human volunteers had already peaked by the time the patients had symptoms (244). It is unknown whether severe or fatal cases had a higher peak viral load because the majority of them did not present early; however, viral load monitoring after patient admission showed that patients had a delayed clearance of viral load in respiratory specimens, with concomitantly higher serum proinflammatory cytokines and chemokines (510). Recently, it has also been shown that agonism of sphingosine-1-phosphate signaling in the endothelium could suppress the influenza virus-induced cytokine storm, suggesting the role of endothelial cells in the modulation of inflammation triggered by influenza virus (504). It is possible that the final clinical outcome of the host was determined by the balance between the amount of tissue damage due to virus-infected cells, which is governed by the innate immune control and the subsequent adaptive immune control, the inflammatory damage inflicted by the innate immune response and the subsequent adaptive immune response, the pulmonary reserve, and regenerative power of the patient.

Infection by the A/2009/H1N1 virus may sometimes induce broadly reactive neutralizing antibodies that target the H stalk and globular head domain in multiple influenza virus strains. In addition, A/2009/H1N1 infection induced a cytotoxic T lymphocyte response that targeted internal type-specific viral core proteins (211, 555). However, patients with severe influenza were shown to have lower serum complement levels and higher titers of antibodies, with a lower avidity for attaching to H, than those with mild cases (391). The immune complex formed between low-avidity antibodies and viral antigens in infected cells fixed complement, which activated the inflammatory cascade and led to the exaggerated inflammation of infected lung tissue. This finding revealed a new phenomenon of pathogenesis for pandemic A/2009/H1N1 influenza virus (391), which requires further confirmation.

Many studies have been conducted on the cross-reactive T lymphocyte-mediated immunity directed against both the A/2009/H1N1 and seasonal H1N1 viruses (Table 6). The amount of cross-reactivity varied from 18.1% to 69% and was directed mainly against the internal proteins, such as M1, NP, and PB1 (213). The prevalence of cross-reactive antibodies against the A/2009/H1N1 and seasonal H1N1 viruses in the sera of healthy individuals younger than 65 years of age was generally less than 6% before 2009 (77). When these healthy individuals were immunized with the seasonal H1N1 vaccine, up to 22% of these vaccinees had a protective neutralizing antibody titer (232). When mice were immunized with the seasonal H1N1 live attenuated vaccine, decreased morbidity and mortality were observed (92). This protection was largely due to cell-mediated immunity.

Using flow cytometry, increased regulatory T (Treg) lymphocyte activity was observed in peripheral blood samples from infected versus noninfected patients at 1 to 2 days after symptom onset, but the increase was more significant in moderate cases than in severe cases (222). In severe cases, B lym-

TABLE 6 Cross-reactive adaptive immune responses against A/2009/H1N1 virus in humans and experimental animals[a]

Type of response	Study design (location)	Subjects tested	Findings	Reference
T cell	Measure the extent of cross-reactivity of seasonal H1N1 influenza A virus-specific CD4 T cells with A/2009/H1N1 epitopes (USA)	11 persons (age NM)	15 A/2009/H1N1 peptides were found to cross-react with seasonal influenza virus-specific T cells	196
	Compare the T cell responses in patients with or without A/2009/H1N1 infection (Australia)	6 healthy donors and 2 patients with laboratory-confirmed A/2009/H1N1 infection	A/2009/H1N1 uninfected patients showed no preexisting T cell response to the 2009-NP418 variant or the 1918-NP418 variant; natural infection with the A/2009/H1N1, however, elicited CD8$^+$ T cells specific for the 2009-NP418 and 1918-NP418 epitopes	211
	Measure the cross-reactivity of memory T cell immunity against A/2009/H1N1 in PBMCs collected before the 2009 pandemic (USA)	20 adults	69% of the epitopes recognized by CD8$^+$ T cells were conserved among the seasonal H1N1 influenza virus and A/2009/H1N1; cross-reactive memory T cell immunity was present in the general population	213
	Measure the T cell responses in a cohort of healthy nonimmunized adults; the blood samples were collected before summer 2009 (USA)	46 healthy nonimmunized adults	5/46 subjects had T cell response <30% of coexistent seasonal response; 19/46 subjects had T cell response greater than seasonal response	491
	Measure the CTL response to A/2009/H1N1 in patients without prior infection with A/2009/H1N1 (Hong Kong)	12 adults	CTL from uninfected individuals could directly lyse A/2009/H1N1-infected target cells and produce IFN-γ and TNF-α; 17/94 (18.1%) of influenza A virus CD8 T-cell epitopes were conserved in A/2009/H1N1, and >1/2 of these conserved epitopes were derived from M1 protein; seasonal influenza vaccination could expand the functional $M1_{58-66}$ epitope-specific CTLs in 20% of HLA-A2$^+$ individuals	518
	Investigate the degree of T-cell cross-reactivity between seasonal influenza A (sH1N1, H3N2) from 1968 to 2009 and A/2009/H1N1 strains (Canada)	NA	T cell cross-reactivity was estimated to be 52%, and maximum conservancy was found between sH1N1 and A/2009/H1N1 with a significant correlation	162
	Assess the cross-reactive T cell immunity in mice (the Netherlands)	Mice	Mice with prior H3N2 infection displayed reduced weight loss after challenge infection and cleared the A/2009/H1N1 more rapidly; virus-specific CD8$^+$ T cells in concert with CD4$^+$ T cells were responsible for the observed protection (by adoptive transfer experiments)	258
	Evaluate the memory T cell repertoire in healthy adults (USA)	9 healthy adults aged 18–50 yr	Most individuals had abundant circulating CD4 T cells that recognized influenza virus-encoded proteins	457
	Examine the production of cytokines in response to virus from CD8$^+$ T cells from subjects who had no evidence of exposure to A/2009/H1N1 and had blood collected prior to the emergence of the pandemic in April of 2009 (USA)	9 healthy adults aged 18–49 yr	Most subjects exhibited cytokine positive CD8$^+$ T cells in response to A/2009/H1N1	464
T cell and antibody	Compare the T cell and antibody responses elicited by trivalent inactivated influenza vaccine and live attenuated influenza vaccine (USA)	30 healthy adults	Both vaccines boosted preexisting T cells to the seasonal and pandemic H, but responses were significantly greater following immunization with LAIV; antibody titers were significantly boosted only by TIV	492
Antibody	Measure cross-reactive antibody response to A/2009/H1N1 before and after seasonal influenza vaccination (2005 to 2009) (USA)	Age 6 mo to 9 yr (n = 28), 18–59 yr (n = 30), >60 years (n = 42)	Before seasonal influenza vaccination (% with VN titer >160): children, no cross-reactive antibody; 18–64 yr, 6–9%; >60 yr, 33%; after seasonal influenza vaccine (increase in cross-reactive antibody titer): children, no increase; 18–64 yr, 2-fold; >60 yr, no increase in cross-reactive antibody	77
	Measure the preexisting cross-reactive antibody response to A/2009/H1N1 before and after seasonal influenza vaccination (USA)	Born before 1950 (n = 115), born after 1980 (n = 107), age 6 mo to 9 yr (n = 55), age 18–64 yr (n = 231), age ≥60 yr (n = 113)	Proportion of individuals with cross-reactive antibody (VN): born before 1950, 34% (neutralizing antibody titer, ≥80); born after 1980, 4% (neutralizing antibody titer, ≥40). Increase in cross-reactive antibody to 2009 H1N1 by 4-fold after seasonal influenza vaccination (VN): 6 mo to 9 yr, 0%; 18–64 yr, 12–22%; >60 yr, <5%	232
	Measure the cross-reactive antibody levels against A/2009/H1N1 in sera collected between 2004 and 2005 by HI assay (Finland)	Born between 1909 and 1919 (n = 27), between 1920 and 1929 (n = 104), between 1930 and 1939 (n = 125), 1940 or after (n = 775)	Proportion of patients with cross-reactive antibody (HI titer, > 10): born between 1909 and 1919, 96%; born between 1920 and 1929, 56.7%; born between 1930 and 1939, 13.6%; born after 1939, <10%	270
	Measure the antibody titer (by HI and VN assay) in sera collected in 2008 (France)	100 adults	Seasonal 2007 H1N1 infection was an independent predictor of elevated preexposure antibody titers against A/2009/H1N1	345
	Study neutralization response to A/2009/H1N1 in patients with prior immunization with 1976 "swine flu" vaccine (USA)	Age ≥55 yr (n = 116), received "swine flu" vaccination in 1976 (n = 46), age 0–18 yr (n = 20)	Receipt of 1976 "swine flu" vaccine enhanced neutralization response to A/2009/H1N1 (VN titers of ≥160: vaccine recipients, 17.4%; vs non-vaccine recipients, 4.3% [P = 0.018])	379
	Animal model (mice, ferrets, minipigs); study the cross-reactive immunity of seasonal influenza vaccine (SW/Korea/CAN01/04; A/Brisbane/59/07) on A/2009/H1N1 (South Korea)	NA	Although receipt of SW/Korea/CAN01/04 induced detectable cross-reactive antibody against A/2009/H1N1, active virus replication and virus shedding were not suppressed	431

(Continued on following page)

TABLE 6 (Continued)

Type of response	Study design (location)	Subjects tested	Findings	Reference
	Measure cross-reactive antibody response to A/2009/H1N1 in serum samples collected in 2004 by HI, SRH, and VN assay (Italy)	Born between 1909 and 1938 (n = 201), 1939 and 1948 (n = 193), 1949 and 2004 (n = 193)	Proportion of patients with HI titer of ≥40: born between 1909 and 1938, 22.4%; born between 1939 and 1948, 12.4%; born between 1949 and 2004, 6.7%	459
	Mouse model; expose mice to virus (1947 virus A/FM/1/47 or 1934 virus A/PR/8/34) and measure the cross-protective immune responses to mouse-adapted A/2009/H1N1 (USA)	NA	Mice exposed to 1934 and 1947 H1N1 viruses were protected against lethal challenge with mouse-adapted A/2009/H1N1	477
	Measure the cross-reactive antibody titers in healthy volunteers mostly born before 1958 (Singapore)	50 adults	HI or VN titer of ≥40: 0% among those 40–80 yr old	502
	Measure the cross-reactive antibody titer (China)	Age 7 to 84 yr (n = 4,043)	HI titer of ≥40, 1.7%; VN titer of ≥40, 0.3%	95
	Measure the cross-reactive antibody titers in sera collected in the prepandemic and early epidemic phases before widespread community transmission (Singapore)	838 members of the community, 1,213 military personnel, 558 hospital staff, 300 from long-term care facilities	HI titer of ≥ 40, 2.6% of community members, 9.4% of military personnel, 6.6% of hospital staff, 6.7% of subjects from long-term care facilities	101
	Measure the VN titer in blood samples collected from donors in 1999 and 2009 (Japan)	315 persons	Most patients born after 1920 had no neutralizing antibody against A/2009/H1N1	274
	Measure the background prepandemic cross-reacting antibodies to the A/2009/H1N1 in older populations (Australia)	259 serum samples from persons aged 60 yr or older	HI titer of ≥40: ≥60 yr, 37.5%; ≥85 yr, 60%; 60–64 yr, 12%	39
	Measure the cross-reactive antibody titer (Taiwan)	229 stored sera from donors born between 1917 and 2008	Neutralizing titer of ≥160: >80 yr, 59%. Neutralizing titer of ≥40: children 6 mo to 9 yr, 4% (only those children with neutralizing titer to pandemic H1N1 virus had high neutralizing titers to seasonal H1N1 virus)	110
	Measure the cross-reactive antibody titer (France)	1,693 serum samples collected in 2007 to 2008	HI titer of ≥40 increased from 40.5% in those 0–24 yr to 70% in elderly	143
	Measure the cross-reactive antibody in IVIG preparation (USA)	6 IVIG samples prepared in prepandemic period, sera from 19 Kawasaki patients treated with IVIG	All the IVIG preparations had significant levels of cross-reactive specific antibody; 18 out of 19 Kawasaki patients treated with IVIG had significant increase in cross-reactive specific antibody	261
Clinical	Ferret model; effect of prior infection with seasonal influenza A virus on the outcome of A/2009/H1N1 challenge (Australia)	NA	Prior infections with seasonal influenza A viruses reduced the incidence of infection, amount and duration of virus shedding, and frequency of transmission following A/2009/H1N1 challenge	335
Vaccine	Measure the neutralizing antibody titers before and after immunization with 2009 Southern Hemisphere seasonal influenza vaccine (Singapore)	Age 19–46 yr (n = 51)	Postvaccination, 12% had 4-fold rise in VN titer to A/2009/H1N1	343
	Mouse model; protection against lethal challenge with A/2009/H1N1 by 1918-like and classical swine H1N1-based vaccine (USA)	NA	Immunization with 1918-like or classical swine H1N1 vaccine completely protected C57B/6 mice from lethal challenge with A/2009/H1N1	373
	Mouse model; evaluate the cross-protection induced by immunization with 2009 pH1N1 vaccines following a lethal challenge with 1918 H1N1 (USA)	NA	Vaccination with A/2009/H1N1 protected mice from lethal challenge with 1918 virus	164
Vaccine/prior infection	Mouse model; evaluate the effect of prior seasonal H1N1 infection and seasonal influenza vaccine on the immune response to pandemic influenza vaccine/infection (USA)	NA	p-LAIV induced a cellular response and nonneutralizing antibody production but only partial protection from A/2009/H1N1 challenge; primary infection with sH1N1 followed by p-LAIV resulted in cross-reactive antibody and robust cellular response and was associated with complete protection	92
	Ferret model; evaluate the effect of prior seasonal H1N1 infection or vaccine (USA)	NA	Seasonal influenza vaccine was unable to alter subsequent morbidity or transmission in ferrets	434
Vaccine	Evaluate the antibody titer to A/2009/H1N1 after seasonal influenza vaccine (Australia)	20 children with median age of 4 yr	Only 2 children were seropositive (HI titer = 40) for A/2009/H1N1	380
	Evaluate the effect of prior seasonal influenza vaccine (USA)	30 healthy subjects between age 18 and 49 yr who received vaccine between October and November 2007 (15 received LAIV and 15 received TIV)	Only preexisting T cells, and not antibody titer, to pandemic H1 boosted after LAIV	492
	Mouse model; evaluate the effect of prior live seasonal influenza vaccine (USA)	NA	Mice immunized with seasonal LAIV had decreased morbidity and mortality from A/2009/H1N1 infection Protective immunity primarily dependent upon $CD4^+$ T cells but not $CD8^+$ T cells	496
	Mouse model; evaluate the effect of a vaccine using synthetic consensus H1 antigen (USA)	NA	80% of mice challenged with A/2009/H1N1 were protected	539
	Evaluate the cross-reactivity after inactivated seasonal influenza vaccine (USA)	120 adults aged 20–64 yr 59 elderly aged 65 yr or above	22% of adults and 34% of elderly showed a ≥4-fold increase in HI titers after TIV vaccination	561
Prior seasonal virus infection	Mouse model; evaluate the effect of prior seasonal influenza virus infection on subsequent pH1N1 infection (USA)	NA	Sequential infection with viral strains with different surface glycosylation can prime the host for immunopathology if a neutralizing antibody response matching the T cell response is not present	538

[a] HI, hemagglutination inhibition; CTL, cytotoxic T lymphocytes; IFN, interferon; IVIG, intravenous immunoglobulin; LAIV, live attenuated influenza vaccine; NA, not applicable; PBMCs, peripheral blood mononuclear cells; p-LAIV, pandemic live attenuated influenza vaccine; SRH, single-radial-hemolysis; TIV, trivalent influenza vaccine; TNF, tumor necrosis factor; VN, virus neutralization.

TABLE 7 Adaptive immune response in patients infected by A/2009/H1N1 virus[a]

Cell type	Study design	Results	Reference
T cell	Compare T cell function in 6 severe and 22 mild cases	T cells from severe cases had impaired effector cell differentiation and failed to respond to mitogenic stimulation; massive expression of CD95 marker found on anergic T cells, suggesting apoptosis-related mechanism	3
T cell and B cell	Compare T cells in 31 A/2009/H1N1 cases, 18 patients with flu-like illness, and 10 healthy volunteers	A/2009/H1N1-infected patients had reduced numbers of CD4 lymphocytes and B lymphocytes but increased numbers of T-regulatory lymphocytes	198
T cell	Compare T cells in 53 A/2009/H1N1 cases and 21 healthy controls	A/2009/H1N1-infected patients had T cell activation and preferential loss of Th17 subset at the early stage of infection; the functional loss of Th17 cells is likely due to upregulated IFN-α	288
	Compare T cells in 36 severe cases, 40 moderate cases, and 20 healthy volunteers	At 1-2 days from onset, the frequency of Treg cells was higher in moderate than in severe cases	222
	Compare T cells in 9 severe and 7 mild cases	Pretreatment and posttreatment $CD4^+$ and $CD8^+$ T cell counts, measured by flow cytometry, did not differ significantly between the groups ($P > 0.05$); analysis by the paired-sample *t* test showed a significant increase in posttreatment $CD4^+$T cells in the severe cases only	541
B cell	Compare B cells in 36 severe cases, 40 moderate cases, and 20 healthy volunteers	B cells were increased in severe cases	222
NK cell	Comparison between A/2009/H1N1, H5N1, and 1918 H1N1, using pseudotyped particles	Much stronger NK activation was triggered by H5N1 and 1918 H1N1 than by A/2009/H1N1	158
	Analyze the interaction of NK-activating receptors NKp46 and A/2009/H1N1 or H5N1 in both *in vitro* and mouse models	NKp46 binds to H of A/2009/H1N1, leading to killing both *in vitro* and in the mouse model; NKp46-H5N1 interactions cannot elicit direct killing of infected cells	1
	Describe the cellular immunology profile in 3 patients with rapidly progressive infection compared with 7 healthy uninfected individuals	NK cells were markedly reduced in 3 patients with progressive infection	148
	Compare NK cells in 36 severe cases, 40 moderate cases, and 20 healthy volunteers	Frequency of NK cells was lowest in severe cases	222

[a] H, hemagglutinin; IFN, interferon; NK, natural killer; Th, T helper; Treg, regulatory T cell.

phocytes were increased, T cells had impaired effector cell differentiation and proliferation, and there were fewer NK cells (Table 7).

CLINICAL MANIFESTATIONS

Similar to the case for seasonal human influenza, which is a common cause of acute upper and lower respiratory tract infections in community and health care settings, A/2009/H1N1 disease ranges from a febrile upper respiratory tract infection to fulminant primary viral pneumonia or secondary bacterial pneumonia with ARDS, requiring intensive care and respiratory support. A study demonstrated that patients who subsequently developed ARDS and succumbed had a slower decline in nasopharyngeal viral loads, had higher plasma levels of proinflammatory cytokines and chemokines, and were more likely to have viremia, bacterial coinfections, or a complication of myocarditis than patients who had mild disease or who survived with ARDS (510). Other studies demonstrated that pneumococcal coinfection played a similar role in causing mortality (200, 378, 423) as it did during the Spanish flu (112). The clinical features of A/2009/H1N1 influenza virus infection were similar to those of seasonal influenza (Table 8). However, there were notable differences between the fatal cases reported in North America and those in Asia (Table 4). The incidences of diarrhea (23.7% versus 4.3%; $P < 0.001$) and myalgia (40.9% versus 13.3%; $P < 0.001$) were significantly higher in North American patients. Regarding hospitalized patients, significantly higher rates of admission to intensive care units (ICUs) ($P < 0.001$), mechanical ventilation ($P < 0.001$), and death ($P < 0.001$) were observed in North America than in Asia (chi-square test) (Table 9) (32, 69, 75, 131, 281, 322, 365, 418, 493, 511). This difference may be due to the different thresholds of hospitalization in the different cultures. However, the lower overall clinical manifestation of diarrhea and myalgia in Asians also suggests that Asians may have a lower rate of severe disease.

In addition to upper and lower respiratory tract diseases, extrapulmonary manifestations were also reported for A/2009/H1N1 infection (Table 10) (42, 49, 61, 224, 323, 370, 376, 419, 485, 510). These manifestations included hepatitis, myocarditis, rhabdomyolysis, renal failure, systemic or pulmonary vascular thrombosis, reactive hemophagocytosis, and, in children, acute necrotizing encephalopathy or encephalopathy. Renal failure was associated with an increased risk of death. Several of these manifestations were similar to those found in patients with H5N1 infection. Influenza-associated encephalopathy has been associated with thermolabile carnitine palmitoyltransferase II variants. The disease can be rapidly fatal within 1 to 4 days of acquisition (370).

CLINICAL MANAGEMENT AND LABORATORY DIAGNOSIS

There are no pathognomonic signs and symptoms of influenza virus infections (169, 511). The clinical, routine laboratory and radiologic findings are not distinguishable from those associated with other causes of influenza-like illness, severe community-acquired pneumonia, or ARDS (577). Patients with risk factors for severe disease who present with a nonsevere influenza-like illness

TABLE 8 Clinical features of patients with laboratory-confirmed A/2009/H1N1 infection

Clinical symptom	No. with symptom/total no. (% with symptom)				
	Asia[a] (n = 2,077)	Oceania[b] (n = 221)	Europe[c] (n = 1,943)	North America[d] (n = 3,857)	South America[e] (n = 3,236)
Fever	1,660/2,077 (79.9)	187/221 (84.6)	1,567/1,943 (80.6)	3,340/3,841 (87.0)	3,032/3,236 (93.7)
Cough	1,473/2,077 (70.9)	153/167 (91.6)	1,397/1,878 (74.4)	2,274/2,701 (84.2)	2,908/3,236 (89.9)
Sore throat	836/1,995 (41.9)	30/55 (54.5)	903/1,931 (46.8)	1,379/3,408 (40.5)	2,215/3,073 (72.1)
Running nose	577/1,938 (29.8)	30/55 (54.5)	518/882 (58.7)	675/2,152 (31.4)	2,407/3,236 (74.4)
Headache	273/1,511 (18.1)	4/12 (33.3)	705/1,753 (40.2)	674/2,203 (30.6)	1,015/1,465 (69.3)
Dyspnea	138/596 (23.2)	16/43 (37.2)	434/1,428 (30.4)	1,444/2,371 (60.9)	192/233 (82.4)
Fatigue or malaise	129/603 (21.4)	23/43 (53.5)	214/273 (78.4)	112/159 (70.4)	1,370/1,782 (76.9)
Nausea or vomiting	89/936 (9.5)	NM[f]	371/1,700 (21.8)	888/2,017 (44.0)	708/3,015 (23.5)
Diarrhea	75/1,742 (4.3)	30/209 (14.4)	231/1,943 (11.9)	716/3,016 (23.7)	318/2,138 (14.9)
Myalgia	201/1,450 (13.9)	58/109 (53.2)	552/1,172 (47.1)	867/2,118 (40.9)	827/1,331 (62.1)
Arthralgia	10/271 (3.7)	NM	353/1,058 (33.4)	20/44 (45.5)	10/11 (90.9)

[a] Including hospitalized patients (1,099 cases), critically ill patients (90 cases), clustering of an outbreak (11 cases), and other (877 cases) (6, 33, 58, 87, 111, 131, 231, 257, 317, 339, 350, 396, 420, 463, 469, 509, 511).
[b] Including hospitalized patients (166 cases), hospitalized pregnant patients (43 cases), and cystic fibrosis patients (12 cases) (142, 147, 183, 255).
[c] Including hospitalized patients (631 cases), clustering of 4 outbreaks in a camp and schools (172 cases), and other (1,140 cases) (53, 154, 223, 226, 246-248, 412, 480).
[d] Including hospitalized patients (1,782 cases), transplant recipients (242 cases), hospitalized pregnant patients (128 cases), clustering of 5 outbreaks in a plane, a camp, and schools (409 cases), and other (1,296 cases) (32, 65, 69, 74, 78, 130, 281, 282, 322, 347, 363, 365, 418, 514, 547, 550).
[e] Including hospitalized patients (204 cases), critically ill patients (29 cases), and other (3,003 cases) (199, 204, 355, 399, 442, 506, 578).
[f] NM, not mentioned.

may be managed as outpatients while waiting for the results of laboratory investigations. A chest radiograph should be performed if pulmonary involvement is suspected. The decision for hospitalization is based on the clinical assessment of disease severity, whether the patient can be readily followed up, and the risk factors for severe influenza infection. Patients with risk factors for severe disease should be empirically treated with oseltamivir or zanamivir, and patients with severe pneumonia should be treated with oseltamivir and broad-spectrum antibacterials for typical and atypical bacterial pneumonia (e.g., a β-lactam plus a macrolide). Influenza-like illness is clinically defined by the abrupt onset of fever and respiratory symptoms, such as rhinorrhea, sore throat, and cough, often with myalgia or headache; therefore, the specificity of this clinical definition can be as low as 10% during noninfluenza seasons and as high as 80% during an outbreak period. Additionally, the syndrome can be caused by other respiratory viruses, such as parainfluenza virus, enterovirus, adenovirus, and metapneumovirus, and several atypical bacteria, which can be differentiated only by laboratory testing.

Specimen Collection

The best specimen for a laboratory diagnosis should contain a high viral titer and a large number of infected cells. The yield is best when the specimens are collected within the first 2 to 3 days after the onset of symptoms during the peak of viral shedding in the respiratory tract (509). Aerosol-generating procedures for specimen collection should be performed using proper infection control measures with droplet precautions and eye shielding. The first specimen should be collected before the commencement of antiviral therapy whenever possible. For upper respiratory tract specimens, a nasopharyngeal aspirate, nasopharyngeal flocked swab, nasopharyngeal rayon swab, nasal wash fluid, throat wash fluid, or throat swab can be collected, in descending order of sensitivity (409). Lower respiratory tract specimens, including sputum, endotracheal aspirate, and bronchoalveolar lavage fluid, may be more sensitive in some cases with predominantly lower respiratory tract involvement (35). Additionally, influenza virus may be detected in blood, stool, or urine (509, 510, 516). Besides respiratory specimens, fecal specimens should also be collected in a viral transport medium, such as Hanks' balanced salt solution supplemented with 0.5% bovine serum albumin or 0.1% gelatin and antibiotics. Although the viral titer decreases with storage, the virus can usually be recovered by cell culture if it is maintained in a viral transport medium at 4°C for up to 5 days. A longer

TABLE 9 Complications in hospitalized patients with laboratory-confirmed A/2009/H1N1 infection

Outcome	No. with outcome/total no. (% with outcome)[a]				
	Asia (n = 1,848)	Oceania (n = 617)	Europe (n = 2,386)	North America (n = 2,097)	South America (n = 251)
Admission to ICU	126 (6.8)	95/617 (15.4)	235 (9.8)	486/1,773 (27)	47 (18.8)
Mechanical ventilation	67 (3.6)	25/222 (11.3)	NM[b]	308/1,773 (17.4)	42 (16.7)
Death	30 (1.6)	14/617 (2.3)	57 (2.4)	145/2,097 (6.9)	13 (5.2)

[a] The chi-square test was used to assess the difference in outcomes (admission to ICU, mechanical ventilation, and death) among hospitalized patients. There were significantly higher rates of admissions to ICU ($P < 0.001$), mechanical ventilation ($P < 0.001$), and death ($P < 0.001$) in North America than in Asia. References are as follows: for patients in Asia, references 131, 493, and 511; for patients in Oceania, references 142, 147, 177, 237, and 527; for patients in Europe, references 132 and 523; for patients in North America, references 32, 69, 75, 281, 322, 365, and 418; and for patients in South America, reference 355.
[b] NM, not mentioned.

TABLE 10 Extrapulmonary manifestations in patients with laboratory-confirmed A/2009/H1N1 infection

System involved (illness)	Description[a]	Reference
Liver (hepatitis)	F/37 yr and M/57 yr with hypertension, had elevated ALT of 1.5–2 times between days 7 and 10 after ICU admission; postmortem liver biopsy of the first patient showed micro- and macrovesicular steatosis, and RT-PCR was positive for influenza A H1N1 virus	61
Heart (myocarditis)	Four H1N1 influenza virus-associated myocarditis cases based on elevated cardiac enzymes ($n = 2$), significant acute decrease in left ventricular systolic function demonstrated by the echocardiogram ($n = 3$), or histologic evidence of severe myocarditis ($n = 1$); three children presented with fulminant myocarditis, 1 with a fatal outcome and 2 requiring ECMO support	42
	Seven patients with H1N1 influenza virus-associated myocarditis, aged 3–52 yr, with 2 fatal outcomes and 3 requiring ECMO, one requiring MCS, and one requiring IABP	323
	6.8% (5/74) of patients had myocarditis, and all died	510
Heart (reversible cardiac dysfunction)	4.9% (6/123) of patients had either new or worsened left ventricular dysfunction; age ranged from 23 to 51 yr; all had preexisting medical conditions; ICU care was required for 83% (5/6); 67% (4/6) improved on follow-up echocardiograms	376
Central nervous system (acute necrotizing encephalopathy)	F/3-yr-old Italian patient had high fever and severe convulsions following 2 days of cough and diarrhea; lumbar puncture showed slightly increased pressure (170 mm H_2O); CSF contained 6 cells/mm^3 and elevated protein, numerous red cells, and normal lactate and pyruvate levels; MRI demonstrated abnormal signal intensity in the supratentorial white matter, splenium of the corpus callosum, dorsal aspect of pons, bilateral thalami, and cerebellar hemispheres with mass effect on the surrounding parenchyma suggestive of acute necrotizing encephalopathy	419
Central nervous system (encephalopathy)	Two unrelated Chinese patients, aged 3 and 4 yr, had thermolabile CPT-II variants that were associated with persistent high-fever-triggered viral infection-associated encephalopathy, multiorgan failure, and death	370
Muscle	M/56 yr receiving therapy for recurrent multiple myeloma had rhabdomyolysis with myoglobinuria that arose during convalescence from severe A/2009/H1N1 pneumonia	224
Kidney	50 critically ill patients with severe respiratory syndrome (47 confirmed cases, 3 probable cases); kidney injury, kidney failure, and need for dialysis occurred in 66.7%, 66%, and 11% of patients, respectively; kidney failure was associated with increased death (OR, 11.29; 95% CI, 1.29–98.9), whereas the need for dialysis was associated with an increase in length of stay (RR, 2.38; 95% CI, 2.13–25.75)	485
Vessel (vascular thrombosis)	7 (5.9%) of patients (18–84 yr), experienced thrombotic vascular event, involving coronary and infrarenal aorta, and femoral and iliac veins with pulmonary embolism, between 3 and 33 days after symptom onset	49
	1.4% (1/74) of patients had thrombosis in branches of pulmonary artery	510
Reticuloendothelial system (reactive hemophagocytosis)	2.7% (2/74) of patients developed reactive hemophagocytosis in lymph node or bone marrow	510

[a] ALT, alanine aminotransferase; CPT-II, carnitine palmitoyltransferase II; CSF, cerebrospinal fluid; ECMO, extracorporeal membrane oxygenation; MRI, magnetic resonance imaging; F, female; IABP, intra-aortic balloon pump; ICU, intensive care unit; M, male; MCS, mechanical circulatory support; OR, odds ratio; CI, confidence interval; RR, relative risk.

period of storage requires a temperature of −70°C to maintain viral viability (21).

Nucleic Acid Amplification

RT-PCR diagnostic tests are often targeted against the M gene, which is abundant and genetically conserved, and the H gene, which is abundant and subtype specific (153, 331, 447). The most useful laboratory test for the clinical management of A/2009/H1N1 influenza is nucleic acid amplification after reverse transcription (Table 11). In 10 studies, the overall reported sensitivity of real-time RT-PCR ranged from 90.5% to 97.6% (60, 202, 240, 286, 321, 336, 404, 422, 432, 559). The majority of the studies reported a specificity of 100%, with four exceptions (60, 404, 432, 559). Many diagnostic laboratories and commercial vendors have rapidly responded to this pandemic by providing in-house or commercially packed real-time nucleic acid amplification assays.

Antigen Detection

All commercially available immunochromatographic assays for the detection of the viral NP can differentiate between influenza A and B viruses but not between the different subtypes. The reported sensitivities ranged from 11% to 83.7% with a median of 53.3%, which is significantly inferior to that of RT-PCR or viral culture. The specificities for immunochromatographic assays ranged from 96% to 100% (Table 12) (115–117, 144, 157, 171, 191, 201, 242, 309, 315, 497, 525, 526, 540). Similarly, the direct immunofluorescent antigen detection test for the influenza virus NP had a sensitivity ranging from 38.7% to 92.8%, with a median of 64.9%, and a specificity of 94.5% to 100% (191, 202, 242, 446). However, the testing of these exfoliated cells must be performed soon after specimen collection. The cells from specimens can be washed in cold buffer and treated with *N*-acetylcysteine to remove mucus before fixation onto slides (82, 83). In some laboratories, the influenza A and B viruses, respiratory syncytial virus (RSV), adenovirus, and parainfluenza (PIF) 1, 2, and 3 viruses are concurrently detected in a common pool before a monoclonal antibody is used to differentiate the respiratory virus that is present (82, 83).

Viral Culture

The gold standard for the diagnosis of influenza virus relies on a positive culture of the respiratory secretions in cell lines, such as

TABLE 11 Evaluation and clinical utilization of molecular diagnostic tests for A/2009/H1N1 virus[a]

Test mode (commercial kit), target	Samples (clinical, RNA extract, or virus isolates), patient demographic (if available), and country	Analytical performance	Reference
Real-time RT-PCR (Luminex xTAG respiratory virus panel; Luminex Molecular Diagnostics, Toronto, Canada)	288 nasopharyngeal specimens (flocked swabs), aged 4 days-to 98 yr, USA	Sensitivity, 97.8%; specificity, 100%; PPV, 100%; NPV, 97.3%; gold standard, real-time RT-PCR by CDC protocol	202
In-house real-time RT-PCR, H1 gene	38 clinical samples,[b] Canada	Detection limit, virus in a 10^6 dilution of 4×10^6 $TCID_{50}$ /ml when 5 μl was used as the template; median (range) of C_T values, 25.9 (17–45); sensitivity, 95.2%; specificity, 100%; gold standard, amplification and direct sequencing of the products	422
In-house real-time RT-PCR, M1 gene	36 clinical samples,[b] Canada	Detection limit, virus in a 10^6 dilution of 4×10^6 $TCID_{50}$ /ml when 5 μl was used as the template; median (range) of C_T values, 29.1 (19.5–45); sensitivity, 90.5%; specificity, 100%; gold standard, amplification and direct sequencing of the products	422
In-house real-time RT-PCR, M2 gene	38 clinical samples,[b] Canada	Detection limit, virus in a 10^6 dilution of 4×10^6 $TCID_{50}$ /ml when 5 μl was used as the template; median (range) of C_T value: 27.8 (18.8–45); sensitivity, 97.6%; specificity, 100%; gold standard, amplification and direct sequencing of the products	422
In-house duplex RT-PCR, annealing temp at 55°C	198 consecutive nasopharyngeal, nasal, and throat swabs, Canada	Sensitivity, 93.9%; specificity, 100%; PPV, 100%; NPV, 97.1%; a modified "gold standard" was used[c]	336
In-house duplex RT-PCR, annealing temp at 50°C	198 consecutive nasopharyngeal, nasal, and throat swabs, Canada	Sensitivity, 77.3%; specificity, 100%; PPV, 100%; NPV, 89.8%; a modified "gold standard" was used[c]	336
In-house monoplex RT-PCR	198 consecutive nasopharyngeal, nasal, and throat swabs, Canada	Sensitivity, 80.3%; specificity, 100%; PPV, 100%; NPV, 91.0%; a modified "gold standard" was used[c]	336
In-house real-time RT-PCR	198 consecutive nasopharyngeal, nasal, and throat swabs, Canada	Sensitivity, 90.9%; specificity, 100%; PPV, 100%; NPV, 95.7%; a modified "gold standard" was used[c]	336
In-house RT-LAMP, H1 gene	260 clinical samples[b] collected from Japanese and Vietnamese patients	Detection limit, 10 RNA copies per reaction volume; sensitivity, 97.8%; specificity, 100%; gold standard, real-time RT-PCR	321
RT-LAMP (Eiken Chemical, Tokyo, Japan), H1 gene and M gene	45 nasal swabs, Japan	Sensitivity, 96.3%; specificity, 88.9%; gold standard, real-time RT-PCR	404
RT-LAMP (Eiken Chemical, Tokyo, Japan), H1 gene	56 nasal swabs, mean age of 31.6 yr (20–51 yr), Japan	Detection limit, 100 copies of virus; sensitivity, 96.3%; gold standard, real-time RT-PCR	240
StepOnePlus real-time RT-PCR (Applied Biosystems, Foster City, CA), H1 gene	50 respiratory specimens (nasopharyngeal aspirates, nasopharyngeal swabs, throat swabs), Hong Kong	Detection limit, 1.252 gene copy equivalent	328
In-house multiplex PCR, H1 and H3 genes	50 respiratory specimens (nasopharyngeal aspirates, nasopharyngeal swabs, throat swabs), Hong Kong	Detection limit, 125.2 gene copy equivalent	328
In-house conventional RT-PCR, H1 gene	50 respiratory specimens (nasopharyngeal aspirates, nasopharyngeal swabs, throat swabs), Hong Kong	Detection limit, 12.52 gene copy equivalent	328
In-house nucleic acid dipstick test with isothermal amplification and visual detection on dipstick, H1 gene	262 nasal or throat swabs; mean age, 22.4 yr (19 days–91 yr), UK	Sensitivity, 95.3%; specificity, 99.4%; PPV, 98.8%; NPV, 97.8%; gold standard, real-time RT-PCR	559
Nucleic acid lateral-flow assay based on rapid amplification and hybridization technology (Analytik Jena AG, Jena, Germany), H1 gene	174 nasal swabs, Japan	Sensitivity, 88%; specificity, 94%; PPV, 96%; NPV, 84%; gold standard: real-time RT-PCR	432
In-house real-time RT-PCR, segment 7 carrying the M1 gene	11 clinical samples,[b] Ireland	Detection limit, detect virus in 10^{-3} dilution of a clinical sample; sensitivity, 100%; specificity, 88.8%; gold standard, confirmation by reference laboratory in Health Protection Agency	60
In-house conventional 1-step RT-PCR, H1 gene	Test evaluation, Hong Kong	Detection limits for the positive control in the range of 1.0×10^{-4} of the $TCID_{50}$ per reaction; specificity, 100%	447
In-house real-time RT-PCR, H1 gene	Test evaluation, Hong Kong	Detection limits for the positive control in the range of 2.0×10^{-3} of the $TCID_{50}$ per reaction; specificity, 100%	447
In-house real-time RT-PCR, H1 gene	Test evaluation, Taiwan	Detection limit, 10 copies of H gene per reaction	568
In-house real-time RT-PCR, H1 gene	Test evaluation, Germany	Detection limit, 100–1,000 genomic copies per ml	542
In-house real-time RT-PCR, N gene	Test evaluation, Germany	Detection limit, 100–1,000 genomic copies per ml	542
In-house real-time RT-PCR, modified primer to detect mutation H275Y	Test evaluation, Israel	Analytical sensitivity, 0.014 $TCID_{50}$ with 97.2% amplification efficiency	260
Commercial POC molecular test (Xpert Flu A Panel nucleic acid amplification-based POC test; Cepheid, Sunnyvale, CA)	Test evaluation of 7 clinical samples[b] positive for influenza virus, the Netherlands	Sensitivity, 71.4%; specificity, 100%; gold standard: real-time RT-PCR	286

[a] H, hemagglutinin; N, neuraminidase; M, matrix; NP, nucleoprotein; PPV, positive predictive value; NPV, negative predictive value; POC, point of care; RT-LAMP, reverse transcription–loop-mediated isothermal amplification; RT-PCR, reverse transcription-PCR; $TCID_{50}$, 50% tissue culture infective dose; C_T, threshold cycle.

[b] The nature of the clinical samples was not specified.

[c] A modified "gold standard" was used to assess the clinical performance of all RT-PCR assays when a positive case was defined by concordant results between at least two RT-PCRs targeting different genomic regions and subsequent sequence analysis to ensure the specificities of the primers.

TABLE 12 Evaluation and clinical utilization of conventional diagnostic tests for A/2009/H1N1 virus[a]

Test mode (commercial kit), target	Samples (clinical, RNA extract, or virus isolates), patient demographic (if available), and country	Analytical performance	Reference
Rapid antigen detection (Binax Now A+B)	144 clinical samples,[b] median age of 18 yr (1–59 yr), Germany	Sensitivity, 11.1%; gold standard, real-time RT-PCR for H gene	157
	84 nasopharyngeal specimens, USA	Sensitivity, 38.3%; specificity, 100%; PPV, 100%; NPV, 88.2%; gold standard, real-time RT-PCR using Luminex xTAG RVP (Luminex)	525
	820 nasopharyngeal specimens (flocked swabs), median age of 3.4 years (1 mo–17 yr), Canada	Sensitivity, 62%; specificity, 99%; gold standard, conventional RT-PCR	242
	354 nasopharyngeal specimens (flocked swabs), median age of 43 yr (18–89 yr), Spain	Sensitivity, 32%; specificity, 100%; PPV, 100%; NPV, 67%; gold standard, real time RT-PCR (Applied Biosystems real-time RT-PCR)	201
	254 nasopharyngeal specimens (flocked swabs), median age of 14.1 yr (7 mo–53 yr), South Korea	Sensitivity, 83.7%; specificity, 100%; gold standard: real-time RT-PCR	117
	1 nasopharyngeal aspirate specimen collected on day 3 of illness, Hong Kong	Detection limit, 6.8 copies M gene/ml	81
Rapid antigen detection (Directigen EZ A+B)	84 nasopharyngeal specimens, USA	Sensitivity, 46.7%; specificity, 100%; PPV, 100%; NPV, 89.6%; gold standard, real-time RT-PCR using Luminex xTAG RVP (Luminex)	525
	1 nasopharyngeal aspirate specimen collected on day 3 of illness, Hong Kong	Detection limit, 6.1 copies M gene/ml	81
Rapid antigen detection (Espline)	1 nasopharyngeal aspirate specimen collected on day 3 of illness, Hong Kong	Detection limit, 5.8 copies M gene/ml	81
Rapid antigen detection (QuickVue A+B)	39 clinical samples,[b] USA	Sensitivity, 51%; specificity, 99%; gold standard, real-time RT-PCR	171
	84 nasopharyngeal specimens, USA	Sensitivity, 53.3%; specificity, 100%; PPV, 100%; NPV, 90.8%; gold standard, real-time RT-PCR using Luminex xTAG RVP (Luminex)	525
	174 nasal or throat swabs, Australia	Sensitivity, 53.4%; specificity, 100%; PPV, 100%; NPV, 76.2%; gold standard, real-time in-house RT-PCR using a TaqMan probe	315
	418 patients (aged 6 mo–14 yr) with nasal and throat swabs, Thailand	Sensitivity, 62.7%; specificity, 99.2%; gold standard, real-time RT-PCR	497
	360 nasal specimens, median age of 13.7 yr (6 mo–73 yr), Philippines	Sensitivity, 63%; specificity, 96%; PPV, 97%; NPV, 57%; gold standard, conventional RT-PCR	526
	526 respiratory specimens (nasopharyngeal swabs, pharyngeal washes, bronchoalveolar lavage fluid samples), Germany	Sensitivity, 18.2%; specificity, 100%; PPV, 100%; NPV, 78.1%; gold standard, real-time RT-PCR	191
	1 nasopharyngeal aspirate specimen collected on day 3 of illness, Hong Kong	Detection limit, 6.5 copies M gene/ml	81
Rapid antigen detection (Binax Now A+B and 3 M Rapid Detection Flu A+B test)	288 nasopharyngeal specimens (flocked swabs), aged 4 days–98 yr, USA	Sensitivity, 17.8%; specificity, 93.6%; PPV, 77.4%; NPV, 47.9%; gold standard, real-time RT-PCR	202
Rapid antigen detection[c]	1,599 clinical samples[b] from pediatric patients, USA	Sensitivity, 17.8%; specificity, 93.6%; gold standard, real-time RT-PCR using Luminex xTAG RVP (Luminex)	540
Rapid antigen detection (SD Bioline Influenza Ag Standard Diagnostics for H)	759 nasopharyngeal specimens (flocked swabs), aged 2 wk–83 yr, South Korea	Sensitivity, 77%; specificity, 100%; PPV, 100%; NPV, 86%; gold standard, real-time RT-PCR	116
Rapid antigen detection [SD Bioline Influenza Ag A/B/(H1N1) Pandemic for H]	260 clinical samples,[b] aged 2 mo–78 yr, South Korea	Sensitivity, 70%; specificity, 98.4%; PPV, 94.3%; NPV, 89.7%; gold standard, real-time RT-PCR	309
Rapid antigen detection (SD Bioline Influenza Ag, Standard Diagnostics for NP)	260 clinical samples,[b] aged 2 mo–78 yr, South Korea	Sensitivity, 58.8%; specificity, 99.6%; PPV, 98.1%; NPV, 86.5%; gold standard, real-time RT-PCR	309
Rapid antigen detection (SD Bioline Influenza Ag)	254 nasopharyngeal specimens (flocked swabs), median age of 14.1 yr (7 mo–53 yr), South Korea	Sensitivity, 69.5%; specificity, 100%; gold standard, real-time RT-PCR	117
	938 throat or nasopharyngeal swabs, all patient over 15 yr, South Korea	Sensitivity, 44%; specificity, 99.9%; gold standard, real-time RT-PCR	115
Rapid antigen detection (Wondfo)	1 nasopharyngeal aspirate specimen collected on day 3 of illness, Hong Kong	Detection limit 5.8 copies M gene/ml	81
Rapid antigen detection (ClearView Exact Influenza A & B)	1,016 oropharyngeal and nasopharyngeal swabs, median age of 45 yr (3 mo–97 yr), Spain	Sensitivity, 19%; specificity, 100%; PPV, 100%; NPV, 75%; gold standard, real time RT-PCR	144
Rapid antigen detection (DFA test)	288 nasopharyngeal specimens (flocked swabs), aged 4 days–98 yr, USA	Sensitivity, 46.7%; specificity, 94.5%; PPV, 91.3%; NPV, 58.9%; gold standard, real time RT-PCR	202
	111 nasopharyngeal specimens (109 flocked swabs and 3 aspirates), median age of 44.1 yr, USA	Sensitivity, 92.8%; specificity, 97.1%; gold standard, real-time RT-PCR	446
	820 nasopharyngeal specimens (flocked swabs), median age of 3.4 yr (1 mo–17 yr), Canada	Sensitivity, 83%; specificity, 96%; gold standard, conventional RT-PCR	242
	526 respiratory specimens (nasopharyngeal swabs, pharyngeal washes, bronchoalveolar lavage fluid samples), Germany	Sensitivity, 38.7%; specificity, 100%; PPV, 100%; NPV, 82.2%; gold standard, real-time RT-PCR	191
ELISA[d] for IgG antibodies	783 serum samples, India	Compared to hemagglutination inhibition test, concordance of 98.4%	14
Immunochromatography, monoclonal antibodies against NP	Clinical samples[b] from 5 patients with PCR-confirmed pandemic influenza A (H1N1), Japan	Detection limit, 2×10^5 copies/kit; sensitivity, 100%; specificity, 100%; gold standard, real time RT-PCR	389
Viral culture (R-mix viral culture)	288 nasopharyngeal specimens (flocked swabs), aged 4 days–98 yr, USA	Sensitivity, 88.9%; specificity, 100%; PPV, 100%; NPV, 87.9%; gold standard, real-time RT-PCR	202
Viral culture, MDCK cells	526 respiratory specimens (nasopharyngeal swabs, pharyngeal washes, bronchoalveolar lavage fluid samples), Germany	Sensitivity, 45.7%; specificity, 99.8%; PPV, 95.5%; NPV, 94.8%; gold standard, real-time RT-PCR	191

[a] DFA, direct fluorescent antibody; H, hemagglutinin; MDCK, Madin-Darby canine kidney; M, matrix; N, neuraminidase; NP, nucleoprotein; PPV, positive predictive value; NPV, negative predictive value; RT-PCR, reverse transcription-PCR; ELISA, enzyme-linked immunosorbent assay.
[b] The nature of the clinical samples was not specified.
[c] The details of rapid antigen detection were not specified.
[d] Recombinant hemagglutinin protein-based enzyme-linked immunosorbent assay.

the Madin-Darby canine kidney (MDCK) or rhesus monkey kidney cell line, in the presence of trypsin (329). Most continuous cell lines do not produce proteases that cleave H to make infectious viral progeny, with the exception of Caco2 cells, which can produce trypsin-like proteases (349). Tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-treated trypsin at 1 to 2 μg/ml is added to the cell culture medium. TPCK treatment will inactivate the chymotrypsin in a pancreatic extract, whose proteolytic activity on H will nullify the trypsin-mediated enhancement of viral infectivity. Fetal calf sera contain inhibitory factors that must be removed by washing with Hanks' buffer prior to infection. Incubation with serum-free culture medium with trypsin at 33 to 35°C will usually produce a cytopathic effect within 1 to 7 days. Additional strains may be recovered with prolonged incubation of up to 14 days after one blind passage. The interval between inoculation and detection ranges from 2 to 14 days, with a median of 3 to 5 days (346). The R-mix cell line (a mixture of mink lung and A549 cell lines) was reported to have a sensitivity of 88.9%, whereas the MDCK cell line had a sensitivity of only 45.7%, compared with real-time RT-PCR as the gold standard (191). In addition to identifying the microscopic changes of cytopathic effects, positive cultures can be detected by hemadsorption. Influenza virus isolates can be typed as type A or B and subtyped by immunofluorescence. In contrast to nucleic acid amplification, antigen detection, and antibody assays, viral culture is less affected by genetic or antigenic changes. Cell culture had a specificity of nearly 100% (191, 202).

Antibody Testing

The detection of specific antibodies in serum may be used when the specimens for viral isolation or RNA or antigen detection are negative, inadequate, or unavailable. Antibody testing has detected up to one-third of severe cases admitted to ICUs when the RT-PCR results were negative (277). However, this testing cannot differentiate different lineages of the same subtype of the influenza A virus. The majority of individuals have already been exposed to the seasonal influenza A virus, which has almost identical NP and matrix protein sequences; therefore, a 4-fold rise in the complement fixation antibody titer with these antigens indicates a recent influenza A virus infection without differentiating between seasonal H1N1 or H3N2 infection and the pandemic 2009 H1N1 infection (14). Although recombinant H has been found to be sensitive and specific for antibody detection by EIA, cross-reactivity between seasonal H1N1 and pandemic H1N1 may still occur, because their overall amino acid identity was 80.1% for H (440 out of 549 amino acids), 72% for HA1, and 92% for HA2 (Fig. 1). Therefore, IgG and IgM detection by EIA is generally not useful, except in the case of avian H5N1 infection in humans, where recombinant H5 was used in a Western blot immunoassay (44). The traditional gold standard techniques for detecting influenza virus-specific antibodies are neutralization and hemagglutination inhibition assays, which may detect subtype-specific or lineage-specific serological responses. Human influenza viruses can agglutinate turkey, human, and guinea pig erythrocytes; however, most laboratories routinely use turkey erythrocytes for hemagglutination inhibition antibody tests. Turkey erythrocytes are small and nucleated and sediment quickly, producing a clear and reproducible endpoint. Turkey erythrocytes express a mixture of α-2,3- and α-2,6-linked sialic acid receptors and can be used for the detection of antibodies against either human or avian influenza viruses, whereas the avian influenza A H5N1, H9N2 and H7N7 viruses agglutinate horse erythrocytes, which almost exclusively express α-2,3-linked sialic acid receptors (273). Antibody levels accurately correlate with protection from (or susceptibility to) disease and vaccination status (84). A definitive serological diagnosis of acute influenza requires the demonstration of a 4-fold increase in antibody titers on paired acute- and convalescent-phase serum samples. A seroprevalence study using the hemagglutination inhibition test was useful in 2009 when the population was immunologically naïve except for elderly patients, who may have been previously exposed to the 1976 swine influenza virus or the 1918 Spanish influenza virus, both of which have cross-reactivity to the A/2009/H1N1 virus (45). Since changes in the H proteins of influenza A H3N2 and influenza B viruses have markedly diminished their ability to agglutinate avian erythrocytes, including turkey erythrocytes since late 1990s, the neutralizing antibody test may be a better option for these viruses.

Though it is more labor-intensive and still could be confounded by cross-reactivity, the neutralizing antibody test is often considered the gold standard serological test for immunity against different strains of influenza virus. A retrospective analysis of the serum antibody responses assayed by neutralizing antibody titers against the A/2009/H1N1 virus in 881 convalescent patients demonstrated that 90% of them had a seroprotective titer of 1:40 or above (265). A multivariate analysis by ordinal regression showed that pneumonia and sputum production were two independent factors associated with higher levels of convalescent-phase neutralizing antibody titers. Patients who were afebrile on presentation were associated with a subsequent poor neutralizing antibody titer ($<$1:40). A positive correlation between the nasopharyngeal viral load on presentation and the convalescent neutralizing antibody titer was demonstrated. Convalescent patients with a high neutralizing antibody titer were suitable candidates for the donation of plasma for passive immunotherapy (265).

Antiviral Susceptibility Testing

Viral susceptibility to adamantanes can be detected by standard cell protection assays, whereas the inhibition of the enzymatic activity of N is the most sensitive and specific phenotypic means of detecting resistance to neuraminidase inhibitors, such as oseltamivir, peramivir, and zanamivir. The standard methods for detecting antiviral resistance based on changes in the viral phenotype in cell culture, such as the plaque reduction and yield reduction assays, were not reliable for the detection of clinical isolates resistant to neuraminidase inhibitors (217, 218). The contradictory findings from cell culture- and enzymatic activity-based assays suggested that the H of clinical isolates may bind suboptimally to the α-2,3-linked sialic receptors of the MDCK cells used in cell protection assays, because human influenza viruses bind preferentially to α-2,6-linked sialic acid. When H binds with a lower affinity to MDCK cell receptors, the virus is less dependent on the neuraminidase activity of N for its release; therefore, the virus appears to be less sensitive to neuraminidase inhibitors *in vitro*. Zanamivir susceptibility in animal challenge models correlated well with *in vitro* susceptibility as determined by the neuraminidase inhibition assay but not by the plaque reduction assay in MDCK cells (507). The majority of laboratories currently use a phenotypic assay with chemiluminescent or fluorescent substrates (NAStar; Applied Biosystems, Foster City, CA) to detect the susceptibility of the enzymatic activity of N to neuraminidase inhibitors.

A rapid genotypic assay for the detection of mutations causing resistance against adamantanes and neuraminidase inhibitors can be performed by standard PCR sequencing and, more recently, by pyrosequencing without the need for viral culture (324). Multiplex PCR can simultaneously detect the presence of the A/2009/H1N1 virus and the mutations responsible for the resistance (29, 368). Methods that can detect the resistant strains among a mixed population include pyrosequencing (146), high-resolution melting curve analysis (102), and, in research laboratories, deep sequencing (197). Other methods include RT-PCR and the restriction fragment length polymorphism assay (415). However, pyrosequencing can detect the emergence of resistant quasispecies in treated individuals without laborious cloning and Sanger sequencing. The clinical significance of viral quasispecies in drug resistance is undetermined except in immunosuppressed hosts in whom resistant viral quasispecies have emerged early in treatment (22). In this group of patients, prolonged viral carriage and shedding provided an opportunity for the viruses to pick up compensatory mutations that maintain the resistance after selective drug pressure is removed.

ANTIVIRAL AND IMMUNOMODULATORY THERAPIES

Antiviral Treatment

The antivirals against influenza A virus that are currently available and commonly used include the adamantanes (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir), but only the latter are active against the A/2009/H1N1 virus. Oseltamivir is available orally. Zanamivir is available either as a dry powder that is delivered by oral inhalation or, more recently, as an intravenous formulation. Nebulized zanamivir is useful in selected cases (135). However, nebulization of the powder form of zanamivir that is dissolved in water has been found to clog the ventilator circuit and should not be used (179). Intravenous peramivir was available only for compassionate use in the United States during the pandemic (34). In addition to neuraminidase inhibitors, the traditional Chinese medicine maxingshigan-yinquaosan has been shown to hasten fever resolution in a randomized trial, but its action may be immunomodulatory rather than antiviral (534). The role of other newer antivirals, such as nucleozin analogs (295), the neuraminidase inhibitor CS-8958 (311), the polymerase inhibitors viramidine (474) and T-705 (312), and sialidase DAS181 (27), requires further evaluation (243). Combination therapy with the protease inhibitor aprotinin and other antivirals may be effective, but the side effects of aprotinin treatment, including stroke, heart attack, kidney failure, thrombosis, and anaphylaxis, must be considered (582).

Randomized controlled trials with patients having seasonal influenza suggested that neuraminidase inhibitors shortened the duration of illness by approximately 1 day (245). However, most authorities would not recommend routine oseltamivir treatment for mild illness because of the risk of fostering drug resistance. For the A/2009/H1N1 virus, oseltamivir therapy has generally been associated with a faster resolution of symptoms and a more rapid clearance of viral shedding in hospitalized patients of all ages, organ transplant recipients, and pregnant women (350, 357, 575) (Table 13). A longer delay of oseltamivir treatment in severe cases was associated with a poorer outcome (281, 574) because viral shedding in the respiratory tract peaks at 24 to 72 h after infection. Since viral shedding is already substantial even before the onset of symptoms, the efficacy of treatment decreases sharply with any delay in antiviral administration.

Intravenous zanamivir and peramivir have been used in patients with severe disease (Table 14). These antivirals have *in vitro* activity against the A/2009/H1N1 virus and have been beneficial in some cases; however, no randomized controlled trials or comparative clinical trials have been reported (238, 303). Oseltamivir-zanamivir combination therapy has not been studied in patients with A/2009/H1N1 influenza, but it has been found to be less effective than oseltamivir monotherapy for the treatment of seasonal influenza (161). The optimal dosing for intravenous zanamivir is not known, but 600 mg every 12 h has been shown to be effective for oseltamivir-resistant strains of the A/2009/H1N1 virus in an *in vitro* hollow-fiber infection model (47).

Ribavirin has been used for the treatment and prophylaxis of influenza (90). The routes of administration include oral, intravenous, and aerosolization routes. Anecdotal reports have demonstrated the efficacy of this therapy against influenza A and B, including infections in immunosuppressed hosts. Unfortunately, a consistent benefit has not been observed in all of the clinical trials, and intravenous ribavirin is currently not considered a drug of choice for the treatment of influenza because of its toxicity. Similar to the case for inhaled zanamivir, inhaled ribavirin may not be able to penetrate the poorly aerated consolidations of pneumonic lungs.

Antiviral Resistance

All A/2009/H1N1 strains are resistant to adamantanes due to the S31N mutation in the M2 protein (79, 554). Globally, oseltamivir resistance due to the H275Y (by N1 numbering) substitution in neuraminidase accounts for fewer than 2% of the strains tested (554). In addition, H275Y strains are resistant to peramivir but remain susceptible to zanamivir. The differential antiviral susceptibility is due to the differences between the binding mechanisms of oseltamivir and zanamivir. The binding of oseltamivir to the active site of neuraminidase requires a conformational change, whereas this change is not required for zanamivir (395). It is interesting to note that the *in vitro* triple combination of oseltamivir, amantadine, and ribavirin is highly synergistic against the A/2009/H1N1 virus (411).

Oseltamivir resistance has been reported in patients without prior exposure to oseltamivir (Table 15), and it can develop quickly after oseltamivir treatment (272). It is especially common in immunosuppressed hosts, in whom a higher viral load is expected due to the poor control by the host immune response. The nosocomial spread of an oseltamivir-resistant influenza virus has been documented in a clinical setting (392). Other mutations (E119G, E119V, and I222V) have been found to confer oseltamivir resistance *in vitro*, but they have not been reported in clinical strains (445).

The S247N mutation, which was found in 30% of the specimens from northern Australia, results in 6-fold and 3-fold reductions in susceptibility to oseltamivir and zanamivir, respectively (266). Despite this low level of resistance *in vitro*, there were reports of clinical treatment failure (410, 521). The combined S247N and H275Y mutations resulted in a >5,000-fold reduction in oseltamivir susceptibility. A structural analysis using computer modeling suggested that the S247N mutation pushes the E277 residue deeper into the drug binding pocket, resulting in reduced oselta-

TABLE 13 Clinical or virological response of different patient groups started on antiviral therapy at different time after onset of A/2009/H1N1 infection[a]

Country(ies)	Population	Day of illness at initiation of antiviral treatment	Findings	Reference
China	Hospitalized patients during containment phase	≤2 vs >2 (no. of patients in each group not mentioned)	Late treatment associated with longer viral shedding duration (OR, 4.46)	58
Singapore	Hospitalized patients during containment phase	≤2 (n = 36) vs >2 (n = 34)	Patients with earlier treatment had shorter viral shedding duration	357
Hong Kong	Hospitalized patients during containment phase	≤2 (n = 83) vs >2 (n = 35)	Patients with earlier treatment had faster viral load reduction and shorter viral shedding duration	350
USA	Hospitalized patients	≤2 (n = 75) vs >2 (n = 120)	Late treatment was associated with ICU admission or death	281
USA	Hospitalized patients	≤2 (n = 36) vs >2 (n = 40)	Early treatment was associated with shorter lengths of stay in hospital (P = 0.03)	75
Spain	Hospitalized patients	≤3 (n = 297) vs >3 (n = 288)	Early treatment was associated with nonsevere disease (OR, 0.32)	529
Canada, USA	Solid-organ transplant recipients	≤2 (n = 90) vs >2 (n = 125)	Late treatment was associated with ICU admission (P = 0.007)	322
Argentina	Solid-organ transplant recipients	NA	Late treatment was associated with more severe disease (P = 0.008)	482
Vietnam	Hospitalized patients	NA	Estimated median clearance times between 2.6 and 2.8 days posttreatment for illness-to-treatment intervals of 1 to 4 days	257
USA	Pregnant women	≤2 (n = 30) vs >2 (n = 30)	Late treatment was associated with ICU admission or death (RR, 4.3)	363
USA	Pregnant women	≤2 (n = 30) vs 3-4 (n = 14) vs ≥5 (n = 9)	Late treatment was associated with severe illness (P = 0.002)	128
France	Pregnant women	<3 (n = 237) vs 3-5 (n = 39) vs >5 (n = 23)	Late treatment was associated with severe disease (3-5 days, adjusted OR = 4.78; >5 days, adjusted OR = 61.24)	159
USA	Children	≤2 (n = 5) vs >2 (n = 6)	No difference in length of ICU stay	361
China	All ages	<5 (n = 2,858) vs ≥5 (n = 3,807)	Increased risk of severe disease (OR, 1.42)	574

[a] ICU, intensive care unit; OR, odds ratio; RR, relative risk; NA, not applicable.

mivir binding. Additionally, the I223R and I223K substitutions in neuraminidase can result in reduced susceptibility to zanamivir (410, 521).

There has been controversy regarding the fitness and virulence of resistant mutants. The H275Y mutation in the A/2009/H1N1 virus has been shown to reduce viral replication *in vitro* (46). However, H275Y mutants were as virulent as wild-type viruses in mouse and ferret models, with no reduction in transmissibility (230, 383).

Safety of Antivirals

Neuraminidase inhibitors are relatively safe. Reports from Japan suggested that oseltamivir may be associated with neurotoxicity in adolescents. Oseltamivir and its metabolites may have excitatory effects on the central nervous systems of rats, which may account for some of the neuropsychiatric side effects that have been observed in the past few years with more widespread use of the drug (278). The safety of these agents in infants and pregnant women is unknown. Oseltamivir and zanamivir are currently approved for children over 1 and 7 years of age, respectively. Limited studies have demonstrated that oseltamivir is safe in infants under 1 year of age (136, 310). Currently, there is no evidence that oseltamivir is teratogenic, but it is classified as FDA category C due to the lack of data. Additional safety data should be collected due to the increasing use of intravenous zanamivir and peramivir (54, 314, 495).

Passive Immunotherapy

There is no definitive evidence that antivirals work in severe cases when patients present late after the onset of symptoms. Thus, modulation of the host immune response by dampening down the proinflammatory damage with steroids, statins, and macrolides has been studied (Table 16); however, none of these drugs were tested in the setting of a randomized controlled trial. Convalescent-phase plasma is another potentially useful form of therapy for severe influenza due to the influenza A virus. Convalescent-phase plasma has been used in one patient with A/H5N1 infection, with good clinical and virological responses (585). A meta-analysis of the use of convalescent-phase blood products during the Spanish influenza pandemic showed that there was a survival benefit in patients who received this treatment, and the benefit was greater if treatment was initiated within 4 days of the onset of pneumonia (367). Based on these findings, a prospective cohort study was conducted on the treatment of patients with severe A/2009/H1N1 infection using convalescent-phase plasma collected from sur-

TABLE 14 Use of intravenous antivirals for A/2009/H1N1 infection[a]

Country	Population	Findings	Reference
USA	Case series; 20 adults and 11 children had rapidly progressing radiologically confirmed viral pneumonia with respiratory failure	Peramivir was administered for 1–14 days (median duration, 10 days); survival rate at 14 days, 76.7%; survival rate at 28 days, 66.7%; survival rate at 56 days, 59%; no reports of serious adverse events	253
USA	33 received peramivir; 8 received i.v. zanamivir	27.7% mortality	186
Germany	2 patients with ARDS and ICU admission (M/39 yr, with no underlying disease, and M/49 yr, a smoker with diabetes mellitus, nephropathy, and hypertension)	Both patients improved after i.v. zanamivir	238
UK	F/22 yr with neutropenic fever after chemotherapy for Hodgkin's disease	High level of H1N1 RNA still detected after 6 days of oseltamivir and nebulized zanamivir; after change to i.v. zanamivir and methylprednisolone, patient improved	303
Germany	M/2 yr, after liver transplant for Caroli's disease	During i.v. zanamivir treatment, no clinical improvement but liver and renal function worsened	151
USA	F/18 mo, undergoing allogeneic matched related stem cell transplant	Regimen was well tolerated and associated with a decrease in viral burden	160
Canada	Case report; M/50s, allogeneic stem cell transplant recipient	Proportion of H275Y mutant increased during peramivir treatment but decreased after treatment stopped	456
Japan	Case report; F/40s, diabetic nephropathy	Rapid improvement in respiratory failure after peramivir	405
USA	Case report; F/40s, multiple myeloma	Respiratory failure despite oseltamivir, amantadine, ribavirin, and IVIG; improvement after peramivir	57
USA	2 critically ill adult patients on CVVHDF; daily infusion of 600 mg over 30 min while on CVVHDF; study to describe pharmacokinetics	C_{max} = 18,400 and 20,300 ng/ml; plasma $t_{1/2}$ = 7.6 and 3.7 h; V_d = 0.51 and 0.54 liters/kg	24

[a] ARDS, acute respiratory distress syndrome; C_{max}, maximum concentration; CVVHDF, continuous venovenous hemodiafiltration; F, female; ICU, intensive care unit; i.v., intravenous; IVIG, intravenous immunoglobulin; M, male; $t_{1/2}$: half-life; V_d, volume of distribution.

viving patients (264, 557). In this study, a number of practical limitations were encountered during the collection of convalescent-phase plasma by apheresis (548). These limitations included the failure of donors to meet blood donation eligibility criteria, failed laboratory tests, insufficient neutralizing antibody titers, and the inability of donors to attend the apheresis appointment. Despite these limitations, 276 liters of convalescent-phase plasma were collected from more than 60% of the potential donors with sufficient neutralizing antibody titers. None of the patients in the treatment group developed adverse effects after convalescent serotherapy. The mortality in the treatment group was significantly lower than that in the nontreatment group. In addition, the reductions of viral load and the corresponding cytokine/chemokine were greater in the treatment group. These two studies demonstrated that passive immunotherapy was feasible (344) and that the treatment of severe A/2009/H1N1 infection with convalescent-phase plasma appeared safe and effective, which has important implications for treatment strategies using passive immunotherapy in future pandemics. However, these findings should be confirmed in randomized controlled trials.

Broadly neutralizing antibodies against the stalk of H and the M2 ectodomain may be important in passive immunization. Humanized neutralizing anti-M2e monoclonal antibodies were shown to protect against lethal challenge by H5N1 and H1N1 viruses (210). A monoclonal antibody that targeted the conserved F subdomain of HA2 has been shown to protect mice and ferrets from both H1N1 and H3N2 infections (122). A randomized controlled trial of treatment with specific human monoclonal antibodies or hyperimmune intravenous immunoglobulin in patients with severe influenza infection is warranted.

Controversial Use of Steroids and Other Immunomodulators

The use of corticosteroids in the treatment of A/2009/H1N1 influenza is controversial because no randomized controlled trials have been conducted. Some studies have shown a beneficial effect of corticosteroids, whereas other studies showed no effect or even a detrimental effect (Table 16). One study demonstrated that the risk of death was higher in the corticosteroid group only in patients who received early administration of corticosteroids within 3 days of mechanical ventilation (48). Case reports have indicated that therapy with etoposide and betamethasone was beneficial in a patient with hemophagocytic lymphohistiocytosis (251). High-dose *N*-acetylcysteine has been shown to reduce the inflammatory response (327). Statins that were started before or after influenza infection were not associated with better outcomes (43, 528). Animal and cell culture models have suggested that agents such as paracetamol and mesalazine may reduce damage, but these findings remain to be confirmed in humans (334, 549, 581). In mice, activation of the innate immune system by PIKA, a chemical analog of dsRNA, is associated with a reduction in viral replication in the respiratory tract (333). Recently, an anti-inflammatory immunomodulator, a sphingosine analog, was successfully used in combination with oseltamivir to improve the survival of A/2009/H1N1-infected mice by significantly blunting the cytokine storm and tissue injury. In contrast to steroids, this novel approach does not suppress the protective adaptive immune response of the host against the invading virus and is a more logical approach for treating patients with severe disease (532).

TABLE 15 Oseltamivir resistance of A/2009/H1N1 virus[a]

Country (total no. of isolates tested)	Population with resistance gene	Mutation(s) (N1 numbering), IC_{50} (nM) (if available)	Change of antiviral; outcome	Reference
Worldwide (close to 1,000)	3 patients (Denmark, Japan, Hong Kong)[b]	NM	No change; survived	552
Worldwide (NA)	39 patients (32 with information); 16 associated with treatment, 7 immunosuppressed, 13 with chemoprophylaxis, 3 with no history of oseltamivir treatment or prophylaxis	H275Y	NM	551
USA (3,359)	0.7% were resistant	H275Y	NA	219
USA (6,740)	0.5% were resistant; among the patients with resistant strains, 76% were immunocompromised and 86% received oseltamivir before specimen collection	H275Y		209
Canada (804)	5 patients (0.6%)	H275Y	NA	362
Australia (71 pretreatment and 25 posttreatment isolates)	3 in posttreatment samples (M/62 yr allogeneic stem cell transplant recipient, M/47 yr immunocompetent, F/19 mo with malignancy)[c]	H275Y	M/47 yr received alternative therapy; all recovered	533
Australia (17 with HSCT, 15 with malignancy)	4 patients[c]	H275Y	Changed to zanamivir in 1 patient; 2 died	513
Scotland (1,640)	15 immunocompromised patients[c]	H275Y	NM	239
UK (2,864)	27 cases	H275Y	NA	325
Italy (186)	F/2 yr with acute lymphoid leukemia[c]	H275Y	No change; survived	56
Italy (31)	3 strains	H275Y	NA	450
Germany (1,570)	8 patients	H275Y, 200-400	NA	163
Spain (1,229)	8 patients (1.93%)	H275Y	NA	338
Spain (14)	2 patients (M/40 yr with HIV, M/49 yr with hematological malignancy)	H275Y	M/49 yr; oseltamivir was changed to zanamivir	7
Asia-Pacific region (1,488)	Singapore, 3.1%; Australia, 1.3%	H275Y	NA	267
Japan (75)	1 strain	H275Y, 46	NA	300
Japan (253)	3 patients	H275Y (2 patients), N295S (1 patient)	NA	394
United Arab Emirates (96)	8-yr-old child who received prophylactic oseltamivir	H275Y	NM	5
Hong Kong (95)	F/16 yr from San Francisco, CA, hospitalized for isolation[d]	H275Y,[e] 197.5	No change; survived	93
Mexico (692)	8-mo-old patient	H275Y, 27.3	NM	454
USA	2 adolescent girls attending summer camps who received oseltamivir prophylaxis[c]	H275Y, I223V	Changed to zanamivir in 1 patient; survived	71
Canada	M/59 yr with COPD and taking prednisolone 5mg daily (emergence during prophylaxis)[c]	H275Y, >400	NM	23
USA (4)	Nosocomial transmission of oseltamivir-resistant A/2009/H1N1 in immunocompromised patients	H275Y	NA	98
Singapore	F/28 yr, developed resistance within 48 h of oseltamivir treatment	H275Y	NA	272

[a] Case reports of oseltamivir-resistant influenza infection not shown in this table are in references 11, 88, 160, 168, 194, 197, 257, 259, 384, 488, 505, and 590). COPD, chronic obstructive pulmonary disease; F, female; HSCT, hematopoietic stem cell transplant; M, male; NA, not applicable; NM, not mentioned; IC_{50}, 50% inhibitory concentration.
[b] History of oseltamivir therapy was unknown.
[c] Oseltamivir resistance developed after oseltamivir therapy.
[d] Oseltamivir resistance occurred before oseltamivir therapy.
[e] Quasispecies.

EXTRACORPOREAL MEMBRANE OXYGENATION

Of the hospitalized patients with A/2009/H1N1 infection, 10% to 44% required intensive care, and a significant proportion of these patients developed ARDS. Moreover, those patients with A/2009/H1N1 infection who required intensive care were significantly younger than and had fewer underlying medical conditions than patients with seasonal influenza infection. Because of the encouraging results from the CESAR (conventional ventilatory support

TABLE 16 Use of immunomodulators for treatment of severe A/2009/H1N1 infection[a]

Country(ies)	Population	Immunomodulator	Concomitant therapy	Findings	Reference
South Korea	245 ICU patients; 107 received corticosteroid	Corticosteroid (given within 2 days of ICU admission): hydrocortisone, 50%; methylprednisolone, 38%; other corticosteroid, 12%	No significant difference in oseltamivir use between steroid group and nonsteroid group	90-day mortality significantly higher in those with corticosteroid (58%) than those without (27%); corticosteroid group more likely to have superinfection	306
France	208 ICU patients with ARDS; 83 received corticosteroid	Corticosteroid (60.2% given within 3 days of ICU admission): hydrocortisone, 57.8%; methylprednisolone, 37.3%; prednisolone, 4.8%	No significant difference in oseltamivir use between steroid group and nonsteroid group	Corticosteroid associated with death (adjusted hazard ratio, 2.82); subgroup analysis showed that only early administration of corticosteroids within 3 days of mechanical ventilation was associated with the increased risk of death, not administration at >3 days	48
Spain, Brazil, UK, Portugal	220 ICU patients; 126 received corticosteroid	Corticosteroid at ICU admission: >24 mg/day methylprednisolone or >30 mg/day prednisolone	No significant difference in oseltamivir use between steroid group and nonsteroid group	Corticosteroid treatment associated with increased risk of hospital acquired pneumonia (OR = 2.2) and ICU mortality (OR = 3.8)	377
China	155 hospitalized patients; 52 received corticosteroid	Corticosteroid: median methylprednisolone dose, 80 mg (or equivalent dose)	80.6% of all patients received oseltamivir	Corticosteroid treatment associated with a trend toward higher hospital mortality ($P = 0.052$)	560
Argentina	13 adult patients with ICU admission; all received corticosteroid	Corticosteroid (intravenous hydrocortisone 100 mg every 8 h until ICU discharge, then 100 mg every 12 h for 7 days and 100 mg daily for 7 days); for patients with ARDS, hydrocortisone was changed to methylprednisolone at 1 mg/kg/day for 14 days, then 0.5 mg/kg/day for 7 days, then tapered over 6 days	High-dose oseltamivir (150 mg twice daily for 5 days, then 75 mg twice daily for 3 to 5 days)	Significant reduction in CRP, APACHEII, lung injury, and SOFA; 15% mortality	453
Hong Kong	93 patients (20 patients with plasma therapy)	Convalescent-phase plasma	All received high-dose oseltamivir	Multivariate analysis showed that plasma treatment reduced mortality (OR = 0.2)	264
Australia	5 patients	IVIG	3 patients received ECMO, 2 patients received intravenous zanamivir	3 patients had clinical improvement; 2 patients had respiratory deterioration following IVIG	207
UK	1,520 patients	Statin	NM	No significant association between preadmission statin use and severity of outcome	43
Spain	197 patients with pneumonia; 68 patients received anti-inflammatory therapy	Corticosteroid (37), macrolide (31), statin (12)	NM	None of the therapies were associated with lower risk of developing severe disease	528

[a] APACHEII, Acute Physiology and Chronic Health Evaluation II score; ARDS, acute respiratory distress syndrome; CRP, C-reactive protein; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; IVIG, intravenous immunoglobulin; NM, not mentioned; OR, odds ratio; SOFA, sequential organ failure assessment score.

versus extracorporeal membrane oxygenation [ECMO] for severe adult respiratory failure) trial, ICUs from different centers around the world (86, 139, 185, 358, 414) have treated patients using ECMO, with promising results. The rate of ECMO use for all mechanically ventilated patients with A/2009/H1N1 pneumonia ranged from 4% to 9%. The survival rates for patients who underwent ECMO and mechanical ventilatory support were similar across the studies and ranged from 66% to 86%, with no severe complications associated with the use of ECMO. In addition, a study in Italy demonstrated the feasibility of transferring critically ill A/2009/H1N1-infected ARDS patients to ECMO centers for treatment, with a high survival rate (433). The roles of different adjunctive therapies that combine immunotherapy and ECMO support for the treatment of patients with severe A/2009/H1N1 infection should be assessed in future studies.

VACCINES

Despite the best available treatments, the mortality from severe A/2009/H1N1 infection is substantial. Prevention by vaccination is crucial. In addition to the prevention of pulmonary complications, influenza vaccination can prevent medical catastrophes triggered by influenza, such as myocardial infarction and stroke, especially when combined with pneumococcal vaccination (263). The greatest setback in the 2009 influenza pandemic was the nonavailability of the vaccine until late 2009 in most countries. Thus, the summer peak in tropical areas and the winter peak in the Southern Hemisphere during August 2009 passed before vaccines were administered. Innovations, such as cell-based whole-virion inactivated vaccines and dose-sparing adjuvants (17, 129, 519), boosted the vaccine supply but were unable to shorten the interval between the first detection of the virus and the availability of the vaccine in the market (249, 479). A hemagglutination inhibition titer of 1:40 has been achieved in more than 90% of vaccinees, although children less than 11 years of age or adults greater than 61 years of age had poorer responses (588). Other factors that are associated with a poor response to vaccination include immunosuppressed states or chronic diseases. Adjuvants and two-dose regimens may improve immunogenicity. Dose sparing by the use of the intradermal route or new adjuvants was shown to have equivalent efficacy to that of a normal dose of vaccine (508). Vaccine effectiveness, as defined by the reduction in the risk of laboratory-confirmed infection between vaccinated and nonvaccinated individuals, has been estimated to be approximately 70% (235). It should be noted that live attenuated vaccines induce a lower antibody response in adults than inactivated vaccines (62),

and vaccine recipients have had lower antibody titers than individuals with natural infection (84).

Prior seasonal influenza vaccination has been found to worsen the outcomes of patients with A/2009/H1N1 infection (283, 478). The increased risk may be due to the lack of cross-reactive immunity induced by natural seasonal influenza infection (385). However, other clinical studies have demonstrated contradictory findings, with no apparent increase in the severity of pandemic A/2009/H1N1 infection in individuals who received a prior seasonal influenza vaccine (126, 192, 289). It has been shown that prior seasonal influenza vaccination can increase the levels of antibodies against neuraminidase in the elderly (374). Furthermore, cross-reactive T cells induced by the seasonal influenza vaccine may confer some degree of protection against pandemic A/2009/H1N1 infection (258). The acceptance of the pandemic influenza vaccine is low, partly due to the concerns regarding safety, such as the development of Guillain-Barre syndrome after vaccination (330, 466, 467, 512). Nevertheless, both premarketing trials and postmarketing surveys showed that the risk of developing Guillain-Barre syndrome in individuals who receive the pandemic influenza vaccine is similar to that in the general population (76, 353).

INFECTION CONTROL IN THE COMMUNITY AND HOSPITALS

Before the emergence of the A/2009/H1N1 pandemic, nonpharmacological interventions with social distancing, such as school closures, had been evaluated in modeling (26, 175) and epidemiological (38, 125, 256, 375) studies. School closures were practiced in North America and Australia during the 2009 pandemic (40, 121, 165) because of a high clinical attack rate that ranged from 10% to 60% (133, 221, 248, 275, 347, 514) (Table 3). School closures were associated with a 65% reduction in the mean total number of contacts for each student as reported in a retrospective questionnaire survey in the United Kingdom (279). However, a late school closure in which 27% of the students already had symptoms had no significant impact on the spread of infection (63). Therefore, school closures should begin once the threshold for daily case counts is exceeded (228). In Hong Kong, kindergartens and primary schools were closed when local transmission of the A/2009/H1N1 virus was identified, followed shortly afterwards by secondary school closures for summer vacations. The transmission of the A/2009/H1N1 virus was estimated to be reduced by 25% (556).

Household transmission of the A/2009/H1N1 virus was reported to have an attack rate of up to 45% (Table 3) (59, 64, 124, 182, 284, 318, 393, 428, 436, 550, 558, 571). The transmissibility of the A/2009/H1N1 virus in the household setting was broadly similar to that of seasonal influenza A viruses (124). The attack rate was highest among children and adolescents. Household contacts less than 18 years of age were approximately 15 times more likely to be infected than older contacts (348). When oseltamivir was used as postexposure prophylaxis, the household secondary attack rate was reduced from 26.1% to 0.6% (318). However, whether the early treatment of the index person with oseltamivir can reduce secondary infections in the household setting remains to be determined (203). Hand hygiene and face masks appeared to prevent household transmission of influenza virus when implemented within 36 h of symptom onset in the index patient (123). The wearing of face masks was well tolerated by children less than 14 years of age (494). Frequent hand washing has been shown to reduce surface contamination by influenza virus in the household (475). Simple nonpharmaceutical interventions may mitigate pandemic influenza if compliance can be ensured.

The nosocomial transmission of the A/2009/H1N1 virus was reported to have an attack rate of 10% to 45% in health care settings (531), including acute-care hospitals and long-term care facilities (10, 74, 114, 172). Among the 30 patients who acquired A/2009/H1N1 infection during hospitalization in the United Kingdom, 8 (27%) of the patients died. Most of them had an underlying malignancy or immunosuppressive conditions (166). For health care workers (HCWs), asymptomatic infection as evidenced by seroconversion was observed in 28 (9.6%) of 290 nurses, especially in nurses who worked in the isolation wards (100). Therefore, an infection control program that consisted of multiple coherent measures was proposed to minimize the nosocomial transmission of the A/2009/H1N1 virus during the early phase of the pandemic in Hong Kong. This program included several open staff forums that achieved high attendance, the early recognition of index cases among inpatients by liberal testing, the early relief of sick staff from work, directly observed hand hygiene practices during outbreaks, and the monitoring of compliance with infection control practices. With these measures, only 4 (0.48%) of 836 persons who were exposed to laboratory-confirmed patients and staff with A/2009/H1N1 infection were virologically confirmed to have A/2009/H1N1 infection. Not wearing a surgical mask, either by exposed persons during contact with index cases (4/4 versus 264/832; $P = 0.010$) or vice versa (4/4 versus 300/832; $P = 0.017$), was found to be a significant risk factor for the nosocomial acquisition of A/2009/H1N1 infection (108). Additionally, this finding was observed in a study in Singapore that demonstrated that the incidence of pandemic A/2009/H1N1 infection remained low in HCWs who wore surgical masks (10). In contrast, surgical mask use was not consistently practiced in the United States, where the Centers for Disease Control and Prevention received reports of 70 HCWs with A/2009/H1N1 infection from 22 states. Of these cases, 35 (50%) were classified as being infected in a health care setting. Of the 23 HCWs infected by patients, only 20% reported using an N95 or surgical mask during all patient care practices (546).

Good adherence to infection control practices may reduce potential occupational exposure to the A/2009/H1N1 virus in the hospital setting. In a seroprevalence study of the A/2009/H1N1 antibodies in 599 HCWs after the first wave in Hong Kong, only 12% of the HCWs who did not receive the pandemic vaccine had antibody titers of ≥1:40 as detected by a viral neutralization assay (587). Influenza vaccination has been strongly advocated to protect both patients and HCWs, especially HCWs who work in ICUs with high-risk aerosol-generating procedures, such as intubation, resuscitation, and the delivery of aerosolized medications (138). However, the A/2009/H1N1 vaccination uptake was less than 50% in a group of HCWs who worked in the critical care and theater settings in the United Kingdom (430), whereas the A/2009/H1N1 vaccination rate of HCWs was just over 30% in France (503) but less than 20% in Spain and Italy (9, 145). The A/2009/H1N1 vaccination uptake rate is higher in the United States. Among the HCWs who worked at a facility where vaccination was required by their employer, 98.1% were vaccinated, whereas only up to 70% of HCWs were vaccinated without this employer requirement (70). In addition, a high level of acceptance with a voluntary uptake rate of up to 70% was observed in Canada (292). However, the A/2009/

H1N1 vaccine appeared to have suboptimal immunogenicity in several HCWs. Of 104 HCWs who received the A/2009/H1N1 vaccine in Hong Kong, only 42% had an antibody titer of ≥1:40 as detected by viral neutralization assay. The proportion of HCWs with an antibody titer of ≥1:40 significantly decreased with age (586). Additional studies are required to confirm whether the A/2009/H1N1 vaccine maintains high efficacy and effectiveness in HCWs and prevents the nosocomial transmission of the A/2009/H1N1 virus.

CONCLUSION

The lessons of the human pandemic of A/2009/H1N1 virus in 2009, the epidemic of SARS coronavirus in 2003, and the poultry epidemic of influenza A H5N1 virus since 1997 suggest that preparedness against agents of emerging infectious diseases that jump from animals to humans must continue. Rapid economic growth in many developing areas of the world has led to an increasing demand for animal proteins, such as pork. Large numbers of swine and other food animals are reared with antibiotic-loaded feeds in overcrowded conditions. Inadequate biosecurity measures allow for the continued jumping of novel influenza virus reassortants or multidrug-resistant bacteria from swine to humans. The potential combination of influenza and antibiotic-resistant bacterial infections could prove disastrous should future pandemics occur. Improved human and animal surveillance programs are essential for a rapid response against this occurrence. Currently, no bioinformatic or *in vitro* tests can reliably predict the virulence or clinical severity of potential new pandemic viruses. Transgenic humanized animal models should be investigated to address this important issue for better resource allocation.

The superiority of the nucleic acid amplification test has been demonstrated in the clinical management of A/2009/H1N1 infection. A multiplex system coupled with microfluidic technology that uses a small amount of clinical samples can enhance the robustness and application of nucleic acid amplification in field settings. More antiviral agents that attack the different parts of the viral life cycle should be developed to overcome antiviral resistance. Novel immunomodulatory agents that dampen detrimental inflammatory responses without hindering the protective immune response of the host should be developed and tried in those late presenters with severe diseases due to overly activated cytokines and chemokines. In future pandemics, instead of waiting for a new vaccine that is based on the pandemic virus, heterologous protection should be achieved with a readily available universal vaccine that is based on the highly conserved stalk region of H and the ectodomain of M2. This universal vaccine will overcome the delay between the detection of the new virus and the availability of the vaccine in the market from mass production. Strategies that accelerate a vaccine-induced immune response should be developed. The influenza virus is highly unpredictable, and we should always be open-minded when facing the unknown.

ACKNOWLEDGMENTS

We acknowledge research funding from the Providence Foundation, Ltd., in memory of the late Lui Hac Minh, the Shaw Foundation for Infectious Diseases Research, the HKSAR Research Fund for the Control of Infectious Diseases of the Health, Welfare and Food Bureau, and the Consultancy Service for Enhancing Laboratory Surveillance of Emerging Infectious Disease for the HKSAR Department of Health.

We thank Chung-Ying Leung, Consultant Pathologist, United Christian Hospital, for providing the histopathology pictures from patients who died of A/2009/H1N1 influenza.

REFERENCES

1. **Achdout H, et al.** 2010. Killing of avian and swine influenza virus by natural killer cells. J. Virol. **84**:3993–4001.
2. **Aggarwal S, Bradel-Tretheway B, Takimoto T, Dewhurst S, Kim B.** 2010. Biochemical characterization of enzyme fidelity of influenza A virus RNA polymerase complex. PLoS One **5**:e10372.
3. **Agrati C, et al.** 2010. Association of profoundly impaired immune competence in H1N1v-infected patients with a severe or fatal clinical course. J. Infect. Dis. **202**:681–689.
4. **Alexander DJ.** 2007. An overview of the epidemiology of avian influenza. Vaccine **25**:5637–5644.
5. **Alfaresi M, Albedwawi S, Hag-Ali M.** 2011. Detection of an oseltamivir-resistant pandemic influenza A/H1N1 virus in the United Arab Emirates. Med. Princ. Pract. **20**:97–99.
6. **AlMazroa MA, Memish ZA, AlWadey AM.** 2010. Pandemic influenza A (H1N1) in Saudi Arabia: description of the first one hundred cases. Ann. Saudi Med. **30**:11–14.
7. **Alonso M, et al.** 2011. Resistance and virulence mutations in patients with persistent infection by pandemic 2009 A/H1N1 influenza. J. Clin. Virol. **50**:114–118.
8. **Altmann M, et al.** 2011. Severe cases of pandemic (H1N1) 2009 in children, Germany. Emerg. Infect. Dis. **17**:186–192.
9. **Amodio E, et al.** 2011. Vaccination against the 2009 pandemic influenza A (H1N1) among healthcare workers in the major teaching hospital of Sicily (Italy). Vaccine **29**:1408–1412.
10. **Ang B, Poh BF, Win MK, Chow A.** 2010. Surgical masks for protection of health care personnel against pandemic novel swine-origin influenza A (H1N1)-2009: results from an observational study. Clin. Infect. Dis. **50**:1011–1014.
11. **Anton A, et al.** 2010. Selection and viral load kinetics of an oseltamivir-resistant pandemic influenza A (H1N1) virus in an immunocompromised patient during treatment with neuraminidase inhibitors. Diagn. Microbiol. Infect. Dis. **68**:214–219.
12. **Anton A, et al.** 2010. D225G mutation in the hemagglutinin protein found in 3 severe cases of 2009 pandemic influenza A (H1N1) in Spain. Diagn. Microbiol. Infect. Dis. **67**:207–208.
13. **Arankalle VA, et al.** 2010. Role of host immune response and viral load in the differential outcome of pandemic H1N1 (2009) influenza virus infection in Indian patients. PLoS One **5**:e13099.
14. **Arankalle VA, Virkar RG, Tandale BV, Ingle NB.** 2010. Utility of pandemic H1N1 2009 influenza virus recombinant hemagglutinin protein-based enzyme-linked immunosorbent assay for serosurveillance. Clin. Vaccine Immunol. **17**:1481–1483.
15. **Archer B, et al.** 2009. Interim report on pandemic H1N1 influenza virus infections in South Africa, April to October 2009: epidemiology and factors associated with fatal cases. Euro Surveill. **14**:19369.
16. **Area E, et al.** 2004. 3D structure of the influenza virus polymerase complex: localization of subunit domains. Proc. Natl. Acad. Sci. U. S. A. **101**:308–313.
17. **Arguedas A, et al.** 2011. Assessment of the safety, tolerability and kinetics of the immune response to A/H1N1v vaccine formulations with and without adjuvant in healthy pediatric subjects from 3 through 17 years of age. Hum. Vaccin. **7**:58–66.
18. **Baker MG, et al.** 2010. Transmission of pandemic A/H1N1 2009 influenza on passenger aircraft: retrospective cohort study. BMJ **340**:c2424.
19. **Balcan D, et al.** 2009. Seasonal transmission potential and activity peaks of the new influenza A(H1N1): a Monte Carlo likelihood analysis based on human mobility. BMC Med. **7**:45.
20. **Barboza P, et al.** 2010. Influenza A(H1N1)2009 in the French Pacific territories: assessment of the epidemic wave during the austral winter. Clin. Microbiol. Infect. **16**:304–308.
21. **Baxter BD, Couch RB, Greenberg SB, Kasel JA.** 1977. Maintenance of viability and comparison of identification methods for influenza and other respiratory viruses of humans. J. Clin. Microbiol. **6**:19–22.
22. **Baz M, Abed Y, McDonald J, Boivin G.** 2006. Characterization of multidrug-resistant influenza A/H3N2 viruses shed during 1 year by an immunocompromised child. Clin. Infect. Dis. **43**:1555–1561.

23. **Baz M, et al.** 2009. Emergence of oseltamivir-resistant pandemic H1N1 virus during prophylaxis. N. Engl. J. Med. **361**:2296–2297.
24. **Bazan JA, et al.** 2010. Peramivir pharmacokinetics in two critically ill adults with 2009 H1N1 influenza A concurrently receiving continuous renal replacement therapy. Pharmacotherapy **30**:1016–1020.
25. **Beigel JH, et al.** 2005. Avian influenza A (H5N1) infection in humans. N. Engl. J. Med. **353**:1374–1385.
26. **Bell DM.** 2006. Non-pharmaceutical interventions for pandemic influenza, national and community measures. Emerg. Infect. Dis. **12**:88–94.
27. **Belser JA, et al.** 2007. DAS181, a novel sialidase fusion protein, protects mice from lethal avian influenza H5N1 virus infection. J. Infect. Dis. **196**:1493–1499.
28. **Belser JA, et al.** 2010. Pathogenesis of pandemic influenza A (H1N1) and triple-reassortant swine influenza A (H1) viruses in mice. J. Virol. **84**: 4194–4203.
29. **Bennett S, Gunson RN, MacLean A, Miller R, Carman WF.** 2011. The validation of a real-time RT-PCR assay which detects influenza A and types simultaneously for influenza A H1N1 (2009) and oseltamivir-resistant (H275Y) influenza A H1N1 (2009). J. Virol. Methods **171**:86–90.
30. **Bermejo-Martin JF, et al.** 2010. Host adaptive immunity deficiency in severe pandemic influenza. Crit. Care **14**:R167.
31. **Bermejo-Martin JF, et al.** 2009. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. Crit. Care. **13**:R201.
32. **Bettinger JA, et al.** 2010. Pandemic influenza in Canadian children: a summary of hospitalized pediatric cases. Vaccine **28**:3180–3184.
33. **Bin C, et al.** 2009. Clinical and epidemiologic characteristics of 3 early cases of influenza A pandemic (H1N1) 2009 virus infection, People's Republic of China, 2009. Emerg. Infect. Dis. **15**:1418–1422.
34. **Birnkrant D, Cox E.** 2009. The emergency use authorization of peramivir for treatment of 2009 H1N1 influenza. N. Engl. J. Med. **361**:2204–2207.
35. **Blyth CC, Iredell JR, Dwyer DE.** 2009. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. N. Engl. J. Med. **361**: 2493.
36. **Boelle PY, Bernillon P, Desenclos JC.** 2009. A preliminary estimation of the reproduction ratio for new influenza A(H1N1) from the outbreak in Mexico, March-April 2009. Euro Surveill. **14**:19205.
37. **Bommakanti G, et al.** 2010. Design of an HA2-based Escherichia coli expressed influenza immunogen that protects mice from pathogenic challenge. Proc. Natl. Acad. Sci. U. S. A. **107**:13701–13706.
38. **Bootsma MC, Ferguson NM.** 2007. The effect of public health measures on the 1918 influenza pandemic in U.S. cities. Proc. Natl. Acad. Sci. U. S. A. **104**:7588–7593.
39. **Booy R, et al.** 2011. Cross-reacting antibodies against the pandemic (H1N1) 2009 influenza virus in older Australians. Med. J. Aust. **194**: 19–23.
40. **Borse RH, et al.** 2011. Closing schools in response to the 2009 pandemic influenza A H1N1 virus in New York City: economic impact on households. Clin. Infect. Dis. **52**(Suppl. 1):S168–S172.
41. **Bradley KC, et al.** 2011. Comparison of the receptor binding properties of contemporary swine isolates and early human pandemic H1N1 isolates (novel 2009 H1N1). Virology **413**:169–182.
42. **Bratincsak A, et al.** 2010. Fulminant myocarditis associated with pandemic H1N1 influenza A virus in children. J. Am. Coll. Cardiol. **55**:928–929.
43. **Brett SJ, et al.** 2011. Pre-admission statin use and in-hospital severity of 2009 pandemic influenza A(H1N1) disease. PLoS One **6**:e18120.
44. **Bridges CB, et al.** 2002. Risk of influenza A (H5N1) infection among poultry workers, Hong Kong, 1997-1998. J. Infect. Dis. **185**:1005–1010.
45. **Broberg E, Nicoll A, Amato-Gauci A.** 2011. Seroprevalence to influenza A(H1N1) 2009 virus—where are we? Clin. Vaccine Immunol. **18**:1205–1212.
46. **Brookes DW, Miah S, Lackenby A, Hartgroves L, Barclay WS.** 2011. Pandemic H1N1 2009 influenza virus with the H275Y oseltamivir resistance neuraminidase mutation shows a small compromise in enzyme activity and viral fitness. J. Antimicrob. Chemother. **66**:466–470.
47. **Brown AN, et al.** 2011. Zanamivir, at 600 milligrams twice daily, inhibits oseltamivir-resistant 2009 pandemic H1N1 influenza virus in an in vitro hollow-fiber infection model system. Antimicrob. Agents Chemother. **55**:1740–1746.
48. **Brun-Buisson C, Richard JC, Mercat A, Thiebaut AC, Brochard L.** 2011. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med. **183**: 1200–1206.
49. **Bunce PE, et al.** 2011. Pandemic H1N1 influenza infection and vascular thrombosis. Clin. Infect. Dis. **52**:e14–17.
50. **Bussey KA, Bousse TL, Desmet EA, Kim B, Takimoto T.** 2010. PB2 residue 271 plays a key role in enhanced polymerase activity of influenza A viruses in mammalian host cells. J. Virol. **84**:4395–4406.
51. **Butler D.** 2009. Patchy pig monitoring may hide flu threat. Nature **459**: 894–895.
52. **Butt KM, et al.** 2005. Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. J. Clin. Microbiol. **43**:5760–5767.
53. **Calatayud L, et al.** 2010. Pandemic (H1N1) 2009 virus outbreak in a school in London, April-May 2009: an observational study. Epidemiol. Infect. **138**:183–191.
54. **Calfee DP, Peng AW, Cass LM, Lobo M, Hayden FG.** 1999. Safety and efficacy of intravenous zanamivir in preventing experimental human influenza A virus infection. Antimicrob. Agents Chemother. **43**:1616–1620.
55. **Campanini G, et al.** 2010. Genetic divergence of influenza A NS1 gene in pandemic 2009 H1N1 isolates with respect to H1N1 and H3N2 isolates from previous seasonal epidemics. Virol. J. **7**:209.
56. **Campanini G, et al.** 2010. First case in Italy of acquired resistance to oseltamivir in an immunocompromised patient with influenza A/H1N1v infection. J. Clin. Virol. **48**:220–222.
57. **Campbell AP, et al.** 2010. Respiratory failure caused by 2009 novel influenza A/H1N1 in a hematopoietic stem-cell transplant recipient: detection of extrapulmonary H1N1 RNA and use of intravenous peramivir. Ann. Intern. Med. **152**:619–620.
58. **Cao B, et al.** 2009. Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. N. Engl. J. Med. **361**:2507–2517.
59. **Carcione D, et al.** 2011. Secondary attack rate of pandemic influenza A(H1N1) 2009 in Western Australian households, 29 May-7 August 2009. Euro Surveill. **16**:19765.
60. **Carr MJ, et al.** 2009. Development of a real-time RT-PCR for the detection of swine-lineage influenza A (H1N1) virus infections. J. Clin. Virol. **45**:196–199.
61. **Carrillo-Esper R, Perez-Bustos E, Ornelas-Arroyo S, Albores-Saavedra J, Uribe M.** 2010. Liver involvement in severe human influenza a H1N1. Ann. Hepatol. **9**:107–111.
62. **Carter NJ, Curran MP.** 2011. Live attenuated influenza vaccine (FluMist(R); Fluenz): a review of its use in the prevention of seasonal influenza in children and adults. Drugs **71**:1591–1622.
63. **Cauchemez S, et al.** 2011. Role of social networks in shaping disease transmission during a community outbreak of 2009 H1N1 pandemic influenza. Proc. Natl. Acad. Sci. U. S. A. **108**:2825–2830.
64. **Cauchemez S, et al.** 2009. Household transmission of 2009 pandemic influenza A (H1N1) virus in the United States. N. Engl. J. Med. **361**: 2619–2627.
65. **Centers for Disease Control and Prevention.** 2009. 2009 pandemic influenza A (H1N1) virus infections—Chicago, Illinois, April-July 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:913–918.
66. **Centers for Disease Control and Prevention.** 2009. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1)—United States, May-August 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:1071–1074.
67. **Centers for Disease Control and Prevention.** 2010. Deaths and hospitalizations related to 2009 pandemic influenza A (H1N1)—Greece, May 2009-February 2010. MMWR Morb. Mortal. Wkly. Rep. **59**:682–686.
68. **Centers for Disease Control and Prevention.** 2009. Deaths related to 2009 pandemic influenza A (H1N1) among American Indian/Alaska natives—12 states, 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:1341–1344.
69. **Centers for Disease Control and Prevention.** 2009. Hospitalized patients with novel influenza A (H1N1) virus infection—California, April-May, 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:536–541.
70. **Centers for Disease Control and Prevention.** 2011. Influenza vaccination coverage among health-care personnel—United States, 2010-11 influenza season. MMWR Morb. Mortal. Wkly. Rep. **60**:1073–1077.
71. **Centers for Disease Control and Prevention.** 2009. Oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infection in two summer campers receiving prophylaxis–North Carolina, 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:969–972.

72. **Centers for Disease Control and Prevention.** 2010. Outbreak of 2009 pandemic influenza A (H1N1) at a school—Hawaii, May 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:1440–1444.
73. **Centers for Disease Control and Prevention.** 2010. Outbreak of 2009 pandemic influenza A (H1N1) on a Peruvian Navy ship—June-July 2009. MMWR Morb. Mortal. Wkly. Rep. **59**:162–165.
74. **Centers for Disease Control and Prevention.** 2010. Outbreaks of 2009 pandemic influenza A (H1N1) among long-term-care facility residents—three states, 2009. MMWR Morb. Mortal. Wkly. Rep. **59**:74–77.
75. **Centers for Disease Control and Prevention.** 2010. Patients hospitalized with 2009 pandemic influenza A (H1N1)—New York City, May 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:1436–1440.
76. **Centers for Disease Control and Prevention.** 2010. Preliminary results: surveillance for Guillain-Barre syndrome after receipt of influenza A (H1N1) 2009 monovalent vaccine—United States, 2009–2010. MMWR Morb. Mortal. Wkly. Rep. **59**:657–661.
77. **Centers for Disease Control and Prevention.** 2009. Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. MMWR Morb. Mortal. Wkly. Rep. **58**:521–524.
78. **Centers for Disease Control and Prevention.** 2009. Swine-origin influenza A (H1N1) virus infections in a school—New York City, April 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:470–472.
79. **Centers for Disease Control and Prevention.** 2009. Update: drug susceptibility of swine-origin influenza A (H1N1) viruses, April 2009. MMWR Morb. Mortal. Wkly. Rep. **58**:433–435.
80. **Chan JF, et al.** 2011. The lower serum immunoglobulin G2 level in severe cases than in mild cases of pandemic H1N1 2009 influenza is associated with cytokine dysregulation. Clin. Vaccine Immunol. **18**:305–310.
81. **Chan KH, et al.** 2009. Analytical sensitivity of rapid influenza antigen detection tests for swine-origin influenza virus (H1N1). J. Clin. Virol. **45**:205–207.
82. **Chan KH, et al.** 2002. Evaluation of the Directigen FluA+B test for rapid diagnosis of influenza virus type A and B infections. J. Clin. Microbiol. **40**:1675–1680.
83. **Chan KH, Peiris JS, Lim W, Nicholls JM, Chiu SS.** 2008. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. J. Clin. Virol. **42**:65–69.
84. **Chan KH, et al.** 2011. Differences in antibody responses of individuals with natural infection and those vaccinated against pandemic H1N1 2009 influenza. Clin. Vaccine Immunol. **18**:867–873.
85. **Chan KH, et al.** 2010. Wild type and mutant 2009 pandemic influenza A (H1N1) viruses cause more severe disease and higher mortality in pregnant BALB/c mice. PLoS One **5**:e13757.
86. **Chan KK, et al.** 2010. Hong Kong's experience on the use of extracorporeal membrane oxygenation for the treatment of influenza A (H1N1). Hong Kong Med. J. **16**:447–454.
87. **Chan M, et al.** 2010. Pandemic (H1N1) 2009: clinical and laboratory findings of the first fifty cases in Singapore. Ann. Acad. Med. Singapore **39**:267–276.
88. **Chan PA, et al.** 2010. Oseltamivir-resistant 2009-2010 pandemic influenza A (H1N1) in an immunocompromised patient. Clin. Microbiol. Infect. **16**:1576–1578.
89. **Chan PK, et al.** 2011. Clinical and virological course of infection with haemagglutinin D222G mutant strain of 2009 pandemic influenza A (H1N1) virus. J. Clin. Virol. **50**:320–324.
90. **Chan-Tack KM, Murray JS, Birnkrant DB.** 2009. Use of ribavirin to treat influenza. N. Engl. J. Med. **361**:1713–1714.
91. **Chen C, Zhuang X.** 2008. Epsin 1 is a cargo-specific adaptor for the clathrin-mediated endocytosis of the influenza virus. Proc. Natl. Acad. Sci. U. S. A. **105**:11790–11795.
92. **Chen GL, Lau YF, Lamirande EW, McCall AW, Subbarao K.** 2011. Seasonal influenza infection and live vaccine prime for a response to the 2009 pandemic H1N1 vaccine. Proc. Natl. Acad. Sci. U. S. A. **108**:1140–1145.
93. **Chen H, et al.** 2009. Oseltamivir-resistant influenza A pandemic (H1N1) 2009 virus, Hong Kong, China. Emerg. Infect. Dis. **15**:1970–1972.
94. **Chen H, et al.** 2006. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc. Natl. Acad. Sci. U. S. A. **103**:2845–2850.
95. **Chen H, et al.** 2009. Serologic survey of pandemic (H1N1) 2009 virus, Guangxi Province, China. Emerg. Infect. Dis. **15**:1849–1850.
96. **Chen H, et al.** 2010. Quasispecies of the D225G substitution in the hemagglutinin of pandemic influenza A(H1N1) 2009 virus from patients with severe disease in Hong Kong, China. J. Infect. Dis. **201**:1517–1521.
97. **Chen J, et al.** 1998. Structure of the hemagglutinin precursor cleavage site, a determinant of influenza pathogenicity and the origin of the labile conformation. Cell **95**:409–417.
98. **Chen LF, et al.** 2011. Cluster of oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infections on a hospital ward among immunocompromised patients—North Carolina, 2009. J. Infect. Dis. **203**:838–846.
99. **Chen LM, et al.** 2011. Receptor specificity of subtype H1 influenza A viruses isolated from swine and humans in the United States. Virology **412**:401–410.
100. **Chen MI, et al.** 2010. Risk factors for pandemic (H1N1) 2009 virus seroconversion among hospital staff, Singapore. Emerg. Infect. Dis. **16**:1554–1561.
101. **Chen MI, et al.** 2010. 2009 influenza A(H1N1) seroconversion rates and risk factors among distinct adult cohorts in Singapore. JAMA **303**:1383–1391.
102. **Chen N, Pinsky BA, Lee BP, Lin M, Schrijver I.** 2011. Ultrasensitive detection of drug-resistant pandemic 2009 (H1N1) influenza A virus by rare-variant-sensitive high-resolution melting-curve analysis. J. Clin. Microbiol. **49**:2602–2609.
103. **Chen X, et al.** 2007. Avian influenza A (H5N1) infection in a patient in China, 2006. Influenza Other Respir. Viruses **1**:207–213.
104. **Cheng VC, et al.** 2011. Infection of immunocompromised patients by avian H9N2 influenza A virus. J. Infect. **62**:394–399.
105. **Cheng VC, et al.** 2004. Viral replication in the nasopharynx is associated with diarrhea in patients with severe acute respiratory syndrome. Clin. Infect. Dis. **38**:467–475.
106. **Cheng VC, Lau SK, Woo PC, Yuen KY.** 2007. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin. Microbiol. Rev. **20**:660–694.
107. **Cheng VC, et al.** 2009. Fatal co-infection with swine origin influenza virus A/H1N1 and community-acquired methicillin-resistant Staphylococcus aureus. J. Infect. **59**:366–370.
108. **Cheng VC, et al.** 2010. Prevention of nosocomial transmission of swine-origin pandemic influenza virus A/H1N1 by infection control bundle. J. Hosp. Infect. **74**:271–277.
109. **Cheng VC, Tang BS, Wu AK, Chu CM, Yuen KY.** 2004. Medical treatment of viral pneumonia including SARS in immunocompetent adult. J. Infect. **49**:262–273.
110. **Chi CY, et al.** 2010. Preexisting antibody response against 2009 pandemic influenza H1N1 viruses in the Taiwanese population. Clin. Vaccine Immunol. **17**:1958–1962.
111. **Chien YS, et al.** 2010. Predictors and outcomes of respiratory failure among hospitalized pneumonia patients with 2009 H1N1 influenza in Taiwan. J. Infect. **60**:168–174.
112. **Chien YW, Klugman KP, Morens DM.** 2009. Bacterial pathogens and death during the 1918 influenza pandemic. N. Engl. J. Med. **361**:2582–2583.
113. **Childs RA, et al.** 2009. Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. Nat. Biotechnol. **27**:797–799.
114. **Chironna M, et al.** 2010. A nosocomial outbreak of 2009 pandemic influenza A(H1N1) in a paediatric oncology ward in Italy, October-November 2009. Euro Surveill. **15**:19454.
115. **Choi WS, et al.** 2011. The clinical usefulness of the SD Bioline Influenza Antigen Test(R) for detecting the 2009 influenza A (H1N1) virus. Yonsei Med. J. **52**:683–685.
116. **Choi YJ, et al.** 2010. Evaluation of new rapid antigen test for detection of pandemic influenza A/H1N1 2009 virus. J. Clin. Microbiol. **48**:2260–2262.
117. **Choi YJ, et al.** 2010. Comparative analysis of the multiple test methods for the detection of pandemic influenza A/H1N1 2009 virus. J. Microbiol. Biotechnol. **20**:1450–1456.
118. **Chou YY, et al.** 2011. The M segment of the 2009 new pandemic H1N1 influenza virus is critical for its high transmission efficiency in the guinea pig model. J. Virol. **85**:11235–11241.
119. **Chutinimitkul S, et al.** 2010. Virulence-associated substitution D222G

in the hemagglutinin of 2009 pandemic influenza A(H1N1) virus affects receptor binding. J. Virol. **84**:11802–11813.
120. **Colizza V, Barrat A, Barthelemy M, Valleron AJ, Vespignani A.** 2007. Modeling the worldwide spread of pandemic influenza: baseline case and containment interventions. PLoS Med. **4**:e13.
121. **Como-Sabetti K, et al.** 2010. The 2009 H1N1 influenza pandemic and Minnesota's K-12 schools: public health lessons learned. Minn. Med. **93**:36–40.
122. **Corti D, et al.** 2011. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science **333**:850–856.
123. **Cowling BJ, et al.** 2009. Facemasks and hand hygiene to prevent influenza transmission in households: a cluster randomized trial. Ann. Intern. Med. **151**:437–446.
124. **Cowling BJ, et al.** 2010. Comparative epidemiology of pandemic and seasonal influenza A in households. N. Engl. J. Med. **362**:2175–2184.
125. **Cowling BJ, et al.** 2008. Effects of school closures, 2008 winter influenza season, Hong Kong. Emerg. Infect. Dis. **14**:1660–1662.
126. **Cowling BJ, et al.** 2010. Protective efficacy of seasonal influenza vaccination against seasonal and pandemic influenza virus infection during 2009 in Hong Kong. Clin. Infect. Dis. **51**:1370–1379.
127. **Cox CM, Blanton L, Dhara R, Brammer L, Finelli L.** 2011. 2009 Pandemic influenza A (H1N1) deaths among children—United States, 2009-2010. Clin. Infect. Dis. **52**(Suppl. 1):S69–S74.
128. **Creanga AA, et al.** 2010. Severity of 2009 pandemic influenza A (H1N1) virus infection in pregnant women. Obstet. Gynecol. **115**:717–726.
129. **Cristiani C, et al.** 2011. Safety of MF-59 adjuvanted vaccine for pandemic influenza: results of the vaccination campaign in an Italian health district. Vaccine **29**:3443–3448.
130. **Crum-Cianflone NF, et al.** 2009. Clinical and epidemiologic characteristics of an outbreak of novel H1N1 (swine origin) influenza A virus among United States military beneficiaries. Clin. Infect. Dis. **49**:1801–1810.
131. **Cui W, et al.** 2010. Factors associated with death in hospitalized pneumonia patients with 2009 H1N1 influenza in Shenyang, China. BMC Infect. Dis. **10**:145.
132. **Cullen G, et al.** 2009. Surveillance of the first 205 confirmed hospitalised cases of pandemic H1N1 influenza in Ireland, 28 April-3 October 2009. Euro Surveill. **14**:19389.
133. **Cutler J, et al.** 2009. Investigation of the first cases of human-to-human infection with the new swine-origin influenza A (H1N1) virus in Canada. CMAJ **181**:159–163.
134. **Cutter JL, et al.** 2010. Outbreak of pandemic influenza A (H1N1-2009) in Singapore, May to September 2009. Ann. Acad. Med. Singapore **39**: 273–310.
135. **Da Dalt L, et al.** 2010. Oseltamivir-resistant pandemic (H1N1) 2009 treated with nebulized zanamivir. Emerg. Infect. Dis. **16**:1813–1815.
136. **Dalvi PS, Singh A, Trivedi HR, Mistry SD, Vyas BR.** 2011. Adverse drug reaction profile of oseltamivir in children. J. Pharmacol. Pharmacother. **2**:100–103.
137. **Das K, Aramini JM, Ma LC, Krug RM, Arnold E.** 2010. Structures of influenza A proteins and insights into antiviral drug targets. Nat. Struct. Mol. Biol. **17**:530–538.
138. **Daugherty EL, Branson RD, Deveraux A, Rubinson L.** 2010. Infection control in mass respiratory failure: preparing to respond to H1N1. Crit. Care Med. **38**:e103–109.
139. **Davies A, et al.** 2009. Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) acute respiratory distress syndrome. JAMA **302**: 1888–1895.
140. **Dawood FS, et al.** 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N. Engl. J. Med. **360**:2605–2615.
141. **De Clercq E.** 2004. Antiviral drugs in current clinical use. J. Clin. Virol. **30**:115–133.
142. **Dee S, Jayathissa S.** 2010. Clinical and epidemiological characteristics of the hospitalised patients due to pandemic H1N1 2009 viral infection: experience at Hutt Hospital, New Zealand. N. Z. Med. J. **123**:45–53.
143. **Delangue J, et al.** 2012. Serological study of the 2009 pandemic due to influenza A H1N1 in the metropolitan French population. Clin. Microbiol. Infect. **18**:177–183.
144. **De la Tabla VO, et al.** 2010. Clinical evaluation of rapid point-of-care testing for detection of novel influenza A (H1N1) virus in a population-based study in Spain. Clin. Microbiol. Infect. **16**:1358–1361.
145. **Del Campo MT, et al.** 2011. 2009-2010 seasonal and pandemic A (H1N1) influenza vaccination among healthcare workers. Vaccine **29**: 3703–3707.
146. **Deng YM, et al.** 2011. A comparison of pyrosequencing and neuraminidase inhibition assays for the detection of oseltamivir-resistant pandemic influenza A(H1N1) 2009 viruses. Antiviral Res. **90**:87–91.
147. **Denholm JT, et al.** 2010. Hospitalised adult patients with pandemic (H1N1) 2009 influenza in Melbourne, Australia. Med. J. Aust. **192**: 84–86.
148. **Denney L, et al.** 2010. Reduction of natural killer but not effector CD8 T lymphocytes in three consecutive cases of severe/lethal H1N1/09 influenza A virus infection. PLoS One **5**:e10675.
149. **De Vries RP, et al.** 2011. Only two residues are responsible for the dramatic difference in receptor binding between swine and new pandemic H1 hemagglutinin. J. Biol. Chem. **286**:5868–5875.
150. **Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C.** 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science **303**:1529–1531.
151. **Dohna-Schwake C, et al.** 2010. Severe H1N1 infection in a pediatric liver transplant recipient treated with intravenous zanamivir: efficiency and complications. Transplantation **90**:223–224.
152. **Donaldson LJ, et al.** 2009. Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. BMJ **339**:b5213.
153. **Dong H, et al.** 2010. Detection of human novel influenza A (H1N1) viruses using multi-fluorescent real-time RT-PCR. Virus Res. **147**:85–90.
154. **D'Ortenzio E, et al.** 2010. A review of the dynamics and severity of the pandemic A(H1N1) influenza virus on Reunion Island, 2009. Clin. Microbiol. Infect. **16**:309–316.
155. **Dowdle WR, et al.** 1975. Orthomyxoviridae. Intervirology **5**:245–251.
156. **Drews SJ, et al.** 2011. Surveillance of autopsy cases for D222G substitutions in haemagglutinin of the pandemic (H1N1) 2009 virus in Alberta, Canada. Clin. Microbiol. Infect. **17**:582–584.
157. **Drexler JF, et al.** 2009. Poor clinical sensitivity of rapid antigen test for influenza A pandemic (H1N1) 2009 virus. Emerg. Infect. Dis. **15**:1662–1664.
158. **Du N, et al.** 2010. Differential activation of NK cells by influenza A pseudotype H5N1 and 1918 and 2009 pandemic H1N1 viruses. J. Virol. **84**:7822–7831.
159. **Dubar G, et al.** 2010. French experience of 2009 A/H1N1v influenza in pregnant women. PLoS One **5**:e13112.
160. **Dulek DE, et al.** 2010. Use of intravenous zanamivir after development of oseltamivir resistance in a critically ill immunosuppressed child infected with 2009 pandemic influenza A (H1N1) virus. Clin. Infect. Dis. **50**:1493–1496.
161. **Duval X, et al.** 2010. Efficacy of oseltamivir-zanamivir combination compared to each monotherapy for seasonal influenza: a randomized placebo-controlled trial. PLoS Med. **7**:e1000362.
162. **Duvvuri VR, et al.** 2010. Highly conserved cross-reactive CD4+ T-cell HA-epitopes of seasonal and the 2009 pandemic influenza viruses. Influenza Other Respir. Viruses **4**:249–258.
163. **Duwe SC, Wedde M, Birkner P, Schweiger B.** 2011. Genotypic and phenotypic resistance of pandemic A/H1N1 influenza viruses circulating in Germany. Antiviral Res. **89**:115–118.
164. **Easterbrook JD, et al.** 2011. Immunization with 1976 swine H1N1- or 2009 pandemic H1N1-inactivated vaccines protects mice from a lethal 1918 influenza infection. Influenza Other Respir. Viruses **5**:198–205.
165. **Effler PV, et al.** 2010. Household responses to pandemic (H1N1) 2009-related school closures, Perth, Western Australia. Emerg. Infect. Dis. **16**: 205–211.
166. **Enstone JE, et al.** 2011. Nosocomial pandemic (H1N1) 2009, United Kingdom, 2009-2010. Emerg. Infect. Dis. **17**:592–598.
167. **Eshima N, et al.** 2011. Sex- and age-related differences in morbidity rates of 2009 pandemic influenza A H1N1 virus of swine origin in Japan. PLoS One **6**:e19409.
168. **Esposito S, et al.** 2010. Oseltamivir-induced resistant pandemic A/H1N1 influenza virus in a child with cystic fibrosis and Pseudomonas aeruginosa infection. J. Clin. Virol. **48**:62–65.
169. **Esposito S, et al.** 2011. Impact of pandemic A/H1N1/2009 influenza on children and their families: comparison with seasonal A/H1N1 and A/H3N2 influenza viruses. J. Infect. **63**:300–307.
170. **Estenssoro E, et al.** 2010. Pandemic 2009 influenza A in Argentina: a study of 337 patients on mechanical ventilation. Am. J. Respir. Crit. Care Med. **182**:41–48.
171. **Faix DJ, Sherman SS, Waterman SH.** 2009. Rapid-test sensitivity for

novel swine-origin influenza A (H1N1) virus in humans. N. Engl. J. Med. **361**:728–729.
172. **Fanella ST, et al.** 2011. Pandemic (H1N1) 2009 influenza in hospitalized children in Manitoba: nosocomial transmission and lessons learned from the first wave. Infect. Control Hosp. Epidemiol. **32**:435–443.
173. **Fang R, Min Jou W, Huylebroeck D, Devos R, Fiers W.** 1981. Complete structure of A/duck/Ukraine/63 influenza hemagglutinin gene: animal virus as progenitor of human H3 Hong Kong 1968 influenza hemagglutinin. Cell **25**:315–323.
174. **Ferguson NM, et al.** 2005. Strategies for containing an emerging influenza pandemic in Southeast Asia. Nature **437**:209–214.
175. **Ferguson NM, et al.** 2006. Strategies for mitigating an influenza pandemic. Nature **442**:448–452.
176. **Flahault A, Vergu E, Boelle PY.** 2009. Potential for a global dynamic of Influenza A (H1N1). BMC Infect. Dis. **9**:129.
177. **Flint SM, et al.** 2010. Disproportionate impact of pandemic (H1N1) 2009 influenza on indigenous people in the top end of Australia's Northern Territory. Med. J. Aust. **192**:617–622.
178. **Fodor E, Pritlove DC, Brownlee GG.** 1994. The influenza virus panhandle is involved in the initiation of transcription. J. Virol. **68**:4092–4096.
179. **Food and Drug Administration.** 9 October 2009, posting date. Relenza (zanamivir) inhalation powder. FDA, Washington, DC. http://www.fda.gov/medwatch/safetyinformation/safetyalertsforhumanmedicalproducts/ucm186081.htm.
180. **Fouchier RA, et al.** 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J. Virol. **79**:2814–2822.
181. **Fowlkes AL, et al.** 2011. Epidemiology of 2009 pandemic influenza A (H1N1) deaths in the United States, April-July 2009. Clin. Infect. Dis. **52**(Suppl. 1):S60–S68.
182. **France AM, et al.** 2010. Household transmission of 2009 influenza A (H1N1) virus after a school-based outbreak in New York City, April-May 2009. J. Infect. Dis. **201**:984–992.
183. **France MW, et al.** 2010. The month of July: an early experience with pandemic influenza A (H1N1) in adults with cystic fibrosis. BMC Pulm. Med. **10**:8.
184. **Fraser C, et al.** 2009. Pandemic potential of a strain of influenza A (H1N1): early findings. Science **324**:1557–1561.
185. **Freed DH, et al.** 2010. Extracorporeal lung support for patients who had severe respiratory failure secondary to influenza A (H1N1) 2009 infection in Canada. Can. J. Anaesth. **57**:240–247.
186. **Fry AM, Perez A, Finelli L.** 2011. Use of intravenous neuraminidase inhibitors during the 2009 pandemic: results from population-based surveillance. JAMA **306**:160–162.
187. **Fuhrman C, et al.** 2010. Severe hospitalised 2009 pandemic influenza A(H1N1) cases in France, 1 July-15 November 2009. Euro Surveill. **15**: 19463.
188. **Fukuzawa K, Omagari K, Nakajima K, Nobusawa E, Tanaka S.** 2011. Sialic acid recognition of the pandemic influenza 2009 H1N1 virus: binding mechanism between human receptor and influenza hemagglutinin. Protein Pept. Lett. **18**:530–539.
189. **Gabriel G, Herwig A, Klenk HD.** 2008. Interaction of polymerase subunit PB2 and NP with importin alpha1 is a determinant of host range of influenza A virus. PLoS Pathog. **4**:e11.
190. **Gack MU, et al.** 2009. Influenza A virus NS1 targets the ubiquitin ligase TRIM25 to evade recognition by the host viral RNA sensor RIG-I. Cell Host Microbe **5**:439–449.
191. **Ganzenmueller T, et al.** 2010. Comparison of the performance of direct fluorescent antibody staining, a point-of-care rapid antigen test and virus isolation with that of RT-PCR for the detection of novel 2009 influenza A (H1N1) virus in respiratory specimens. J. Med. Microbiol. **59**:713–717.
192. **Garcia-Garcia L, et al.** 2009. Partial protection of seasonal trivalent inactivated vaccine against novel pandemic influenza A/H1N1 2009: case-control study in Mexico City. BMJ **339**:b3928.
193. **Garten RJ, et al.** 2009. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science **325**:197–201.
194. **Gaur AH, et al.** 2010. Intravenous zanamivir for oseltamivir-resistant 2009 H1N1 influenza. N. Engl. J. Med. **362**:88–89.
195. **Gdynia G, et al.** 2011. Sudden death of an immunocompetent young adult caused by novel (swine origin) influenza A/H1N1-associated myocarditis. Virchows Arch. **458**:371–376.
196. **Ge X, et al.** 2010. Assessment of seasonal influenza A virus-specific CD4 T-cell responses to 2009 pandemic H1N1 swine-origin influenza A virus. J. Virol. **84**:3312–3319.
197. **Ghedin E, et al.** 2011. Deep sequencing reveals mixed infection with 2009 pandemic influenza A (H1N1) virus strains and the emergence of oseltamivir resistance. J. Infect. Dis. **203**:168–174.
198. **Giamarellos-Bourboulis EJ, et al.** 2009. Effect of the novel influenza A (H1N1) virus in the human immune system. PLoS One **4**:e8393.
199. **Gianella A, et al.** 2009. Epidemiological analysis of the influenza A(H1N1)v outbreak in Bolivia, May-August 2009. Euro Surveill. **14**: 19323.
200. **Gill JR, et al.** 2010. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Arch. Pathol. Lab. Med. **134**: 235–243.
201. **Gimeno C, et al.** 2010. Comparison of BinaxNOW Influenza A&B assay and real-time reverse transcription polymerase chain reaction for diagnosis of influenza A pandemic (H1N1) 2009 virus infection in adult patients. Diagn. Microbiol. Infect. Dis. **68**:456–458.
202. **Ginocchio CC, et al.** 2009. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J. Clin. Virol. **45**:191–195.
203. **Goldstein E, et al.** 2010. Oseltamivir for treatment and prevention of pandemic influenza A/H1N1 virus infection in households, Milwaukee, 2009. BMC Infect. Dis. **10**:211.
204. **Gomez-Gomez A, et al.** 2010. Severe pneumonia associated with pandemic (H1N1) 2009 outbreak, San Luis Potosi, Mexico. Emerg. Infect. Dis. **16**:27–34.
205. **Gordon A, et al.** 2010. Clinical attack rate and presentation of pandemic H1N1 influenza versus seasonal influenza A and B in a pediatric cohort in Nicaragua. Clin. Infect. Dis. **50**:1462–1467.
206. **Gordon CL, et al.** 2010. Association between severe pandemic 2009 influenza A (H1N1) virus infection and immunoglobulin G(2) subclass deficiency. Clin. Infect. Dis. **50**:672–678.
207. **Gordon CL, et al.** 2011. Pooled human immunoglobulin therapy in critically ill patients with pandemic 2009 influenza A(H1N1) pneumonitis and immunoglobulin G2 subclass (IgG2) deficiency. Clin. Infect. Dis. **52**:422–426.
208. **Goto H, Wells K, Takada A, Kawaoka Y.** 2001. Plasminogen-binding activity of neuraminidase determines the pathogenicity of influenza A virus. J. Virol. **75**:9297–9301.
209. **Graitcer SB, et al.** 2011. Characteristics of patients with oseltamivir-resistant pandemic (H1N1) 2009, United States. Emerg. Infect. Dis. **17**: 255–257.
210. **Grandea AG, III, et al.** 2010. Human antibodies reveal a protective epitope that is highly conserved among human and nonhuman influenza A viruses. Proc. Natl. Acad. Sci. U. S. A. **107**:12658–12663.
211. **Gras S, et al.** 2010. Cross-reactive CD8+ T-cell immunity between the pandemic H1N1-2009 and H1N1-1918 influenza A viruses. Proc. Natl. Acad. Sci. U. S. A. **107**:12599–12604.
212. **Gray GC, Kayali G.** 2009. Facing pandemic influenza threats: the importance of including poultry and swine workers in preparedness plans. Poult. Sci. **88**:880–884.
213. **Greenbaum JA, et al.** 2009. Pre-existing immunity against swine-origin H1N1 influenza viruses in the general human population. Proc. Natl. Acad. Sci. U. S. A. **106**:20365–20370.
214. **Guan Y, et al.** 2007. A model to control the epidemic of H5N1 influenza at the source. BMC Infect. Dis. **7**:132.
215. **Guan Y, et al.** 2004. H5N1 influenza: a protean pandemic threat. Proc. Natl. Acad. Sci. U. S. A. **101**:8156–8161.
216. **Guan Y, et al.** 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science **302**:276–278.
217. **Gubareva LV, Kaiser L, Matrosovich MN, Soo-Hoo Y, Hayden FG.** 2001. Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. J. Infect. Dis. **183**:523–531.
218. **Gubareva LV, Matrosovich MN, Brenner MK, Bethell RC, Webster RG.** 1998. Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. J. Infect. Dis. **178**:1257–1262.
219. **Gubareva LV, et al.** 2010. Comprehensive assessment of 2009 pandemic influenza A (H1N1) virus drug susceptibility in vitro. Antivir. Ther. **15**: 1151–1159.
220. **Guillot L, et al.** 2005. Involvement of Toll-like receptor 3 in the immune

response of lung epithelial cells to double-stranded RNA and influenza A virus. J. Biol. Chem. **280**:5571–5580.
221. **Guinard A, Grout L, Durand C, Schwoebel V.** 2009. Outbreak of influenza A(H1N1)v without travel history in a school in the Toulouse district, France, June 2009. Euro Surveill. **14**:19265.
222. **Guo X, et al.** 2011. Dynamic variations in the peripheral blood lymphocyte subgroups of patients with 2009 pandemic H1N1 swine-origin influenza A virus infection. Virol. J. **8**:215.
223. **Gutierrez I, et al.** 2009. Community transmission of influenza A (H1N1)v virus at a rock festival in Belgium, 2-5 July 2009. Euro Surveill. **14**:19294.
224. **Gutierrez RL, Ellis MW, Decker CF.** 2010. Rhabdomyolysis and pandemic (H1N1) 2009 pneumonia in adult. Emerg. Infect. Dis. **16**:565.
225. **Hagau N, et al.** 2010. Clinical aspects and cytokine response in severe H1N1 influenza A virus infection. Crit. Care **14**:R203.
226. **Hahne S, et al.** 2009. Epidemiology and control of influenza A(H1N1)v in the Netherlands: the first 115 cases. Euro Surveill. **14**:19267.
227. **Hai R, et al.** 2010. PB1-F2 expression by the 2009 pandemic H1N1 influenza virus has minimal impact on virulence in animal models. J. Virol. **84**:4442–4450.
228. **Halder N, Kelso JK, Milne GJ.** 2010. Developing guidelines for school closure interventions to be used during a future influenza pandemic. BMC Infect. Dis. **10**:221.
229. **Hale BG, et al.** 2010. Inefficient control of host gene expression by the 2009 pandemic H1N1 influenza A virus NS1 protein. J. Virol. **84**:6909–6922.
230. **Hamelin ME, et al.** 2010. Oseltamivir-resistant pandemic A/H1N1 virus is as virulent as its wild-type counterpart in mice and ferrets. PLoS Pathog. **6**:e1001015.
231. **Han K, et al.** 2009. Lack of airborne transmission during outbreak of pandemic (H1N1) 2009 among tour group members, China, June 2009. Emerg. Infect. Dis. **15**:1578–1581.
232. **Hancock K, et al.** 2009. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N. Engl. J. Med. **361**:1945–1952.
233. **Hanshaoworakul W, et al.** 2009. Severe human influenza infections in Thailand: oseltamivir treatment and risk factors for fatal outcome. PLoS One **4**:e6051.
234. **Hardelid P, Andrews N, Pebody R.** 2011. Excess mortality monitoring in England and Wales during the influenza A(H1N1) 2009 pandemic. Epidemiol. Infect. **139**:1431–1439.
235. **Hardelid P, et al.** 2011. Effectiveness of pandemic and seasonal influenza vaccine in preventing pandemic influenza A(H1N1)2009 infection in England and Scotland 2009–2010. Euro Surveill. **16**:19763.
236. **Harms PW, et al.** 2010. Autopsy findings in eight patients with fatal H1N1 influenza. Am. J. Clin. Pathol. **134**:27–35.
237. **Harris PN, et al.** 2010. Pandemic influenza H1N1 2009 in north Queensland—risk factors for admission in a region with a large indigenous population. Commun. Dis. Intell. **34**:102–109.
238. **Harter G, et al.** 2010. Intravenous zanamivir for patients with pneumonitis due to pandemic (H1N1) 2009 influenza virus. Clin. Infect. Dis. **50**:1249–1251.
239. **Harvala H, et al.** 2010. The emergence of oseltamivir-resistant pandemic influenza A (H1N1) 2009 virus amongst hospitalised immunocompromised patients in Scotland, November-December, 2009. Euro Surveill. **15**:19536.
240. **Hatano B, et al.** 2011. Mobile and accurate detection system for infection by the 2009 pandemic influenza A (H1N1) virus with a pocket-warmer reverse-transcriptase loop-mediated isothermal amplification. J. Med. Virol. **83**:568–573.
241. **Hatta M, et al.** 2007. Growth of H5N1 influenza A viruses in the upper respiratory tracts of mice. PLoS Pathog. **3**:1374–1379.
242. **Hawkes M, et al.** 2010. Sensitivity of rapid influenza diagnostic testing for swine-origin 2009 a (H1N1) influenza virus in children. Pediatrics **125**:e639–e644.
243. **Hayden F.** 2009. Developing new antiviral agents for influenza treatment: what does the future hold? Clin. Infect. Dis. **48**(Suppl. 1):S3–S13.
244. **Hayden FG, et al.** 1998. Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. J. Clin. Invest. **101**:643–649.
245. **Hayden FG, et al.** 1999. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. JAMA **282**:1240–1246.
246. **Health Protection Agency, Health Protection Scotland, National Public Health Service for Wales, and HPA Northern Ireland Swine Influenza Investigation Teams.** 2009. Epidemiology of new influenza A (H1N1) virus infection, United Kingdom, April-June 2009. Euro Surveill. **14**:19232.
247. **Health Protection Agency and Health Protection Scotland New Influenza A(H1N1) Investigation Teams.** 2009. Epidemiology of new influenza A(H1N1) in the United Kingdom, April-May 2009. Euro Surveill. **14**:19213.
248. **Health Protection Agency West Midlands H1N1v Investigation Team.** 2009. Preliminary descriptive epidemiology of a large school outbreak of influenza A(H1N1)v in the West Midlands, United Kingdom, May 2009. Euro Surveill. **14**:19264.
249. **Hendriks J, Holleman M, de Boer O, de Jong P, Luytjes W.** 2011. An international technology platform for influenza vaccines. Vaccine **29**(Suppl. 1):A8–A11.
250. **Henle W, Lief FS, Fabiyi A.** 1958. Strain-specific complement-fixation test in antigenic analysis and serodiagnosis of influenza. Lancet **i**:818–820.
251. **Henter JI, Palmkvist-Kaijser K, Holzgraefe B, Bryceson YT, Palmer K.** 2010. Cytotoxic therapy for severe swine flu A/H1N1. Lancet **376**:2116.
252. **Herfst S, et al.** 2010. Introduction of virulence markers in PB2 of pandemic swine-origin influenza virus does not result in enhanced virulence or transmission. J. Virol. **84**:3752–3758.
253. **Hernandez JE, et al.** 2011. Clinical experience in adults and children treated with intravenous peramivir for 2009 influenza A (H1N1) under an emergency IND program in the United States. Clin. Infect. Dis. **52**:695–706.
254. **Herz C, Stavnezer E, Krug R, Gurney T, Jr.** 1981. Influenza virus, an RNA virus, synthesizes its messenger RNA in the nucleus of infected cells. Cell **26**:391–400.
255. **Hewagama S, et al.** 2010. 2009 H1N1 influenza A and pregnancy outcomes in Victoria, Australia. Clin. Infect. Dis. **50**:686–690.
256. **Heymann A, Chodick G, Reichman B, Kokia E, Laufer J.** 2004. Influence of school closure on the incidence of viral respiratory diseases among children and on health care utilization. Pediatr. Infect. Dis. J. **23**:675–677.
257. **Hien TT, et al.** 2010. Early pandemic influenza (2009 H1N1) in Ho Chi Minh City, Vietnam: a clinical virological and epidemiological analysis. PLoS Med. **7**:e1000277.
258. **Hillaire ML, et al.** 2011. Cross-protective immunity to influenza pH1N1 2009 viruses induced by seasonal A(H3N2) virus is mediated by virus-specific T cells. J. Gen. Virol. **92**:2339–2349.
259. **Hill-Cawthorne GA, Schelenz S, Lawes M, Dervisevic S.** 2010. Oseltamivir-resistant pandemic (H1N1) 2009 in patient with impaired immune system. Emerg. Infect. Dis. **16**:1185–1186.
260. **Hindiyeh M, et al.** 2010. Rapid detection of influenza A pandemic (H1N1) 2009 virus neuraminidase resistance mutation H275Y by real-time reverse transcriptase PCR. J. Clin. Microbiol. **48**:1884–1887.
261. **Hong DK, Tremoulet AH, Burns JC, Lewis DB.** 2011. Cross-reactive neutralizing antibody against pandemic 2009 H1N1 influenza a virus in intravenous immunoglobulin preparations. Pediatr. Infect. Dis. J. **30**:67–69.
262. **Hsieh YH.** 2010. Pandemic influenza A (H1N1) during winter influenza season in the southern hemisphere. Influenza Other Respir. Viruses **4**:187–197.
263. **Hung IF, et al.** 2010. Prevention of acute myocardial infarction and stroke among elderly persons by dual pneumococcal and influenza vaccination: a prospective cohort study. Clin. Infect. Dis. **51**:1007–1016.
264. **Hung IF, et al.** 2011. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin. Infect. Dis. **52**:447–456.
265. **Hung IF, et al.** 2010. Effect of clinical and virological parameters on the level of neutralizing antibody against pandemic influenza A virus H1N1 2009. Clin. Infect. Dis. **51**:274–279.
266. **Hurt A, et al.** 2011. Increased detection in Australia and Singapore of a novel influenza A(H1N1)2009 variant with reduced oseltamivir and zanamivir sensitivity due to a S247N neuraminidase mutation. Euro Surveill. **16**:19884.
267. **Hurt AC, et al.** 2011. Oseltamivir-resistant influenza viruses circulating during the first year of the influenza A(H1N1) 2009 pandemic in the Asia-Pacific region, March 2009 to March 2010. Euro Surveill. **16**:19770.
268. **Hutchinson EC, Curran MD, Read EK, Gog JR, Digard P.** 2008.

Mutational analysis of cis-acting RNA signals in segment 7 of influenza A virus. J. Virol. **82**:11869–11879.
269. **Huttunen R, Syrjanen J.** 2010. Obesity and the outcome of infection. Lancet Infect. Dis. **10**:442–443.
270. **Ikonen N, et al.** 2010. High frequency of cross-reacting antibodies against 2009 pandemic influenza A(H1N1) virus among the elderly in Finland. Euro Surveill. **15**:19478.
271. **Ilyushina NA, et al.** 2010. Adaptation of pandemic H1N1 influenza viruses in mice. J. Virol. **84**:8607–8616.
272. **Inoue M, et al.** 2010. Emergence of oseltamivir-resistant pandemic (H1N1) 2009 virus within 48 hours. Emerg. Infect. Dis. **16**:1633–1636.
273. **Ito T, et al.** 1997. Receptor specificity of influenza A viruses correlates with the agglutination of erythrocytes from different animal species. Virology **227**:493–499.
274. **Itoh Y, et al.** 2009. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature **460**:1021–1025.
275. **Iuliano AD, et al.** 2009. Notes from the field: outbreak of 2009 pandemic influenza A (H1N1) virus at a large public university in Delaware, April-May 2009. Clin. Infect. Dis. **49**:1811–1820.
276. **Iwai A, et al.** 2010. Influenza A virus polymerase inhibits type I interferon induction by binding to interferon beta promoter stimulator 1. J. Biol. Chem. **285**:32064–32074.
277. **Iwasenko JM, et al.** 2010. Enhanced diagnosis of pandemic (H1N1) 2009 influenza infection using molecular and serological testing in intensive care unit patients with suspected influenza. Clin. Infect. Dis. **51**:70–72.
278. **Izumi Y, Tokuda K, O'Dell AK, Zorumski CF, Narahashi T.** 2007. Neuroexcitatory actions of Tamiflu and its carboxylate metabolite. Neurosci. Lett. **426**:54–58.
279. **Jackson C, et al.** 2011. School closures and student contact patterns. Emerg. Infect. Dis. **17**:245–247.
280. **Jagger BW, et al.** 2010. The PB2-E627K mutation attenuates viruses containing the 2009 H1N1 influenza pandemic polymerase. mBio **1**(1): e00067-10. doi:10.1128/mBio.00067-10.
281. **Jain S, et al.** 2009. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N. Engl. J. Med. **361**:1935–1944.
282. **Jamieson DJ, et al.** 2009. H1N1 2009 influenza virus infection during pregnancy in the U. S. A. Lancet **374**:451–458.
283. **Janjua NZ, et al.** 2010. Seasonal influenza vaccine and increased risk of pandemic A/H1N1-related illness: first detection of the association in British Columbia, Canada. Clin. Infect. Dis. **51**:1017–1027.
284. **Janusz KB, et al.** 2011. Influenza-like illness in a community surrounding a school-based outbreak of 2009 pandemic influenza A (H1N1) virus-Chicago, Illinois, 2009. Clin. Infect. Dis. **52**(Suppl. 1):S94–S101.
285. **Jayaraman A, et al.** 2011. A single base-pair change in 2009 H1N1 hemagglutinin increases human receptor affinity and leads to efficient airborne viral transmission in ferrets. PLoS One **6**:e17616.
286. **Jenny SL, Hu Y, Overduin P, Meijer A.** 2010. Evaluation of the Xpert Flu A Panel nucleic acid amplification-based point-of-care test for influenza A virus detection and pandemic H1 subtyping. J. Clin. Virol. **49**:85–89.
287. **Jeong EK, Bae JE, Kim IS.** 2010. Inactivation of influenza A virus H1N1 by disinfection process. Am. J. Infect. Control **38**:354–360.
288. **Jiang TJ, et al.** 2010. Preferential loss of Th17 cells is associated with CD4 T cell activation in patients with 2009 pandemic H1N1 swine-origin influenza A infection. Clin. Immunol. **137**:303–310.
289. **Johns MC, et al.** 2010. Seasonal influenza vaccine and protection against pandemic (H1N1) 2009-associated illness among US military personnel. PLoS One **5**:e10722.
290. **Jones IM, Reay PA, Philpott KL.** 1986. Nuclear location of all three influenza polymerase proteins and a nuclear signal in polymerase PB2. EMBO J. **5**:2371–2376.
291. **Jongkon N, Sangma C.** 2012. Receptor recognition mechanism of human influenza A H1N1 (1918), avian influenza A H5N1 (2004), and pandemic H1N1 (2009) neuraminidase. J. Mol. Model. **18**:285–293.
292. **Kaboli F, et al.** 2010. Influenza vaccination and intention to receive the pandemic H1N1 influenza vaccine among healthcare workers of British Columbia, Canada: a cross-sectional study. Infect. Control Hosp. Epidemiol. **31**:1017–1024.
293. **Kainov DE, et al.** 2011. Differential effects of NS1 proteins of human pandemic H1N1/2009, avian highly pathogenic H5N1, and low pathogenic H5N2 influenza A viruses on cellular pre-mRNA polyadenylation and mRNA translation. J. Biol. Chem. **286**:7239–7247.
294. **Kang YM, Song BM, Lee JS, Kim HS, Seo SH.** 2011. Pandemic H1N1 influenza virus causes a stronger inflammatory response than seasonal H1N1 influenza virus in ferrets. Arch. Virol. **156**:759–767.
295. **Kao RY, et al.** 2010. Identification of influenza A nucleoprotein as an antiviral target. Nat. Biotechnol. **28**:600–605.
296. **Kapelusznik L, et al.** 2009. Severe pandemic (H1N1) 2009 influenza with false negative direct fluorescent antibody assay: case series. J. Clin. Virol. **46**:279–281.
297. **Karlas A, et al.** 2010. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. Nature **463**:818–822.
298. **Karlsson EA, Beck MA.** 2010. The burden of obesity on infectious disease. Exp. Biol. Med. **235**:1412–1424.
299. **Kato H, et al.** 2006. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature **441**:101–105.
300. **Kawano H, et al.** 2011. Genetic analysis and phylogenetic characterization of pandemic (H1N1) 2009 influenza viruses that found in Nagasaki, Japan. Jpn. J. Infect. Dis. **64**:195–203.
301. **Kawaoka Y, Krauss S, Webster RG.** 1989. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. J. Virol. **63**:4603–4608.
302. **Khan G, Al-Mutawa J, Hashim MJ.** 2011. Pandemic (H1N1) 2009, Abu Dhabi, United Arab Emirates, May 2009-March 2010. Emerg. Infect. Dis. **17**:292–295.
303. **Kidd IM, et al.** 2009. H1N1 pneumonitis treated with intravenous zanamivir. Lancet **374**:1036.
304. **Kilander A, Rykkvin R, Dudman SG, Hungnes O.** 2010. Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009–2010. Euro Surveill. **15**:19498.
305. **Kim HS, et al.** 2011. Fatal cases of 2009 pandemic influenza A (H1N1) in Korea. J. Korean Med. Sci. **26**:22–27.
306. **Kim SH, et al.** 2011. Corticosteroid treatment in critically ill patients with pandemic influenza A/H1N1 2009 infection: analytic strategy using propensity scores. Am. J. Respir. Crit. Care Med. **183**:1207–1214.
307. **Kim SH, Samal SK.** 2010. Inhibition of host innate immune responses and pathogenicity of recombinant Newcastle disease viruses expressing NS1 genes of influenza A viruses. J. Gen. Virol. **91**:1996–2001.
308. **Kim YH, Kim JE, Hyun MC.** 2011. Cytokine response in pediatric patients with pandemic influenza H1N1 2009 virus infection and pneumonia: Comparison with pediatric pneumonia without H1N1 2009 infection. Pediatr. Pulmonol. **46**:1233–1239.
309. **Kim YK, Uh Y, Chun JK, Kim C, Kim HY.** 2010. Evaluation of new hemagglutinin-based rapid antigen test for influenza A pandemic (H1N1) 2009. J. Clin. Virol. **49**:69–72.
310. **Kimberlin DW, et al.** 2010. Safety of oseltamivir compared with the adamantanes in children less than 12 months of age. Pediatr. Infect. Dis. J. **29**:195–198.
311. **Kiso M, et al.** 2010. Efficacy of the new neuraminidase inhibitor CS-8958 against H5N1 influenza viruses. PLoS Pathog. **6**:e1000786.
312. **Kiso M, et al.** 2010. T-705 (favipiravir) activity against lethal H5N1 influenza A viruses. Proc. Natl. Acad. Sci. U. S. A. **107**:882–887.
313. **Koegelenberg CF, et al.** 2010. High mortality from respiratory failure secondary to swine-origin influenza A (H1N1) in South Africa. QJM. **103**:319–325.
314. **Kohno S, Kida H, Mizuguchi M, Shimada J.** 2010. Efficacy and safety of intravenous peramivir for treatment of seasonal influenza virus infection. Antimicrob. Agents Chemother. **54**:4568–4574.
315. **Kok J, et al.** 2010. Comparison of a rapid antigen test with nucleic acid testing during cocirculation of pandemic influenza A/H1N1 2009 and seasonal influenza A/H3N2. J. Clin. Microbiol. **48**:290–291.
316. **Kok KH, et al.** 2011. The double-stranded RNA-binding protein PACT functions as a cellular activator of RIG-I to facilitate innate antiviral response. Cell Host Microbe **9**:299–309.
317. **Koliou M, Soteriades ES, Toumasi MM, Demosthenous A, Hadjidemetriou A.** 2009. Epidemiological and clinical characteristics of influenza A(H1N1)v infection in children: the first 45 cases in Cyprus, June-August 2009. Euro Surveill. **14**:19312.
318. **Komiya N, et al.** 2010. Household transmission of pandemic 2009 influenza A (H1N1) virus in Osaka, Japan in May 2009. J. Infect. **61**:284–288.
319. **Konig R, et al.** 2010. Human host factors required for influenza virus replication. Nature **463**:813–817.
320. **Krug RM, Broni BA, Bouloy M.** 1979. Are the 5′ ends of influenza viral mRNAs synthesized in vivo donated by host mRNAs? Cell **18**:329–334.

321. **Kubo T, Agoh M, QMai le, et al.** 2010. Development of a reverse transcription-loop-mediated isothermal amplification assay for detection of pandemic (H1N1) 2009 virus as a novel molecular method for diagnosis of pandemic influenza in resource-limited settings. J. Clin. Microbiol. **48**:728–735.
322. **Kumar D, et al.** 2010. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplants: a multicentre cohort study. Lancet Infect. Dis. **10**:521–526.
323. **Kumar K, et al.** 2011. Influenza myocarditis and myositis: case presentation and review of the literature. Can. J. Cardiol. **27**:514–522.
324. **Lackenby A, Democratis J, Siqueira MM, Zambon MC.** 2008. Rapid quantitation of neuraminidase inhibitor drug resistance in influenza virus quasispecies. Antivir. Ther. **13**:809–820.
325. **Lackenby A, et al.** 2011. Continued emergence and changing epidemiology of oseltamivir-resistant influenza A(H1N1)2009 virus, United Kingdom, winter 2010/11. Euro Surveill. **16**:19784.
326. **Lai CK, Cheng KL, Lee SY, Siu HK, Tsang DN.** 2010. Outbreak of influenza A (H1N1) virus infection in a nursing school in Hong Kong. Infect. Control Hosp. Epidemiol. **31**:653–655.
327. **Lai KY, Ng WY, Osburga Chan PK, Wong KF, Cheng F.** 2010. High-dose N-acetylcysteine therapy for novel H1N1 influenza pneumonia. Ann. Intern. Med. **152**:687–688.
328. **Lam WY, et al.** 2010. Development and comparison of molecular assays for the rapid detection of the pandemic influenza A (H1N1) 2009 virus. J. Med. Virol. **82**:675–683.
329. **Landry ML.** 2011. Diagnostic tests for influenza infection. Curr. Opin. Pediatr. **23**:91–97.
330. **Lau JT, et al.** 2009. Acceptability of A/H1N1 vaccination during pandemic phase of influenza A/H1N1 in Hong Kong: population based cross sectional survey. BMJ **339**:b4164.
331. **Lau SK, et al.** 2009. Confirmation of the first Hong Kong case of human infection by novel swine origin influenza A (H1N1) virus diagnosed using ultrarapid, real-time reverse transcriptase PCR. J. Clin. Microbiol. **47**:2344–2346.
332. **Lau SK, et al.** 2005. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc. Natl. Acad. Sci. U. S. A. **102**: 14040–14045.
333. **Lau YF, Tang LH, Ooi EE, Subbarao K.** 2010. Activation of the innate immune system provides broad-spectrum protection against influenza A viruses with pandemic potential in mice. Virology **406**:80–87.
334. **Lauder SN, et al.** 2011. Paracetamol reduces influenza-induced immunopathology in a mouse model of infection without compromising virus clearance or the generation of protective immunity. Thorax **66**:368–374.
335. **Laurie KL, et al.** 2010. Multiple infections with seasonal influenza A virus induce cross-protective immunity against A(H1N1) pandemic influenza virus in a ferret model. J. Infect. Dis. **202**:1011–1020.
336. **LeBlanc JJ, et al.** 2009. Switching gears for an influenza pandemic: validation of a duplex reverse transcriptase PCR assay for simultaneous detection and confirmatory identification of pandemic (H1N1) 2009 influenza virus. J. Clin. Microbiol. **47**:3805–3813.
337. **Ledesma J, et al.** 2011. Substitutions in position 222 of haemagglutinin of pandemic influenza A (H1N1) 2009 viruses in Spain. J. Clin. Virol. **51**:75–78.
338. **Ledesma J, et al.** 2011. Oseltamivir-resistant pandemic influenza A (H1N1) 2009 viruses in Spain. J. Clin. Virol. **51**:205–208.
339. **Lee CS, Lee JH.** 2010. Dynamics of clinical symptoms in patients with pandemic influenza A (H1N1). Clin. Microbiol. Infect. **16**:389–390.
340. **Lee N, et al.** 2011. Viral clearance and inflammatory response patterns in adults hospitalized for pandemic 2009 influenza A(H1N1) virus pneumonia. Antivir. Ther. **16**:237–247.
341. **Lee SM, et al.** 2010. Systems-level comparison of host responses induced by pandemic and seasonal influenza A H1N1 viruses in primary human type I-like alveolar epithelial cells in vitro. Respir. Res. **11**:147.
342. **Lee VJ, Lye DC, Wilder-Smith A.** 2009. Combination strategies for pandemic influenza response—a systematic review of mathematical modeling studies. BMC Med. **7**:76.
343. **Lee VJ, et al.** 2010. Inactivated trivalent seasonal influenza vaccine induces limited cross-reactive neutralizing antibody responses against 2009 pandemic and 1934 PR8 H1N1 strains. Vaccine **28**:6852–6857.
344. **Leider JP, Brunker PA, Ness PM.** 2010. Convalescent transfusion for pandemic influenza: preparing blood banks for a new plasma product? Transfusion **50**:1384–1398.
345. **Lemaitre M, et al.** 2011. Seasonal H1N1 2007 influenza virus infection is associated with elevated pre-exposure antibody titers to the 2009 pandemic influenza A (H1N1) virus. Clin. Microbiol. Infect. **17**:732–737.
346. **Leonardi GP, Mitrache I, Pigal A, Freedman L.** 2010. Public hospital-based laboratory experience during an outbreak of pandemic influenza A (H1N1) virus infections. J. Clin. Microbiol. **48**:1189–1194.
347. **Lessler J, et al.** 2009. Outbreak of 2009 pandemic influenza A (H1N1) at a New York City school. N. Engl. J. Med. **361**:2628–2636.
348. **Leung YH, Li MP, Chuang SK.** 2011. A school outbreak of pandemic (H1N1) 2009 infection: assessment of secondary household transmission and the protective role of oseltamivir. Epidemiol. Infect. **139**:41–44.
349. **Li IW, et al.** 2009. Differential susceptibility of different cell lines to swine-origin influenza A H1N1, seasonal human influenza A H1N1, and avian influenza A H5N1 viruses. J. Clin. Virol. **46**:325–330.
350. **Li IW, et al.** 2010. The natural viral load profile of patients with pandemic 2009 influenza A(H1N1) and the effect of oseltamivir treatment. Chest **137**:759–768.
351. **Li KS, et al.** 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature **430**:209–213.
352. **Li S, Min JY, Krug RM, Sen GC.** 2006. Binding of the influenza A virus NS1 protein to PKR mediates the inhibition of its activation by either PACT or double-stranded RNA. Virology **349**:13–21.
353. **Liang XF, et al.** 2011. Safety of influenza A (H1N1) vaccine in postmarketing surveillance in China. N. Engl. J. Med. **364**:638–647.
354. **Liao HY, et al.** 2010. Differential receptor binding affinities of influenza hemagglutinins on glycan arrays. J. Am. Chem. Soc. **132**:14849–14856.
355. **Libster R, et al.** 2010. Pediatric hospitalizations associated with 2009 pandemic influenza A (H1N1) in Argentina. N. Engl. J. Med. **362**:45–55.
356. **Lin T, et al.** 2009. The hemagglutinin structure of an avian H1N1 influenza A virus. Virology **392**:73–81.
357. **Ling LM, et al.** 2010. Effects of early oseltamivir therapy on viral shedding in 2009 pandemic influenza A (H1N1) virus infection. Clin. Infect. Dis. **50**:963–969.
358. **Liong T, et al.** 2009. The first novel influenza A (H1N1) fatality despite antiviral treatment and extracorporeal membrane oxygenation in Hong Kong. Hong Kong Med. J. **15**:381–384.
359. **Liu W, et al.** 2010. Community transmission of pandemic influenza A (H1N1) in China. Infect. Control Hosp. Epidemiol. **31**:961–963.
360. **Liu Y, et al.** 2010. Altered receptor specificity and cell tropism of D222G hemagglutinin mutants isolated from fatal cases of pandemic A(H1N1) 2009 influenza virus. J. Virol. **84**:12069–12074.
361. **Lockman JL, Fischer WA, Perl TM, Valsamakis A, Nichols DG.** 2010. The critically ill child with novel H1N1 influenza A: a case series. Pediatr. Crit. Care Med. **11**:173–178.
362. **Longtin J, et al.** 2011. Neuraminidase-inhibitor resistance testing for pandemic influenza A (H1N1) 2009 in Ontario, Canada. J. Clin. Virol. **50**:257–261.
363. **Louie JK, Acosta M, Jamieson DJ, Honein MA.** 2010. Severe 2009 H1N1 influenza in pregnant and postpartum women in California. N. Engl. J. Med. **362**:27–35.
364. **Louie JK, et al.** 2011. A novel risk factor for a novel virus: obesity and 2009 pandemic influenza A (H1N1). Clin. Infect. Dis. **52**:301–312.
365. **Louie JK, et al.** 2009. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. JAMA **302**:1896–1902.
366. **Lowen AC, Mubareka S, Steel J, Palese P.** 2007. Influenza virus transmission is dependent on relative humidity and temperature. PLoS Pathog. **3**:1470–1476.
367. **Luke TC, Kilbane EM, Jackson JL, Hoffman SL.** 2006. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? Ann. Intern. Med. **145**:599–609.
368. **Mahony JB, Chong S, Luinstra K, Petrich A, Smieja M.** 2010. Development of a novel bead-based multiplex PCR assay for combined subtyping and oseltamivir resistance genotyping (H275Y) of seasonal and pandemic H1N1 influenza A viruses. J. Clin. Virol. **49**:277–282.
369. **Maines TR, et al.** 2009. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. Science **325**:484–487.
370. **Mak CM, et al.** 2011. Fatal viral infection-associated encephalopathy in two Chinese boys: a genetically determined risk factor of thermolabile carnitine palmitoyltransferase II variants. J. Hum. Genet. **56**:617–621.
371. **Mak GC, et al.** 2010. Association of D222G substitution in haemagglutinin of 2009 pandemic influenza A (H1N1) with severe disease. Euro Surveill. **15**:19534.

372. **Malato L, et al.** 2011. Pandemic influenza A(H1N1) 2009: molecular characterisation and duration of viral shedding in intensive care patients in Bordeaux, south-west France, May 2009 to January 2010. Euro Surveill. **16**:19776.
373. **Manicassamy B, et al.** 2010. Protection of mice against lethal challenge with 2009 H1N1 influenza A virus by 1918-like and classical swine H1N1 based vaccines. PLoS Pathog. **6**:e1000745.
374. **Marcelin G, et al.** 2010. Inactivated seasonal influenza vaccines increase serum antibodies to the neuraminidase of pandemic influenza A(H1N1) 2009 virus in an age-dependent manner. J. Infect. Dis. **202**:1634–1638.
375. **Markel H, et al.** 2007. Nonpharmaceutical interventions implemented by US cities during the 1918-1919 influenza pandemic. JAMA **298**:644–654.
376. **Martin SS, Hollingsworth CL, Norfolk SG, Wolfe CR, Hollingsworth JW.** 2010. Reversible cardiac dysfunction associated with pandemic 2009 influenza A(H1N1). Chest **137**:1195–1197.
377. **Martin-Loeches I, et al.** 2011. Use of early corticosteroid therapy on ICU admission in patients affected by severe pandemic (H1N1)v influenza A infection. Intensive Care Med. **37**:272–283.
378. **Mauad T, et al.** 2010. Lung pathology in fatal novel human influenza A (H1N1) infection. Am. J. Respir. Crit. Care Med. **181**:72–79.
379. **McCullers JA, et al.** 2010. Recipients of vaccine against the 1976 "swine flu" have enhanced neutralization responses to the 2009 novel H1N1 influenza virus. Clin. Infect. Dis. **50**:1487–1492.
380. **McVernon J, et al.** 2011. Absence of cross-reactive antibodies to influenza A (H1N1) 2009 before and after vaccination with 2009 Southern Hemisphere seasonal trivalent influenza vaccine in children aged 6 months-9 years: a prospective study. Influenza Other Respir. Viruses. **5**:7–11.
381. **Mehle A, Doudna JA.** 2009. Adaptive strategies of the influenza virus polymerase for replication in humans. Proc. Natl. Acad. Sci. U. S. A. **106**:21312–21316.
382. **Melidou A, et al.** 2010. Molecular and phylogenetic analysis of the haemagglutinin gene of pandemic influenza H1N1 2009 viruses associated with severe and fatal infections. Virus Res. **151**:192–199.
383. **Memoli MJ, et al.** 2011. Multidrug-resistant 2009 pandemic influenza A(H1N1) viruses maintain fitness and transmissibility in ferrets. J. Infect. Dis. **203**:348–357.
384. **Memoli MJ, Hrabal RJ, Hassantoufighi A, Eichelberger MC, Taubenberger JK.** 2010. Rapid selection of oseltamivir- and peramivir-resistant pandemic H1N1 virus during therapy in 2 immunocompromised hosts. Clin. Infect. Dis. **50**:1252–1255.
385. **Mercer G, Kelly H.** 2011. Seasonal influenza vaccination and the 2009 pandemic. Clin. Infect. Dis. **52**:828–829. (Author reply, **52**:830–831.)
386. **Meroz D, et al.** 2011. Putative amino acid determinants of the emergence of the 2009 influenza A (H1N1) virus in the human population. Proc. Natl. Acad. Sci. U. S. A. **108**:13522–13527.
387. **Miller E, et al.** 2010. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. Lancet **375**: 1100–1108.
388. **Miller RR, MacLean AR, Gunson RN, Carman WF.** 2010. Occurrence of haemagglutinin mutation D222G in pandemic influenza A(H1N1) infected patients in the West of Scotland, United Kingdom, 2009-10. Euro Surveill. **15**:19546.
389. **Miyoshi-Akiyama T, et al.** 2010. Development of an immunochromatographic assay specifically detecting pandemic H1N1 (2009) influenza virus. J. Clin. Microbiol. **48**:703–708.
390. **Moltedo B, et al.** 2009. Stealth influenza virus replication precedes the initiation of adaptive immunity. J. Immunol. **183**:3569–3573.
391. **Monsalvo AC, et al.** 2011. Severe pandemic 2009 H1N1 influenza disease due to pathogenic immune complexes. Nat. Med. **17**:195–199.
392. **Moore C, et al.** 2011. Evidence of person-to-person transmission of oseltamivir-resistant pandemic influenza A(H1N1) 2009 virus in a hematology unit. J. Infect. Dis. **203**:18–24.
393. **Morgan OW, et al.** 2010. Household transmission of pandemic (H1N1) 2009, San Antonio, Texas, U. S. A., April-May 2009. Emerg. Infect. Dis. **16**:631–637.
394. **Morlighem JE, et al.** 2011. Mutation analysis of 2009 pandemic influenza A(H1N1) viruses collected in Japan during the peak phase of the pandemic. PLoS One **6**:e18956.
395. **Moscona A.** 2009. Global transmission of oseltamivir-resistant influenza. N. Engl. J. Med. **360**:953–956.
396. **Mu YP, et al.** 2010. Clinical features, treatments and prognosis of the initial cases of pandemic influenza H1N1 2009 virus infection in Shanghai China. QJM **103**:311–317.
397. **Mukaigawa J, Nayak DP.** 1991. Two signals mediate nuclear localization of influenza virus (A/WSN/33) polymerase basic protein 2. J. Virol. **65**: 245–253.
398. **Mukhopadhyay S, Philip AT, Stoppacher R.** 2010. Pathologic findings in novel influenza A (H1N1) virus ("swine flu") infection: contrasting clinical manifestations and lung pathology in two fatal cases. Am. J. Clin. Pathol. **133**:380–387.
399. **Munayco CV, et al.** 2009. Epidemiological and transmissibility analysis of influenza A(H1N1)v in a southern hemisphere setting: Peru. Euro Surveill. **14**:19299.
400. **Munster VJ, et al.** 2009. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. Science **325**:481–483.
401. **Muramoto Y, et al.** 2006. Hierarchy among viral RNA (vRNA) segments in their role in vRNA incorporation into influenza A virions. J. Virol. **80**:2318–2325.
402. **Murti KG, Webster RG.** 1986. Distribution of hemagglutinin and neuraminidase on influenza virions as revealed by immunoelectron microscopy. Virology **149**:36–43.
403. **Nakajima N, et al.** 2010. The first autopsy case of pandemic influenza (A/H1N1pdm) virus infection in Japan: detection of a high copy number of the virus in type II alveolar epithelial cells by pathological and virological examination. Jpn. J. Infect. Dis. **63**:67–71.
404. **Nakauchi M, et al.** 2011. Evaluation of reverse transcription loop-mediated isothermal amplification assays for rapid diagnosis of pandemic influenza A/H1N1 2009 virus. J. Med. Virol. **83**:10–15.
405. **Nasu T, Ogawa D, Wada J, Makino H.** 2010. Peramivir for severe influenza infection in a patient with diabetic nephropathy. Am. J. Respir. Crit. Care Med. **182**:1209–1210.
406. **Nath ST, Nayak DP.** 1990. Function of two discrete regions is required for nuclear localization of polymerase basic protein 1 of A/WSN/33 influenza virus (H1 N1). Mol. Cell. Biol. **10**:4139–4145.
407. **Nave H, Beutel G, Kielstein JT.** 2011. Obesity-related immunodeficiency in patients with pandemic influenza H1N1. Lancet Infect. Dis. **11**:14–15.
408. **Newman AP, et al.** 2008. Human case of swine influenza A (H1N1) triple reassortant virus infection, Wisconsin. Emerg. Infect. Dis. **14**: 1470–1472.
409. **Ngaosuwankul N, et al.** 2010. Influenza A viral loads in respiratory samples collected from patients infected with pandemic H1N1, seasonal H1N1 and H3N2 viruses. Virol. J. **7**:75.
410. **Nguyen HT, Fry AM, Loveless PA, Klimov AI, Gubareva LV.** 2010. Recovery of a multidrug-resistant strain of pandemic influenza A 2009 (H1N1) virus carrying a dual H275Y/I223R mutation from a child after prolonged treatment with oseltamivir. Clin. Infect. Dis. **51**:983–984.
411. **Nguyen JT, et al.** 2010. Triple combination of amantadine, ribavirin, and oseltamivir is highly active and synergistic against drug resistant influenza virus strains in vitro. PLoS One **5**:e9332.
412. **Nguyen-Van-Tam JS, et al.** 2010. Risk factors for hospitalisation and poor outcome with pandemic A/H1N1 influenza: United Kingdom first wave (May-September 2009). Thorax **65**:645–651.
413. **Nieto A, et al.** 1992. Nuclear transport of influenza virus polymerase PA protein. Virus Res. **24**:65–75.
414. **Noah MA, et al.** 2011. Referral to an extracorporeal membrane oxygenation center and mortality among patients with severe 2009 influenza A(H1N1). JAMA **306**:1659–1668.
415. **Nukiwa N, et al.** 2010. Simplified screening method for detecting oseltamivir resistant pandemic influenza A (H1N1) 2009 virus by a RT-PCR/restriction fragment length polymorphism assay. J. Virol. Methods **170**: 165–168.
416. **Odaira F, et al.** 2009. Assessment of secondary attack rate and effectiveness of antiviral prophylaxis among household contacts in an influenza A(H1N1)v outbreak in Kobe, Japan, May-June 2009. Euro Surveill. **14**: 19320.
417. **Olsen CW.** 2002. The emergence of novel swine influenza viruses in North America. Virus Res. **85**:199–210.
418. **O'Riordan S, et al.** 2010. Risk factors and outcomes among children admitted to hospital with pandemic H1N1 influenza. CMAJ **182**:39–44.
419. **Ormitti F, Ventura E, Summa A, Picetti E, Crisi G.** 2010. Acute necrotizing encephalopathy in a child during the 2009 influenza A(H1N1) pandemia: MR imaging in diagnosis and follow-up. AJNR Am. J. Neuroradiol. **31**:396–400.

420. **Ou Q, Lu Y, Huang Q, Cheng X.** 2009. Clinical analysis of 150 cases with the novel influenza A (H1N1) virus infection in Shanghai, China. Biosci. Trends **3**:127–130.
421. **Ozawa M, et al.** 2011. Impact of amino acid mutations in PB2, PB1-F2, and NS1 on the replication and pathogenicity of pandemic (H1N1) 2009 influenza viruses. J. Virol. **85**:4596–4601.
422. **Pabbaraju K, et al.** 2009. Design and validation of real-time reverse transcription-PCR assays for detection of pandemic (H1N1) 2009 virus. J. Clin. Microbiol. **47**:3454–3460.
423. **Palacios G, et al.** 2009. Streptococcus pneumoniae coinfection is correlated with the severity of H1N1 pandemic influenza. PLoS One **4**:e8540.
424. **Palese P, Shaw ML.** 2007. Orthomyxoviridae: the viruses and their replication, p 1647–1689. *In* Knipe DM, Howley PM (ed), Fields virology, 6th ed. Lippincott Williams & Wilkins, Hagerstown, MD.
425. **Palese P, Tobita K, Ueda M, Compans RW.** 1974. Characterization of temperature sensitive influenza virus mutants defective in neuraminidase. Virology **61**:397–410.
426. **Pan C, et al.** 2010. Genomic signature and mutation trend analysis of pandemic (H1N1) 2009 influenza A virus. PLoS One **5**:e9549.
427. **Pan C, Jiang S.** 2009. E14-F55 combination in M2 protein: a putative molecular determinant responsible for swine-origin influenza A virus transmission in humans. PLoS Curr. **1**:RRN1044.
428. **Papenburg J, et al.** 2010. Household transmission of the 2009 pandemic A/H1N1 influenza virus: elevated laboratory-confirmed secondary attack rates and evidence of asymptomatic infections. Clin. Infect. Dis. **51**:1033–1041.
429. **Park AW, et al.** 2009. Quantifying the impact of immune escape on transmission dynamics of influenza. Science **326**:726–728.
430. **Parry HM, et al.** 2011. Pandemic influenza A (H1N1) 2009 in a critical care and theatre setting: beliefs and attitudes towards staff vaccination. J. Hosp. Infect. **78**:302–307.
431. **Pascua PN, et al.** 2009. Evaluation of the efficacy and cross-protectivity of recent human and swine vaccines against the pandemic (H1N1) 2009 virus infection. PLoS One **4**:e8431.
432. **Patel P, et al.** 2011. rapidSTRIPE H1N1 test for detection of the pandemic swine origin influenza A (H1N1) virus. J. Clin. Microbiol. **49**: 1591–1593.
433. **Patroniti N, et al.** 2011. The Italian ECMO network experience during the 2009 influenza A(H1N1) pandemic: preparation for severe respiratory emergency outbreaks. Intensive Care Med. **37**:1447–1457.
434. **Pearce MB, Belser JA, Houser KV, Katz JM, Tumpey TM.** 2011. Efficacy of seasonal live attenuated influenza vaccine against virus replication and transmission of a pandemic 2009 H1N1 virus in ferrets. Vaccine **29**:2887–2894.
435. **Pebody RG, et al.** 2010. Pandemic influenza A (H1N1) 2009 and mortality in the United Kingdom: risk factors for death, April 2009 to March 2010. Euro Surveill. **15**:19571.
436. **Pedroni E, et al.** 2010. Outbreak of 2009 pandemic influenza A(H1N1), Los Lagos, Chile, April-June 2009. Euro Surveill. **15**:19456.
437. **Peiris JS, et al.** 2003. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet **361**:1767–1772.
438. **Peiris JS, et al.** 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet **361**:1319–1325.
439. **Peiris JS, et al.** 2004. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet **363**:617–619.
440. **Peiris M, et al.** 1999. Human infection with influenza H9N2. Lancet **354**:916–917.
441. **Perales B, et al.** 2000. The replication activity of influenza virus polymerase is linked to the capacity of the PA subunit to induce proteolysis. J. Virol. **74**:1307–1312.
442. **Perez-Padilla R, et al.** 2009. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N. Engl. J. Med. **361**:680–689.
443. **Pichlmair A, et al.** 2006. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science **314**:997–1001.
444. **Pinto LH, Lamb RA.** 2006. The M2 proton channels of influenza A and B viruses. J. Biol. Chem. **281**:8997–9000.
445. **Pizzorno A, Bouhy X, Abed Y, Boivin G.** 2011. Generation and characterization of recombinant pandemic influenza A(H1N1) viruses resistant to neuraminidase inhibitors. J. Infect. Dis. **203**:25–31.
446. **Pollock NR, et al.** 2009. Ruling out novel H1N1 influenza virus infection with direct fluorescent antigen testing. Clin. Infect. Dis. **49**:e66–68.
447. **Poon LL, et al.** 2009. Molecular detection of a novel human influenza (H1N1) of pandemic potential by conventional and real-time quantitative RT-PCR assays. Clin. Chem. **55**:1555–1558.
448. **Portela A, Digard P.** 2002. The influenza virus nucleoprotein: a multifunctional RNA-binding protein pivotal to virus replication. J. Gen. Virol. **83**:723–734.
449. **Presanis AM, et al.** 2011. Changes in severity of 2009 pandemic A/H1N1 influenza in England: a Bayesian evidence synthesis. BMJ **343**:d5408.
450. **Puzelli S, et al.** 2011. Evaluation of the antiviral drug susceptibility of influenza viruses in Italy from 2004/05 to 2009/10 epidemics and from the recent 2009 pandemic. Antiviral Res. **90**:205–212.
451. **Puzelli S, et al.** 2010. Transmission of hemagglutinin D222G mutant strain of pandemic (H1N1) 2009 virus. Emerg. Infect. Dis. **16**:863–865.
452. **Qi L, et al.** 2011. The ability of pandemic influenza virus hemagglutinins to induce lower respiratory pathology is associated with decreased surfactant protein D binding. Virology **412**:426–434.
453. **Quispe-Laime AM, et al.** 2010. H1N1 influenza A virus-associated acute lung injury: response to combination oseltamivir and prolonged corticosteroid treatment. Intensive Care Med. **36**:33–41.
454. **Ramirez-Gonzalez JE, et al.** 2011. Oseltamivir-resistant pandemic (H1N1) 2009 virus, Mexico. Emerg. Infect. Dis. **17**:283–286.
455. **Rello J, et al.** 2009. Intensive care adult patients with severe respiratory failure caused by influenza A (H1N1)v in Spain. Crit. Care **13**:R148.
456. **Renaud C, et al.** 2010. Early emergence of an H275Y mutation in a hematopoietic cell transplant recipient treated with intravenous peramivir. Transpl. Infect. Dis. **12**:513–517.
457. **Richards KA, Topham D, Chaves FA, Sant AJ.** 2010. CD4 T cells generated from encounter with seasonal influenza viruses and vaccines have broad protein specificity and can directly recognize naturally generated epitopes derived from the live pandemic H1N1 virus. J. Immunol. **185**:4998–5002.
458. **Riquelme R, et al.** 2011. Predicting mortality in hospitalized patients with 2009 H1N1 influenza pneumonia. Int. J. Tuberc. Lung Dis. **15**:542–546.
459. **Rizzo C, et al.** 2010. Cross-reactive antibody responses to the 2009 A/H1N1v influenza virus in the Italian population in the pre-pandemic period. Vaccine **28**:3558–3562.
460. **Robertson JS, Schubert M, Lazzarini RA.** 1981. Polyadenylation sites for influenza virus mRNA. J. Virol. **38**:157–163.
461. **Rowe T, et al.** 2010. Modeling host responses in ferrets during A/California/07/2009 influenza infection. Virology **401**:257–265.
462. **Russell CA, et al.** 2008. The global circulation of seasonal influenza A (H3N2) viruses. Science **320**:340–346.
463. **Saha A, Jha N, Dubey NK, Gupta VK, Kalaivani M.** 2010. Swine-origin influenza A (H1N1) in Indian children. Ann. Trop. Paediatr. **30**:51–55.
464. **Scheible K, et al.** 2011. CD8+ T cell immunity to 2009 pandemic and seasonal H1N1 influenza viruses. Vaccine **29**:2159–2168.
465. **Schnell JR, Chou JJ.** 2008. Structure and mechanism of the M2 proton channel of influenza A virus. Nature **451**:591–595.
466. **Schwarzinger M, Flicoteaux R, Cortarenoda S, Obadia Y, Moatti JP.** 2010. Low acceptability of A/H1N1 pandemic vaccination in French adult population: did public health policy fuel public dissonance? PLoS One **5**:e10199.
467. **Seale H, et al.** 2011. Acceptance of a vaccine against pandemic influenza A (H1N1) virus amongst healthcare workers in Beijing, China. Vaccine **29**:1605–1610.
468. **Shapira SD, et al.** 2009. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. Cell **139**:1255–1267.
469. **Shen Y, Lu H.** 2010. Pandemic (H1N1) 2009, Shanghai, China. Emerg. Infect. Dis. **16**:1011–1013.
470. **Shieh WJ, et al.** 2010. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. Am. J. Pathol. **177**:166–175.
471. **Shimizu T, Takizawa N, Watanabe K, Nagata K, Kobayashi N.** 2011. Crucial role of the influenza virus NS2 (NEP) C-terminal domain in M1 binding and nuclear export of vRNP. FEBS Lett. **585**:41–46.
472. **Shinde V, et al.** 2009. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. N. Engl. J. Med. **360**:2616–2625.
473. **Shinya K, et al.** 2006. Avian flu: influenza virus receptors in the human airway. Nature **440**:435–436.
474. **Sidwell RW, Bailey KW, Wong MH, Barnard DL, Smee DF.** 2005. In

vitro and in vivo influenza virus-inhibitory effects of viramidine. Antiviral Res. **68**:10–17.

475. **Simmerman JM, et al.** 2010. Influenza virus contamination of common household surfaces during the 2009 influenza A (H1N1) pandemic in Bangkok, Thailand: implications for contact transmission. Clin. Infect. Dis. **51**:1053–1061.
476. **Skehel JJ, Wiley DC.** 2000. Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu. Rev. Biochem. **69**:531–569.
477. **Skountzou I, et al.** 2010. Immunity to pre-1950 H1N1 influenza viruses confers cross-protection against the pandemic swine-origin 2009 A (H1N1) influenza virus. J. Immunol. **185**:1642–1649.
478. **Skowronski DM, et al.** 2010. Association between the 2008-09 seasonal influenza vaccine and pandemic H1N1 illness during spring-summer 2009: four observational studies from Canada. PLoS Med. **7**:e1000258.
479. **Skowronski DM, et al.** 2011. Effectiveness of AS03 adjuvanted pandemic H1N1 vaccine: case-control evaluation based on sentinel surveillance system in Canada, autumn 2009. BMJ **342**:c7297.
480. **Smith A, et al.** 2009. An outbreak of influenza A(H1N1)v in a boarding school in South East England, May-June 2009. Euro Surveill. **14**:19263.
481. **Smith GJ, et al.** 2009. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature **459**:1122–1125.
482. **Smud A, et al.** 2010. Pandemic influenza A/H1N1 virus infection in solid organ transplant recipients: a multicenter study. Transplantation **90**: 1458–1462.
483. **Song JM, Van Rooijen N, Bozja J, Compans RW, Kang SM.** 2011. Vaccination inducing broad and improved cross protection against multiple subtypes of influenza A virus. Proc. Natl. Acad. Sci. U. S. A. **108**: 757–761.
484. **Song MS, et al.** 2011. Virulence and genetic compatibility of polymerase reassortant viruses derived from the pandemic (H1N1) 2009 influenza virus and circulating influenza A viruses. J. Virol. **85**:6275–6286.
485. **Sood MM, et al.** 2010. Acute kidney injury in critically ill patients infected with 2009 pandemic influenza A(H1N1): report from a Canadian province. Am. J. Kidney Dis. **55**:848–855.
486. **Soto-Abraham MV, et al.** 2009. Pathological changes associated with the 2009 H1N1 virus. N. Engl. J. Med. **361**:2001–2003.
487. **Soundararajan V, et al.** 2009. Extrapolating from sequence—the 2009 H1N1 'swine' influenza virus. Nat. Biotechnol. **27**:510–513.
488. **Speers DJ, et al.** 2010. Oseltamivir-resistant pandemic (H1N1) 2009 influenza in a severely ill patient: the first Australian case. Med. J. Aust. **192**:166–168.
489. **Steel J, Lowen AC, Mubareka S, Palese P.** 2009. Transmission of influenza virus in a mammalian host is increased by PB2 amino acids 627K or 627E/701N. PLoS Pathog. **5**:e1000252.
490. **Steel J, Palese P, Lowen AC.** 2011. Transmission of a 2009 pandemic influenza virus shows a sensitivity to temperature and humidity similar to that of an H3N2 seasonal strain. J. Virol. **85**:1400–1402.
491. **Subbramanian RA, et al.** 2010. Age-related changes in magnitude and diversity of cross-reactive CD4+ T-cell responses to the novel pandemic H1N1 influenza hemagglutinin. Hum. Immunol. **71**:957–963.
492. **Subbramanian RA, Basha S, Shata MT, Brady RC, Bernstein DI.** 2010. Pandemic and seasonal H1N1 influenza hemagglutinin-specific T cell responses elicited by seasonal influenza vaccination. Vaccine **28**:8258–8267.
493. **Subramony H, et al.** 2010. An epidemiological study of 1348 cases of pandemic H1N1 influenza admitted to Singapore Hospitals from July to September 2009. Ann. Acad. Med. Singapore. **39**:283–288.
494. **Suess T, et al.** 2011. Facemasks and intensified hand hygiene in a German household trial during the 2009/2010 influenza A(H1N1) pandemic: adherence and tolerability in children and adults. Epidemiol. Infect. **139**:1895–1901.
495. **Sugaya N, Kohno S, Ishibashi T, Wajima T, Takahashi T.** 2012. Efficacy, safety, and pharmacokinetics of intravenous peramivir in children with 2009 pandemic A (H1N1) influenza virus infection. Antimicrob. Agents Chemother. **56**:369–377.
496. **Sun K, Ye J, Perez DR, Metzger DW.** 2011. Seasonal FluMist vaccination induces cross-reactive T cell immunity against H1N1 (2009) influenza and secondary bacterial infections. J. Immunol. **186**:987–993.
497. **Suntarattiwong P, et al.** 2010. Clinical performance of a rapid influenza test and comparison of nasal versus throat swabs to detect 2009 pandemic influenza A (H1N1) infection in Thai children. Pediatr. Infect. Dis. J. **29**:366–367.
498. **Takano T, Tajiri H, Kashiwagi Y, Kimura S, Kawashima H.** 2011. Cytokine and chemokine response in children with the 2009 pandemic influenza A (H1N1) virus infection. Eur. J. Clin. Microbiol. Infect. Dis. **30**:117–120.
499. **Takeda M, Leser GP, Russell CJ, Lamb RA.** 2003. Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. Proc. Natl. Acad. Sci. U. S. A. **100**:14610–14617.
500. **Takiyama A, et al.** 2010. Sudden death of a patient with pandemic influenza (A/H1N1pdm) virus infection by acute respiratory distress syndrome. Jpn. J. Infect. Dis. **63**:72–74.
501. **Tambunan US, Ramdhan.** 2010. Identification of sequence mutations affecting hemagglutinin specificity to sialic acid receptor in influenza A virus subtypes. Bioinformation **5**:244–249.
502. **Tang JW, et al.** 2010. Cross-reactive antibodies to pandemic (H1N1) 2009 virus, Singapore. Emerg. Infect. Dis. **16**:874–876.
503. **Tanguy M, et al.** 2011. Acceptance of seasonal and pandemic A (H1N1) 2009 influenza vaccination by healthcare workers in a French teaching hospital. Vaccine **29**:4190–4194.
504. **Teijaro JR, et al.** 2011. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. Cell **146**:980–991.
505. **Thabet AA, Al-Bahlooli SH, Al-Kohlani A, Shoja'A A.** 2010. Oseltamivir-resistant pandemic (H1N1)2009 in Yemen—case report. Virol. J. **7**:88.
506. **Tinoco Y, et al.** 2009. Preliminary population-based epidemiological and clinical data on 2009 pandemic H1N1 influenza A (pH1N1) from Lima, Peru. Influenza Other Respir. Viruses. **3**:253–256.
507. **Tisdale M.** 2000. Monitoring of viral susceptibility: new challenges with the development of influenza NA inhibitors. Rev. Med. Virol. **10**:45–55.
508. **To KK.** 2010. Intradermal 2009 pandemic influenza A(H1N1) vaccination as a strategy for dose and adjuvant sparing. Abstr. IDSA Annu. Meet., Vancouver, Canada, abstr. LB-19.
509. **To KK, et al.** 2010. Viral load in patients infected with pandemic H1N1 2009 influenza A virus. J. Med. Virol. **82**:1–7.
510. **To KK, et al.** 2010. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin. Infect. Dis. **50**:850–859.
511. **To KK, et al.** 2010. Concurrent comparison of epidemiology, clinical presentation and outcome between adult patients suffering from the pandemic influenza A (H1N1) 2009 virus and the seasonal influenza A virus infection. Postgrad Med. J. **86**:515–521.
512. **Torun SD, Torun F.** 2010. Vaccination against pandemic influenza A/H1N1 among healthcare workers and reasons for refusing vaccination in Istanbul in last pandemic alert phase. Vaccine **28**:5703–5710.
513. **Tramontana AR, et al.** 2010. Oseltamivir resistance in adult oncology and hematology patients infected with pandemic (H1N1) 2009 virus, Australia. Emerg. Infect. Dis. **16**:1068–1075.
514. **Tsalik EL, et al.** 2010. Clinical presentation and response to treatment of novel influenza A H1N1 in a university-based summer camp population. J. Clin. Virol. **47**:286–288.
515. **Tse H, et al.** 2011. Structural basis and sequence co-evolution analysis of the hemagglutinin protein of pandemic influenza A/H1N1 (2009) virus. Exp. Biol. Med. **236**:915–925.
516. **Tse H, et al.** 2011. Clinical and virological factors associated with viremia in pandemic influenza A/H1N1/2009 virus infection. PLoS One **6**:e22534.
517. Reference deleted.
518. **Tu W, et al.** 2010. Cytotoxic T lymphocytes established by seasonal human influenza cross-react against 2009 pandemic H1N1 influenza virus. J. Virol. **84**:6527–6535.
519. **Vajo Z, Tamas F, Sinka L, Jankovics I.** 2010. Safety and immunogenicity of a 2009 pandemic influenza A H1N1 vaccine when administered alone or simultaneously with the seasonal influenza vaccine for the 2009-10 influenza season: a multicentre, randomised controlled trial. Lancet **375**:49–55.
520. **Van den Brand JM, et al.** 2010. Severity of pneumonia due to new H1N1 influenza virus in ferrets is intermediate between that due to seasonal H1N1 virus and highly pathogenic avian influenza H5N1 virus. J. Infect. Dis. **201**:993–999.
521. **Van der Vries E, Stelma FF, Boucher CA.** 2010. Emergence of a multidrug-resistant pandemic influenza A (H1N1) virus. N. Engl. J. Med. **363**:1381–1382.
522. **Van Kerkhove MD, et al.** 2011. Risk factors for severe outcomes follow-

ing 2009 influenza A (H1N1) infection: a global pooled analysis. PLoS Med. **8**:e1001053.

523. **Van't Klooster TM, et al.** 2010. Surveillance of hospitalisations for 2009 pandemic influenza A(H1N1) in the Netherlands, 5 June-31 December 2009. Euro Surveill. **15**:19461.
524. **Varga ZT, et al.** 2011. The influenza virus protein PB1-F2 inhibits the induction of type I interferon at the level of the MAVS adaptor protein. PLoS Pathog. **7**:e1002067.
525. **Vasoo S, Stevens J, Singh K.** 2009. Rapid antigen tests for diagnosis of pandemic (swine) influenza A/H1N1. Clin. Infect. Dis. **49**:1090–1093.
526. **Velasco JM, et al.** 2010. Evaluation of QuickVue influenza A+B rapid test for detection of pandemic influenza A/H1N1 2009. J. Clin. Virol. **48**:120–122.
527. **Verrall A, et al.** 2010. Hospitalizations for pandemic (H1N1) 2009 among Maori and Pacific Islanders, New Zealand. Emerg. Infect. Dis. **16**:100–102.
528. **Viasus D, et al.** 2011. Effect of immunomodulatory therapies in patients with pandemic influenza A (H1N1) 2009 complicated by pneumonia. J. Infect. **62**:193–199.
529. **Viasus D, et al.** 2011. Factors associated with severe disease in hospitalized adults with pandemic (H1N1) 2009 in Spain. Clin. Microbiol. Infect. **17**:738–746.
530. **Vijaykrishna D, et al.** 2010. Reassortment of pandemic H1N1/2009 influenza A virus in swine. Science **328**:1529.
531. **Voirin N, Barret B, Metzger MH, Vanhems P.** 2009. Hospital-acquired influenza: a synthesis using the Outbreak Reports and Intervention Studies of Nosocomial Infection (ORION) statement. J. Hosp. Infect. **71**:1–14.
532. **Walsh KB, et al.** 2011. Suppression of cytokine storm with a sphingosine analog provides protection against pathogenic influenza virus. Proc. Natl. Acad. Sci. U. S. A. **108**:12018–12023.
533. **Wang B, et al.** 2010. Detection of the rapid emergence of the H275Y mutation associated with oseltamivir resistance in severe pandemic influenza virus A/H1N1 09 infections. Antiviral Res. **87**:16–21.
534. **Wang C, et al.** 2011. Oseltamivir compared with the Chinese traditional therapy maxingshigan-yinqiaosan in the treatment of H1N1 influenza: a randomized trial. Ann. Intern. Med. **155**:217–225.
535. **Wang W, et al.** 2010. A mutation in the receptor binding site enhances infectivity of 2009 H1N1 influenza hemagglutinin pseudotypes without changing antigenicity. Virology **407**:374–380.
536. **Wang X, et al.** 2011. A critical role of IL-17 in modulating the B-cell response during H5N1 influenza virus infection. Cell Mol. Immunol. **8**:462–468.
537. **Wanitchang A, Jengarn J, Jongkaewwattana A.** 2011. The N terminus of PA polymerase of swine-origin influenza virus H1N1 determines its compatibility with PB2 and PB1 subunits through a strain-specific amino acid serine 186. Virus Res. **155**:325–333.
538. **Wanzeck K, Boyd KL, McCullers JA.** 2011. Glycan shielding of the influenza virus hemagglutinin contributes to immunopathology in mice. Am. J. Respir. Crit. Care Med. **183**:767–773.
539. **Weaver EA, Rubrum AM, Webby RJ, Barry MA.** 2011. Protection against divergent influenza H1N1 virus by a centralized influenza hemagglutinin. PLoS One **6**:e18314.
540. **Welch DF, Ginocchio CC.** 2010. Role of rapid immunochromatographic antigen testing in diagnosis of influenza A virus 2009 H1N1 infection. J. Clin. Microbiol. **48**:22–25.
541. **Wen Y, et al.** 2011. Immunological features in patients with pneumonitis due to influenza A H1N1 infection. J. Invest. Allergol. Clin. Immunol. **21**:44–50.
542. **Wenzel JJ, et al.** 2009. Library of prefabricated locked nucleic acid hydrolysis probes facilitates rapid development of reverse-transcription quantitative real-time PCR assays for detection of novel influenza A/H1N1/09 virus. Clin. Chem. **55**:2218–2222.
543. **White LF, et al.** 2009. Estimation of the reproductive number and the serial interval in early phase of the 2009 influenza A/H1N1 pandemic in the U. S. A. Influenza Other Respir. Viruses. **3**:267–276.
544. **Wilking H, et al.** 2010. Mortality of 2009 pandemic influenza A(H1N1) in Germany. Euro Surveill. **15**:19741.
545. **Wise HM, et al.** 2009. A complicated message: identification of a novel PB1-related protein translated from influenza A virus segment 2 mRNA. J. Virol. **83**:8021–8031.
546. **Wise ME, et al.** 2011. Transmission of pandemic (H1N1) 2009 influenza to healthcare personnel in the United States. Clin. Infect. Dis. **52**(Suppl. 1):S198–S204.
547. **Witkop CT, et al.** 2010. Novel influenza A (H1N1) outbreak at the U.S. Air Force Academy: epidemiology and viral shedding duration. Am. J. Prev. Med. **38**:121–126.
548. **Wong HK, et al.** 2010. Practical limitations of convalescent plasma collection: a case scenario in pandemic preparation for influenza A (H1N1) infection. Transfusion **50**:1967–1971.
549. **Woo PC, et al.** 2010. Cytokine profiles induced by the novel swine-origin influenza A/H1N1 virus: implications for treatment strategies. J. Infect. Dis. **201**:346–353.
550. **World Health Organization.** 2009. Epidemiological summary of pandemic influenza A (H1N1) 2009 virus—Ontario, Canada, June 2009. Wkly. Epidemiol. Rec. **84**:485–491.
551. **World Health Organization.** 2009. Oseltamivir-resistant pandemic (H1N1) 2009 influenza virus, October 2009. Wkly. Epidemiol. Rec. **84**: 453–459.
552. **World Health Organization.** 2009. Pandemic (H1N1) 2009 briefing note 1. Viruses resistant to oseltamivir (Tamiflu) identified. Wkly. Epidemiol. Rec. **84**:299–399.
553. **World Health Organization.** 2010. Preliminary review of D222G amino acid substitution in the haemagglutinin of pandemic influenza A (H1N1) 2009 viruses. Wkly. Epidemiol. Rec. **85**:21–22.
554. **World Health Organization.** 2011. Review of the 2010-2011 winter influenza season, northern hemisphere. Wkly. Epidemiol. Rec. **86**:222–227.
555. **Wrammert J, et al.** 2011. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. J. Exp. Med. **208**:181–193.
556. **Wu JT, et al.** 2010. School closure and mitigation of pandemic (H1N1) 2009, Hong Kong. Emerg. Infect. Dis. **16**:538–541.
557. **Wu JT, Lee CK, Cowling BJ, Yuen KY.** 2010. Logistical feasibility and potential benefits of a population-wide passive-immunotherapy program during an influenza pandemic. Proc. Natl. Acad. Sci. U. S. A. **107**: 3269–3274.
558. **Wu JT, et al.** 2010. The infection attack rate and severity of 2009 pandemic H1N1 influenza in Hong Kong. Clin. Infect. Dis. **51**:1184–1191.
559. **Wu LT, et al.** 2010. Nucleic acid dipstick test for molecular diagnosis of pandemic H1N1. J. Clin. Microbiol. **48**:3608–3613.
560. **Xi X, et al.** 2010. Hospitalized adult patients with 2009 influenza A(H1N1) in Beijing, China: risk factors for hospital mortality. BMC Infect. Dis. **10**:256.
561. **Xie H, et al.** 2011. Immunogenicity and cross-reactivity of 2009-2010 inactivated seasonal influenza vaccine in US adults and elderly. PLoS One **6**:e16650.
562. **Xu L, et al.** 2010. A single-amino-acid substitution in the HA protein changes the replication and pathogenicity of the 2009 pandemic A (H1N1) influenza viruses in vitro and in vivo. Virol. J. **7**:325.
563. **Xu R, et al.** 2010. Structural basis of preexisting immunity to the 2009 H1N1 pandemic influenza virus. Science **328**:357–360.
564. **Xu R, McBride R, Nycholat CM, Paulson JC, Wilson IA.** 2012. Structural characterization of the hemagglutinin receptor specificity from the 2009 H1N1 influenza pandemic. J. Virol. **86**:982–990.
565. **Xu W, Han L, Lin Z.** 2011. Screening of random peptide library of hemagglutinin from pandemic 2009 A(H1N1) influenza virus reveals unexpected antigenically important regions. PLoS One **6**:e18016.
566. **Yamada S, et al.** 2010. Biological and structural characterization of a host-adapting amino acid in influenza virus. PLoS Pathog. **6**:e1001034.
567. **Yang H, Carney P, Stevens J.** 2010. Structure and receptor binding properties of a pandemic H1N1 virus hemagglutinin. PLoS Curr. **2**:RRN1152.
568. **Yang JR, et al.** 2009. Rapid SYBR green I and modified probe real-time reverse transcription-PCR assays identify influenza H1N1 viruses and distinguish between pandemic and seasonal strains. J. Clin. Microbiol. **47**:3714–3716.
569. **Yang L, et al.** 2011. Excess mortality associated with the 2009 pandemic of influenza A(H1N1) in Hong Kong. Epidemiol. Infect. **November 11**: 1–9.
570. **Yang P, et al.** 2010. Severe, critical and fatal cases of 2009 H1N1 influenza in China. J. Infect. **61**:277–283.
571. **Yang Y, et al.** 2009. The transmissibility and control of pandemic influenza A (H1N1) virus. Science **326**:729–733.
572. **Ye J, et al.** 2010. Variations in the hemagglutinin of the 2009 H1N1

pandemic virus: potential for strains with altered virulence phenotype? PLoS Pathog. **6**:e1001145.

573. **Ye Z, Liu T, Offringa DP, McInnis J, Levandowski RA.** 1999. Association of influenza virus matrix protein with ribonucleoproteins. J. Virol. **73**:7467–7473.
574. **Yu H, et al.** 2011. Risk factors for severe illness with 2009 pandemic influenza A (H1N1) virus infection in China. Clin. Infect. Dis. **52**:457–465.
575. **Yu H, et al.** 2010. Effectiveness of oseltamivir on disease progression and viral RNA shedding in patients with mild pandemic 2009 influenza A H1N1: opportunistic retrospective study of medical charts in China. BMJ **341**:c4779.
576. **Yuen KY, et al.** 1998. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet **351**:467–471.
577. **Yun TJ, et al.** 2011. Clinical and radiological features of pandemic H1N1 2009 influenza virus infection manifesting as acute febrile respiratory illness at their initial presentations: comparison with contemporaneous non-H1N1 patients. Acta Radiol. **52**:410–416.
578. **Zepeda HM, et al.** 2010. Identification of influenza A pandemic (H1N1) 2009 variants during the first 2009 influenza outbreak in Mexico City. J. Clin. Virol. **48**:36–39.
579. **Zhang AJ, et al.** 2011. High incidence of severe influenza among individuals over 50 years of age. Clin. Vaccine Immunol. **18**:1918–1924.
580. **Zheng B, et al.** 2010. D225G mutation in hemagglutinin of pandemic influenza H1N1 (2009) virus enhances virulence in mice. Exp. Biol. Med. **235**:981–988.
581. **Zheng BJ, et al.** 2008. Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. Proc. Natl. Acad. Sci. U. S. A. **105**:8091–8096.
582. **Zhirnov OP, Klenk HD, Wright PF.** 2011. Aprotinin and similar protease inhibitors as drugs against influenza. Antiviral Res. **92**:27–36.
583. **Zhou B, et al.** 2010. NS-based live attenuated H1N1 pandemic vaccines protect mice and ferrets. Vaccine **28**:8015–8025.
584. **Zhou B, et al.** 2011. PB2 residue 158 is a pathogenic determinant of pandemic H1N1 and H5 influenza a viruses in mice. J. Virol. **85**:357–365.
585. **Zhou B, Zhong N, Guan Y.** 2007. Treatment with convalescent plasma for influenza A (H5N1) infection. N. Engl. J. Med. **357**:1450–1451.
586. **Zhou Y, et al.** 2011. Seroprevalence of pandemic H1N1 antibody among health care workers in Hong Kong following receipt of monovalent 2009 H1N1 influenza vaccine. PLoS One **6**:e27169.
587. **Zhou Y, et al.** 2011. Seroprevalence of antibody to pandemic influenza A (H1N1) 2009 among healthcare workers after the first wave in Hong Kong. J. Hosp. Infect. **78**:308–311.
588. **Zhu FC, et al.** 2009. A novel influenza A (H1N1) vaccine in various age groups. N. Engl. J. Med. **361**:2414–2423.
589. **Zhu H, et al.** 2010. Substitution of lysine at 627 position in PB2 protein does not change virulence of the 2009 pandemic H1N1 virus in mice. Virology **401**:1–5.
590. **Zonis Z, et al.** 2010. Community-acquired oseltamivir-resistant pandemic (H1N1) 2009 in child, Israel. Emerg. Infect. Dis. **16**:1045–1046.
591. **Zuniga J, et al.** 7 July 2011. Genetic variants associated with severe pneumonia in A/H1N1 influenza infection. Eur. Respir. J. doi:10.1183/09031936.00020611.

Vincent C. C. Cheng is a Consultant Microbiologist and Infection Control Officer at Queen Mary Hospital, Hospital Authority, Hong Kong Special Administrative Region, and an Honorary Associate Professor at the Department of Microbiology, The University of Hong Kong. He graduated from the Faculty of Medicine at The University of Hong Kong in 1994 with distinction in medicine and trained in the field of internal medicine. After acquisition of membership from The Royal Colleges of Physicians of the United Kingdom in 1997, he pursued further training in clinical microbiology and infectious diseases at Queen Mary Hospital. He also received training in clinical infectious diseases and HIV medicine under the tutelage of Davidson Hamer, David Snydman, and Sherwood Gorbach at Tufts Medical Center, Boston, MA, in 2001. He obtained his membership and fellowship from the Royal College of Pathologists in 2004 and 2007, respectively. He has published over 120 international peer-reviewed original articles in the areas of clinical infectious disease, including SARS and influenza, diagnostic clinical microbiology, proactive infection control for multiple-drug-resistant bacteria and respiratory and gastroenteritis viruses, and hospital outbreak investigations.

Kelvin K. W. To is currently Clinical Assistant Professor in the Department of Microbiology, The University of Hong Kong. He received his B.Sc. from The University of British Columbia in 1998 and his M.B.B.S. from The University of Hong Kong in 2003. He then pursued specialist training in clinical microbiology and infectious diseases at Queen Mary Hospital. He obtained his membership from the Royal College of Physicians (United Kingdom) in 2007 and fellowship from the Royal College of Pathologists (United Kingdom) and the Hong Kong College of Pathologists in 2008 and 2010, respectively. He has published more than 35 international peer-reviewed articles. His research interests include influenza virus and other respiratory pathogens.

Herman Tse is currently Clinical Assistant Professor in the Department of Microbiology, The University of Hong Kong. He graduated from the Faculty of Medicine at The University of Hong Kong in 2004. He specializes in the application of microbial genomics and bioinformatics to the study of clinically important organisms, with particular interest in *Staphylococcus* species. He is an editorial board member of PLoS ONE and has published over 50 international peer-reviewed articles.

Ivan F. N. Hung is currently Clinical Assistant Professor and Honorary Associate Consultant in the Department of Medicine, Queen Mary Hospital, The University of Hong Kong. He graduated from the Medical School, University of Bristol, England, in 1996 and returned to the Department of Medicine, Queen Mary Hospital, Hong Kong, for further training in 1999. He obtained his membership from the Royal College of Physicians of the United Kingdom in 2002 and then his fellowship from the Royal College of Physicians of Edinburgh in 2010. He is a dual specialist in gastroenterology and infectious diseases. He received further training in clinical infectious diseases under the tutelage of David Snydman and Sherwood Gorbach at Tufts Medical Center, Boston, MA, in 2005. He obtained his Doctor of Medicine from The University of Hong Kong in 2011. He has published more than 80 international peer-reviewed original articles. His research interests include vaccines, innovative treatment of viral and bacterial infections, and treatment of resistant *Helicobacter pylori* infection. He is a regular reviewer for journals, including the *Lancet* and *Annals of Internal Medicine.* He is an editorial board member of the *Journal of Gastroenterology and Hepatology* and the *World Scientific Journal*.

Kwok-Yung Yuen works on emerging infections and microbial discovery at the Department of Microbiology of The University of Hong Kong. Besides his work on avian and pandemic influenza viruses, he and his team have discovered and characterized over 40 novel viruses in animals and human, including the human coronavirus HKU1 and the bat and human SARS coronaviruses. He has published over 500 research articles, including articles in the *Nature* series, *Proceedings of the National Academy of Sciences of the United States of America*, *Journal of Virology*, and others, with over 10,000 citations. He is presently the Henry Fok Professor in Infectious Diseases and Chair of Microbiology. He also serves as the Director of the Clinical Diagnostic Microbiology Service at the Queen Mary Hospital of Hong Kong and as the Co-Director of the State Key Laboratory of Emerging Infectious Disease of China in the Hong Kong Special Administrative Region of the People's Republic of China. He is an elected Academician of the Chinese Academy of Engineering (Basic Medicine and Health) and a fellow of the Royal Colleges of Physicians (London, Edinburgh, and Ireland), Surgeons (Glasgow), and Pathologists (United Kingdom). He was also the founding Co-Director of The Hong Kong University-Pasteur Research Centre.