

## Original Research

## Swine Flu (novel H1N1) transmission, control, diagnosis and reemergence: An overview

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**ABSTRACT:**

Swine flu (A H1N1), a quadruple reassortant virus, causes respiratory illness in people. It is a new influenza virus, first detected in people in the United States in April 2009. It is a global threat, affecting more than 70 countries. The case fatality rate (CFR) of the pandemic strain has been estimated at 0.4%. In India, 1927 laboratory confirmed cases of novel influenza A H1N1 have been reported with 25 deaths. The novel H1N1 virus spreads from human to human, in similar way that of regular seasonal influenza viruses, and their clinical presentation imitate seasonal flu; sometimes diarrhea, vomiting, severe illnesses and death have also been reported. The virus is resistant to amantadine and rimantadine and sensitive to oseltamivir and zanamivir. However, oseltamivir resistant strains have also emerged. Use of approved N95 filtering face piece respirators, powered air purifying respirators during flu patient care and medical procedures have been recommended. ASO3 adjuvant pandemic (H1N1) 2009 vaccine have been found effective in preventing pandemic (H1N1) 2009 disease. Different technologies such as intranasal administered SAP, multimeric forms of the 2009 A(H1N1) HA (sHA) and NA (sNA) surface glycoproteins, ty/04 att-based vaccines, licochalcone G and chalcones from the acetone extract of *Glycyrrhiza inflata* are being thought of and explored to control and treat influenza. Generalized lymphopenia, preferential loss of Th17 population and T cell activation and radiological imaging have been indicated for earlier diagnosis. The phylogenetic studies indicate 2009 novel H1N1 origin from that of the 1918 pandemic strain. Undoubtedly we are heading towards unraveling the mysteries of the influenza virus, but it seems to be far more tactical to prevail, emerge or re-emerge. Thus, potentially threatening challenge still surrounds us.

**Keywords:**

Novel H1N1, transmission, resistance, re-emergence, Glycyrrhiza.

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**INTRODUCTION:**

Swine flu (A H1N1), a quadruple reassortant virus, causes respiratory illness in people. The WHO update stated world's pandemic flu total as 94,152 cases with 429 becoming fatal (Pandemic (H1N1), 2009). The case fatality rate (CFR) of the pandemic strain has been estimated at 0.4%.(Fraser *et al.*, 2009). In India, 1927 laboratory confirmed cases of Novel influenza A H1N1 have been reported up to Aug, 2009 with 25 deaths. (Swine Flu India).

Novel H1N1 is a new influenza virus, first detected in people in the United States in April 2009. (WHO, 2009). In June 2009, the World Health Organization declared a pandemic of the new virus. The laboratory confirmed cases have now been found in more than 70 countries. The World Health Organization has determined that this virus has shown "sustained" human to human transmission and has upgraded the pandemic alert level to 6 on a scale of 6. (A Phase 6 designation indicates that a global pandemic is underway).(Cal/OSHA, 2009)It is threat to global public health.(CDC, 2009).

The Novel H1N1 virus spreads from human to human, in similar way that of regular seasonal influenza viruses. This virus was originally referred to as "swine flu" because many of the genes in this new virus were similar to influenza virus occurring in pigs in North America. But, this new virus differs from the virus that is normally present in North American pigs. It has two genes from flu viruses that normally circulate in pigs in Europe and Asia, along with avian genes and human genes. (Deadly new flu virus in US and Mexico may go pandemic, 2009). This virus is resistant to the antiviral medications amantadine and rimantadine, but is sensitive to oseltamivir and zanamivir.(Mayo Clinic Staff , 2009).

**Clinical Presentation and Transmission:**

The clinical presentation of novel H1N1 (swine) flu virus in people imitate seasonal flu, and include fever, cough, sore throat, runny or stuffy nose, body aches,

headache, chills and fatigue. A significant number of people who have been infected with this virus also have reported diarrhea and vomiting (CDC). Also, like seasonal flu, severe illnesses and death has occurred as a result of illness associated with this virus.

The difficulty in breathing or shortness of breath, pain or pressure in the chest or abdomen, sudden dizziness, confusion, severe or persistent vomiting are signs calling for emergency attention. In children the infection manifests as fast breathing or troubled breathing, bluish skin color, inadequate drinking of fluids, not waking up or not interacting, being highly irritable. (Pandemic H1N1 flu).

Pandemic H1N1 flu spreads from person to person through coughing or sneezing by infected people. It is not caused from eating pork or pork products. Sometimes people may become infected by touching flu contaminated surfaces/materials, followed by touching their mouth or nose.(CDC)

The pregnant women, infants, children and young adults up to 24 years of age forms the major risk groups. The patients with asthma, diabetes, kidney disease, heart disease, haematological disorder, neurological disorders or compromised immunity are at risk.(Centers for Disease Control and Prevention, 2009). Children on long term aspirin therapy are at risk.(Bell, 2009) Smokers, especially those who breathe second hand smoke are largely at risk of acquiring this infection. (Centers for Disease Control and Prevention, 2009).

**Control and Prophylaxis:**

"Prevention is better than cure" seems to befit the current scenario. The strict compliance to preventive measures need to be followed to protect health care professionals and general masses. Centre for Disease Control (CDC) recommends the use of approved N95 filtering face piece respirators during flu patient care and medical procedures. It is recommended that hospitalized H1N1 flu patients (suspected or confirmed) be housed in an airborne infection isolation room. Higher levels of

respiratory protection, such as powered air purifying respirators, should be considered for employees who perform high hazard procedures, such as endotracheal intubation, nebulizer treatment, bronchoscopy, and resuscitation involving emergency intubation or cardiac pulmonary. In general, in health care operations and other higher risk environments, approved respiratory protection should be used.(Pandemic H1N1 flu)

Application of surgical or procedure masks, tissue and hand hygiene materials should be used in risk areas. Facility cleaning, disinfection, hand hygiene can curb the spread of infection. Alcohol-based hand cleaners are also effective. Close contact with sick people needs to be avoided. (US Centers for Disease Control. 2008)

Thorough symptomatic as well as diagnostic screening for flu infection is a potent tool to curb the transmission of infection.

Persons with swine influenza A (H1N1) virus infection have been considered potentially contagious for up to seven days following illness onset or until symptoms are resolved. The duration of infectiousness might vary by swine influenza A (H1N1) virus strain. (Novel H1N1 Novel Influenza (Swine flu). 2009).

The chemoprophylaxis for novel A H1N1 includes antiviral agents, oseltamivir (Tamiflu) and zanamivir, given at early stage in disease progression so as to prevent the release of virion to infect other cells. Both these antivirals inhibit neuraminidase (eluting enzyme). However, the prevalence of oseltamivir resistant (0.5%) pandemic (H1N1) 2009 has been reported. The novel strains with this resistance have been reported in US, Canada, Japan, Denmark and Hongkong. (Graitcer *et al.*, 2009).

As a part of pandemic planning, split virion vaccine grown in eggs and adjuvanted with ASO3, an oil-in-water adjuvant containing squalene and whole-cell, unadjuvanted vaccine grown in Vero cells have been tried in UK.(Andrews *et al.*, 2011). The ASO3 adjuvant

pandemic (H1N1) 2009 vaccine have been found effective in preventing pandemic (H1N1) 2009 disease. Influenza A (H1N1) 2009 Monovalent Vaccine by Sanofi Pasteur, Novartis, CSL Limited and Influenza A (H1N1) 2009 Monovalent Vaccine Live, Intranasal by MedImmune, LLC have been approved by CDC.

The efforts to curtail and curb the rapid global spread of influenza virus, has been relatively ineffective. The widespread use of antiviral agents has led to the emergence of drug resistance in AH1N1. Thus, development of effective and specific vaccines against this virus is a key concern.(Tang *et al.*, 2010). Different technologies are being thought of and explored in this context.

In a study, researchers observed inhibitory effect of Serum Amyloid P component (SAP) on influenza A virus.(Horváth *et al.*, 2001) SAP binds in vitro Ca(2+)-dependently to several ligands including oligosaccharides with terminal mannose and galactose, suggestive of their role as SAP binding sites. The studies evidenced the potential of intranasal administered SAP for prophylactic treatment of influenza.

Further, the soluble, multimeric forms of the 2009 A(H1N1) HA (sHA) and NA (sNA) surface glycoproteins using a virus-free mammalian expression system have also been evaluated for their efficacy as vaccines in ferrets. The co-administration of both antigens were found to increase the HA-specific but not the NA-specific responses. The investigations demonstrated the vaccine potential of multimeric HA and NA ectodomains. The mass production of these domains has been documented to be easy, rapid, flexible, and safe. The inclusion of NA in influenza vaccine is thought to reduce the HA dose and broaden the protective immunity.(Bosch *et al.*, 2010).

As it is evident that the pandemic H1N1 has crossed the species barriers and infected turkeys and swine in several countries, the development of effective

multiple animal species vaccine, a plausible remediation tool has been advocated. It has been previously demonstrated that introduction of temperature-sensitive mutations in the PB2 and PB1 genes of an avian H9N2 combined with the insertion of an HA tag in PB1 resulted in an attenuated (att) vaccine backbone for both chickens and mice. The genetically modified backbone with impaired polymerase activity was found to restrict the growth of virus at elevated temperatures. The ty/04 att backbone vaccine candidate demonstrated that this vaccine is highly attenuated in mice as evident from the absence of signs of disease, limited replication and minimum histopathological alterations in the respiratory tract. A single immunization with the ty/04 att-based vaccines conferred complete protection against a lethal H1N1pdm infection in mice. Moreover, it has been reported that the vaccination of pigs with a ty/04 att-H1N1 vaccine candidate led to sterilizing immunity upon an aggressive intratracheal challenge with the 2009 H1N1 pandemic virus, the studies were provided with a potential vaccination candidate for humans and animals. (Pena *et al.*, 2010).

The fast evolving virus has given way to the emergence of some drug-resistant strains. This calls for alternative treatments and in depth studies to understand

the mechanisms involved in the drug resistance. Various current approaches such as steered molecular dynamics (SMD) approach to estimate the binding affinity of ligands to the glycoprotein neuraminidase have been applied. Different ligands which are better than the existing commercial drugs have been observed, and thought to be useful for the therapeutic applications. (Mai *et al.*, 2010).

The studies on screening and evaluation of antiviral agents has also been extended to natural products. One new licochalcone G and seven known chalcones were isolated as active principles from the acetone extract of *Glycyrrhiza inflata*. Amongst these compounds, compounds without prenyl group showed strong inhibitory effects on various neuraminidases from influenza viral strains, H1N1, H9N2, novel H1N1 (WT), and oseltamivir-resistant novel H1N1 (H274Y) expressed in 293T cells. Furthermore, it was observed that the efficacy of Oseltamivir with the presence of one of these compounds increased against H274Y neuraminidase. Thus, pointing towards synergistic effect. Obviously, the investigations give a hope for control of pandemic infection by oseltamivir-resistant influenza virus. (Dao *et al.*, 2010).

Confirmed Case	Probable Case	Suspected Case
1. Acute febrile Respiratory illness	1. Acute febrile respiratory illness	1. Acute febrile respiratory illness
2. Laboratory confirmation at CDC by one or more ie Real Time RT-PCR and Viral Culture	2. Positive for influenza A, but negative for H1 and H3 by influenza RT-PCR, or 3. Positive for influenza A by an influenza rapid test or an influenza immunofluorescence assay (IFA) & meets criteria for a suspected case	2. Within seven days of close contact with a person who is a confirmed case of swine influenza A (H1N1) virus infection, or 3. Within seven days of travel to community either within the United States or internationally where there are one or more confirmed swine influenza A(H1N1) cases, or Resides in a community where there are one or more confirmed swine influenza cases.

However, more elaborate investigations are required to come out with targeted effective gunshot to eliminate the pandemic virus.

**Diagnosis Criteria:**

There is a defined criteria to diagnose and confirm the case of swine influenza A (H1N1) as illustrated by CDC.(Centers for Disease Control and Prevention. 2010)

In the wake of the ever-changing tactics of the influenza virus, the studies are underway to explore methods for earlier diagnosis. As an effort towards this, the investigations have shown that the rapidly generalized lymphopenia, preferential loss of Th17 population and T cell activation presented as characteristics of the early immune response in S-OIV-infected patients. These findings have been advocated to be helpful for an earlier diagnosis and further studies of immune pathogenesis of S-OIV infection. (Jiang *et al.*, 2010).

Further, it is documented that the broad range of current imaging capabilities, would make it possible to study influenza at the cellular level, in animal models, and in human clinical trials to elucidate the pathogenesis of severe illness and improve clinical outcomes, thus, would provide with significant opportunities to respond to the current H1N1 pandemic and future epidemics through interdisciplinary strategies that integrate imaging science with pathology, virology, and clinical studies. (Mollura *et al.*, 2010)

**Re-emergence gimmicks:**

The first influenza pandemic dates back to 1918. Since then, four major outbreaks were reported in 1957, 1968, 1998 and 2009, with genomes H1N1, H2N2, H3N2 and triple reassortant H3N2 swine viruses respectively. Subsequent reassortment with classical H1N1 swine virus is thought to have resulted in the emergence of further triple reassorted swine A (H1N1) virus. It is thought to be a reassortment of four known strains of influenza A virus subtype H1N1: one endemic

in humans, one endemic in birds and two endemic in pigs (swine). The genomic data and subsequent phylogenetic tree construction points towards the origin of this novel A(H1N1) 2009.(Sinha *et al.*, 2009)

Influenza virus has high potential for reassortment. H1N1 sequences are evolutionary similar to the most ancient sequence reported in the NCBL database collected in 1918. New H1N1 2009 virus contains a combination of genes previously present in swine or human influenza virus. The NA and M genes are thought to have their origin in the avian influenza virus that entered in Eurasian avian like swine in 1979. The other genes are derived from triple reassorted swine viruses where these genes are thought to have entered in 1998. (Smith *et al.*, 2009).

This new virus is characterized by, a previously unknown constellation of gene segments derived from North American and Eurasian swine lineages and with the absence of common markers predictive of human adaptation. (Safronetz *et al.*, 2010).

The heterogeneity among the two SOIV isolates in virus replication, host transcriptional and cytokine responses, and disease progression, demonstrating a higher pathogenic potential for A/Mexico/InDRE4487/2009. has been documented. The nonhuman primate model has been found to closely mimic influenza in humans.(Safronetz *et al.*, 2010).

A comparative study of the RNA genome of the 1918 strain to other influenza strains using relative synonymous codon usage (RSCU), effective number of codons (ENC), and phylogenetic relationship has been carried out. It has been found that the PB1 gene of the 1918 pandemic virus had ENC values similar to the H1N1 classical swine and human viruses, but different ENC values from avian as well as H2N2 and H3N2 human viruses. Moreover, according to the RSCU of the PB1 gene, the 1918 virus grouped with all human isolates and "classical" swine H1N1 viruses. The

phylogenetic studies of all eight RNA gene segments of influenza A viruses may indicate that the 1918 pandemic strain originated from a H1N1 swine virus, which itself might be derived from a H1N1 avian precursor, which was separated from the bulk of other avian viruses in toto a long time ago. The high stability of the RSCU pattern of the PB1 gene indicated that the integrity of RNA structure is more important for influenza virus evolution than previously thought. (Anhlan *et al.*, 2010)

With the advent of newer tools and techniques, undoubtedly we are heading towards unraveling the mysteries of the influenza virus, but the virus seems to be far more tactical to prevail, emerge or re- emerge. Thus, potentially threatening challenge still surrounds us.

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