

## Review Article



## Influenza A Pandemic (P- H1N1) Flu and its Circulation Trend in Northern India: A Review

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Accepted on: 19-02-2014; Finalized on: 31-03-2014.

### ABSTRACT

The respiratory viral infections remain a major cause of morbidity and mortality in both children and adults. The notable among these is influenza virus which is responsible for large scale annual epidemics. The H1N1 pandemic strain which is in circulation since 2009 seems to be a reassortment of human influenza and swine influenza viruses. Here we review the outbreaks of influenza A (H1N1) virus and we discuss the clinical features, laboratory diagnosis, influenza vaccines and antivirals. This review here focuses the need for instituting precautionary measures to curtail the disease.

**Keywords:** Diagnosis, H1N1, Swine influenza A, Treatment, Vaccines.

### INTRODUCTION

Influenza is a serious respiratory illness of humans which leads to debilitating complications and prolonged hospitalization and death especially in the elderly.<sup>1</sup> Influenza pandemics have been associated with high morbidity and mortality worldwide. Influenza A (H1N1) virus is a subtype of influenza A virus and the most common cause of influenza (flu) in humans.<sup>2,3</sup> Some strains of H1N1 are endemic in human and cause a small fraction of all influenza-like illness and a small fraction of all seasonal influenza. H1N1 strains caused a few percent of all human flu infections in 2004-2005.<sup>4</sup> In 2009, the World Health Organization (WHO) declared the new strain of swine-origin H1N1 as a pandemic which is often referred as swine flu.<sup>5,6</sup>

### H1N1 PANDEMIC EPIDEMIOLOGY

In the 2009 flu pandemic, the virus isolated from patients in the United States (U.S.) was found to be made up of genetic elements from four different flu viruses – North American swine influenza, North American avian influenza, human influenza, and swine influenza virus typically found in Asia and Europe.<sup>7</sup> This new strain appears to be a result of reassortment of human influenza and swine influenza viruses, in all four different strains of subtype H1N1.<sup>8</sup>

Preliminary genetic characterization found that the hemagglutinin (HA) gene was similar to that of swine flu viruses present in U.S. pigs since 1999, but the neuraminidase (NA) and matrix protein (M) genes resembled versions present in European swine flu isolates.<sup>9</sup> The six genes from American swine flu are themselves mixtures of swine flu, bird flu, and human flu viruses.<sup>10</sup>

In April 2009, an outbreak of Influenza-like illness occurred in Mexico and the USA the Centers for Disease

Control and Prevention (CDC) reported seven cases of novel A/H1N1 influenza.<sup>11,12</sup> In 2009 it became clear that the outbreak of ILI in Mexico and the confirmed cases of novel influenza A in the southwest US were related.<sup>13</sup> The disease then spread rapidly, with the number of confirmed cases rising to 2,099 in mid year of 2009, despite aggressive measures taken by the Mexican government to curb the spread of the disease.<sup>14</sup>

On 11<sup>th</sup> June, 2009, the WHO declared an H1N1 pandemic, moving the alert level to Phase 6, marking the first global pandemic since the 1968 Hong Kong flu.<sup>15</sup> On 29<sup>th</sup> November, 2009 worldwide update by the WHO stated that 207 countries and overseas territories/communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009<sup>16</sup>, including at least 8,768 deaths. In 2010 worldwide update by the WHO stated that 208 countries and overseas territories or communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including at least 13,554 deaths.<sup>17</sup> The various pandemics of influenza have been depicted in the Table 1.

**Table 1:** Influenza A Pandemics in the Past Hundred Years

Year of origin	Sub-type in circulation
1890	H2N8
1900	H3N8
1918	H1N1(Spanish Flu, Swine Flu)
1957	H2N2 (Asian Flu)
1968	H3N2 (Hongkong Flu)
1977	H3N2 & H1N1
2007-2008	H5N1 ( Bird Flu)
-	H7N7 (Zoonotic potential)
-	H1N2 (Endemic in Human and Pig)
2009-2013	H1N1(Spanish Flu, Swine Flu)



## Transmission

### Person-to-person transmission

Influenza virus can be transmitted through sneezing and coughing via large-particle droplets.<sup>18</sup> In addition to respiratory secretions, certain other body fluids (e.g. diarrheal stool) should also be considered potentially infectious. In contrast to previous outbreaks of swine influenza viruses, the pandemic of H1N1 influenza A infection that began in March 2009 appears to involve sustained human-to-human transmission, as suggested by the large numbers of patients with respiratory illnesses identified within a short period of time at various locations around the world.<sup>19</sup> Several of the isolates causing disease in the U.S. have been found to be nearly genetically identical to isolates in Mexico, supportive of person-to-person transmission.<sup>20</sup>

### Viral shedding

Influenza shedding begins the day prior to symptom onset and can persist for five to seven days in immunocompetent individuals.<sup>21</sup> Longer periods of shedding may occur in children (especially young infants), elderly adults, patients with chronic illnesses, and immunocompromised hosts.<sup>22</sup> The amount of virus shed is greatest during the first two to three days of illness.<sup>23</sup> Although it is thought that immunocompetent patients with pandemic H1N1 influenza A virus infection are likely to be contagious from one day prior to the development of signs and symptoms until resolution of fever, longer periods of shedding were also detected several cases.<sup>24</sup>

### Secondary attack rates

There are conflicting data regarding whether secondary attack rates are higher with H1N1 influenza infection compared to seasonal influenza.<sup>25</sup>

### Environmental transmission

Transmission via contact with surfaces that have been contaminated with respiratory droplets or by aerosolized small-particle droplets may also occur.<sup>26</sup>

### Role of pigs

Pigs play an important role in interspecies transmission of influenza virus. Susceptible pig cells possess receptors for both avian (alpha 2-3-linked sialic acids) and human influenza strains (alpha 2-6-linked sialic acids), which allow reassortment of influenza virus genes from different species if a pig cell is infected with more than one strain.<sup>27</sup>

### Clinical Manifestations

The signs and symptoms of influenza caused by pandemic H1N1 influenza A virus are similar to those of seasonal influenza, although gastrointestinal manifestations (vomiting) appear to be more common with pandemic H1N1 influenza A.<sup>28</sup>

## Adults and adolescents

The most common clinical findings of the 2009 H1N1 influenza A pandemic have been fever, cough, sore throat, malaise, and headache, vomiting and diarrhea have also been common, both of which are unusual features of seasonal influenza.<sup>29</sup> Other frequent findings have included chills, myalgias, and arthralgias. Rhabdomyolysis has been reported rarely.<sup>30</sup>

## Children

Young children are less likely to have the usual influenza signs and symptoms, such as fever and cough. Infants may present with fever and lethargy, and may not have cough or other respiratory symptoms.<sup>31</sup> Symptoms of severe disease in infants and young children may include apnea, tachypnea, dyspnea, cyanosis, dehydration, altered mental status, and extreme irritability.<sup>32</sup>

## Definitions of illness severity

The U.S. CDC has categorized the severity of illness from influenza as follows:<sup>33</sup>

- A. Mild or uncomplicated illness is characterized by fever, cough, sore throat, rhinorrhea, muscle pain, headache, chills, malaise, and sometimes diarrhea and vomiting, but no shortness of breath and little change in chronic health conditions.
- B. Progressive illness is characterized by typical symptoms plus signs or symptoms such as chest pain, poor oxygenation (e.g. tachypnea, hypoxia, labored breathing in children), cardiopulmonary insufficiency (e.g. hypotension), CNS impairment (e.g. confusion, altered mental status), severe dehydration, or exacerbations of chronic conditions (e.g. asthma, chronic obstructive pulmonary disease, chronic renal failure, diabetes or other cardiovascular conditions).
- C. Severe or complicated illness is characterized by signs of lower respiratory tract disease (e.g. hypoxia requiring supplemental oxygen, abnormal chest radiograph, mechanical ventilation), CNS findings (encephalitis, encephalopathy), complications of hypotension (shock, organ failure), myocarditis or rhabdomyolysis, or invasive secondary bacterial infection based on laboratory testing or clinical signs (e.g. persistent high fever and other symptoms beyond three days).

## Laboratory Diagnosis

### Collection, storage and transport of specimen

Clinical specimens that are to be tested for influenza viruses can be collected either as part of routine patient care (through sentinel surveillance) or during outbreak investigations.<sup>34</sup> The successful isolation of an influenza virus depends upon the prompt collection of high-quality specimens, the rapid transportation of specimens to the testing laboratory, and appropriate transport and storage conditions prior to testing. Ideally, respiratory specimens and acute-phase serological specimens should be



collected within 3 days of the onset of clinical symptoms.<sup>35,36</sup>

### **Processing of clinical specimens for virus isolation**

Virus isolation is a highly sensitive and very useful procedure for the diagnosis of viral infection.<sup>37</sup> One important advantage of virus isolation is that it amplifies the amount of virus in the original specimen, thus producing a sufficient quantity for further antigenic and genetic characterization, and for drug susceptibility testing if required.<sup>38</sup>

### **Virus isolation in cell culture**

The isolation of viruses in cell cultures is increasingly becoming the gold standard for virus diagnosis. However a laboratory must maintain several cell lines to allow for the detection of a variety of respiratory pathogens. Since standard virus-isolation procedures take several days before results are available they are usually of limited use in clinical settings for the prompt diagnosis of influenza.<sup>39</sup>

### **Virus isolation in embryonated chicken eggs**

In recent years, the use of cell cultures has surpassed the use of embryonated eggs to isolate and culture influenza viruses. However, only viruses grown in embryonated eggs can be used as seed viruses for the production of the majority of influenza vaccines. For this reason, laboratories that have the capability to isolate influenza viruses in eggs are encouraged to maintain this capacity.<sup>40</sup>

### **Identification of the haemagglutinin subtype of viral isolates by haemagglutination inhibition testing**

The haemagglutination inhibition (HAI) test is an extremely reliable assay for typing, subtyping and further determining the antigenic characteristics of influenza viral isolates provided that the reference antisera used contain antibodies to currently circulating viruses. The antisera used are based on antigen preparations derived from either the wildtype strain or a high-growth reassortant made using the wild-type strain or an antigenically equivalent strain. The disadvantages of the HAI test include the need to remove nonspecific inhibitors of haemagglutination that occur naturally in sera; the need to standardize reference and test antigens each time a test is performed; and the need for specialized expertise in reading the results of the test. Nevertheless, the HAI test remains the assay of choice for global influenza surveillance and for determining the antigenic characteristics of influenza viral isolates.

### **Serological diagnosis of influenza by haemagglutination inhibition testing**

Diagnosing influenza by virus isolation in cell culture definitively identifies the infecting strain and is usually more rapid than serological diagnosis. However, serological diagnosis is an important approach when clinical specimens are unobtainable or when a laboratory does not have the resources required for virus isolation. Serological methods such as the HAI test are essential for

many epidemiological and immunological studies and for evaluation of the antibody response following vaccination. Serological methods are also very useful in situations where identification of the virus is not feasible (e.g. after viral shedding has stopped). Demonstration of an acute influenza infection using serology requires a significant increase in antibody titres (i.e. 4-fold or greater) between acute-phase and convalescent phase serum samples. The demonstration of such a significant increase may establish the diagnosis of a recent infection even when attempts to detect the virus are negative.

### **Serological diagnosis of influenza by microneutralization assay**

Serological methods such as the HAI test rarely yield an early diagnosis of acute influenza virus infection. Although conventional neutralization tests for influenza viruses (based on the inhibition of cytopathogenic effect formation in MDCK cell culture) are laborious and rather slow, a microneutralization assay using microtitre plates in combination with an ELISA to detect virus-infected cells can yield results within two days. The microneutralization assay is a highly sensitive and specific assay for detecting virus-specific neutralizing antibodies to influenza viruses in human and animal sera, potentially including the detection of human antibodies to avian subtypes. Testing can be carried out quickly once a novel virus is identified and often before purified viral proteins become available for use in other assays.<sup>41</sup>

### **Identification of neuraminidase subtype by neuraminidase assay and neuraminidase inhibition test**

There are two basic forms of assay for influenza virus neuraminidase (NA) based on the use of different substrate molecules, a long-standing assay based on the use of a large substrate such as fetuin and newer assays which utilize small substrate molecules. Although the older assay is more cumbersome and difficult to perform it remains useful because antibody to the neuraminidase will block access to a large but not necessarily to a small substrate molecule. The fetuin-based method is used to determine the potency of the viral NA and thus the standardized NA dose for use in the NA inhibition (NAI) test. Once determined, the standardized dose is added to serial dilutions of test antisera, negative control serum and reference anti-NA serum. Any inhibitory effect of the sera on NA activity can then be determined and the NAI titre calculated.<sup>36</sup>

### **Molecular identification of influenza isolates**

The direct molecular identification of influenza isolates is a rapid and powerful technique. The reverse-transcription polymerase chain reaction (RT-PCR) allows template viral RNA to be reverse transcribed producing complementary DNA (cDNA) which can then be amplified and detected.<sup>42</sup> This method can be used directly on clinical samples and the rapid nature of the results can greatly facilitate investigation of outbreaks of respiratory illness. For example, genetic analysis of influenza virus genes



(especially the HA and NA genes) can be used to identify an unknown influenza virus when the antigenic characteristics cannot be defined. Genetic analyses also can be used to monitor the evolution of influenza viruses and to determine the degree of relatedness between viruses from different geographical areas and those collected at different times of the year.<sup>43</sup>

### **Virus identification by immunofluorescence antibody staining**

Immunofluorescence antibody (IFA) staining of virus-infected cells in original clinical specimens and field isolates is a rapid and sensitive method for diagnosing respiratory and other viral infections. During recent years, monoclonal antibodies against several clinically important respiratory viruses have become commercially available and have been thoroughly evaluated in many laboratories. It is preferable for IFA staining to be performed on isolates rather than original clinical specimens as this allows any virus that is present to first be amplified, and if required used in other studies. However, where rapid diagnosis is needed, this procedure is often carried out on clinical specimens. Because commercially available rapid tests for diagnosing influenza infection differ with regard to the type of specimen required, as well as their complexity, specificity and sensitivity, WHO recommends that such assays should be used in conjunction with other laboratory.<sup>44</sup>

### **Use of neuraminidase inhibition assays to determine the susceptibility of influenza viruses to antiviral drugs**

The emergence of marked resistance to oseltamivir among seasonal A (H1N1) viruses during late 2007 to early 2008 has made it imperative to conduct NA inhibitor susceptibility surveillance among circulating influenza viruses worldwide. A number of different methods have been developed for this purpose, and the two procedures presented in this section have been based upon the Centers for Disease Control and Prevention (CDC) chemiluminescent NAI assay, and the National Institute for Medical Research (NIMR) NAI MUNANA assay. Whichever method is selected, a local risk assessment should be conducted and a suitable level of biosafety containment used – especially for viruses with pandemic potential.<sup>36</sup>

### **Treatment of H1N1 Infection**

Treatment should be initiated as soon as possible since antiviral therapy is most likely to provide benefit when initiated within the first 48 hours of illness. Treatment should not be delayed while awaiting the results of diagnostic testing, nor should it be withheld in patients with indications for therapy who present >48 after the onset of symptoms.<sup>45</sup> Furthermore, patients who have a negative rapid antigen test for influenza but in whom the clinical suspicion for influenza infection is high should be treated with antivirals since the sensitivity of these tests is generally low.<sup>46</sup>

### **Choice of Antiviral**

For patients requiring treatment, the recommended drugs are zanamivir or oseltamivir.<sup>47</sup> Peramivir, an investigational neuraminidase inhibitor that is administered intravenously, is recommended under certain very specific circumstances.<sup>48</sup> Zanamivir is contraindicated in patients with asthma or chronic obstructive pulmonary disease. 75 mg dose must be given to patient twice a day for 5 days. Prophylactic dose is 75 mg once a day for ten days.<sup>49</sup>

### **Vaccination Strategies**

Vaccination against the prevalent wild-type influenza virus is recommended for all individuals in high-risk groups, including those aged 65 years or older (CDC 2005) and those with chronic illness, particularly diabetes, chronic respiratory and cardiac disease, and persons immunocompromised from disease or concomitant therapy.<sup>50</sup> In addition, it is generally recommended that all healthcare personnel be vaccinated annually against influenza (CDC 2006). Influenza vaccines are efficacious in children older than two years but little evidence is available for children under two.<sup>51</sup> Nasal spray of live vaccines seemed to be better at preventing influenza illness than inactivated vaccines. In an ideal world, we would have 6.5 billion vaccine doses the day after the pandemic starts; in addition, we would have 6.5 billion syringes to inject the vaccine; and finally, we would have an unlimited number of health personnel to administer the vaccine.<sup>52</sup> At present, the world has a production capacity of about 300 million trivalent influenza vaccines per year, most of which is produced in nine countries. 300 million trivalent influenza doses translate into 900 million univalent doses, enough to vaccinate 450 million people with an initial vaccination and a booster dose- if the H5N1 vaccine is sufficiently immunogenic.<sup>53</sup>

Influenza vaccines are currently prepared in fertilized chicken eggs, a process which was developed over 50 years ago. New technologies may one day be able to develop vaccines more. A dream vaccine would provide broad-spectrum protection against all influenza subtypes.<sup>54</sup>

Influenza vaccine comprises mainly 2 types a trivalent inactivated subunit (TIV) which is administered intramuscular and trivalent live attenuated vaccine (LAIV) given intranasal. The composition of the 2009-2019 Influenza vaccine is A/California/7/2009 (H1N1), A/Brisbane/60/2007 (H3N2) and B/Florida/4/2006.<sup>55</sup>

Every year, the WHO predicts which strains of the virus are most likely to be circulating in the next year, allowing pharmaceutical companies to develop vaccines that will provide the best immunity against these strains. These vaccines can be effective against multiple strains and are used either as part of a preventive strategy, or combined with culling in attempts to eradicate outbreaks (Table 2).

**Table 2:** Inactivated Influenza Vaccines available during 2009-2014.

Vaccine	Package	Dose	Age
NASOVAC Serum Inst.	Multi-dose vial <sup>+</sup> 5 dose Intranasal	0.5 mL Int Nasal	≥4 yrs
VaxiFlu (Zydus) Inactivated Inf vaccine	Multidose 10 dose vial	0.5 mL I.M	All age group
Influvac Solvay Pharma Inactivated trivalent sub unit vaccine	Single dose syringe packed	0.5 mL I.M.	All age group
Vaxigrip Inactivated split vaccine H1N1, H3N2, type B	Single dose syringe	0.5 mL	2 doses required

### H1N1 Infectivity in Lucknow, Uttar Pradesh

The first case of swine flu was reported from Lucknow on 8<sup>th</sup> August 2009. In Lucknow, Uttar Pradesh, the current situation regarding the outbreak of swine influenza A (H1N1) is evolving rapidly. In Uttar Pradesh, the cases have been reported by Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow.

The throat swab of patients suspected with influenza virus was collected in VTM (Viral Transport Media). Viral RNA was extracted from all samples by using the Qi Aamp Viral RNA mini kit (Qiagen) according to the manufacturer's guidelines. Real Time-PCR of the extracts was performed by using Agpath-ID™ One-Step Real Time-PCR kit (Ambion U.S.A) according to the manufacturer's instructions.

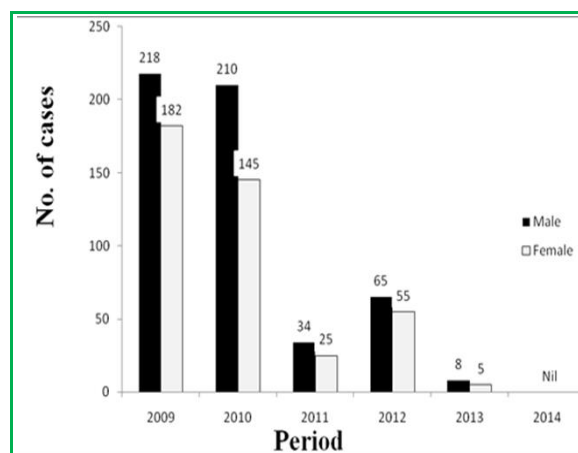
Briefly, the 25µl reaction volume contained 5µl of 5X PCR buffer, 13µl of RNase-free water, 1µl of 10mmol/L dNTPs, 1.5µl of 10 nmol/L reverse primer, 1.5µl of 10nmol/L forward primer, 1 µl of enzyme mix (Taq DNA polymerase and reverse transcriptase), and 2µl of viral RNA extract. Amplification was carried out in an Applied Biosystems Step One Real Time PCR with a single reverse transcription step of 50°C for 30 min, activation of hot start Taq at 95°C for 15 Sec followed by cycling step (95°C for 15 Sec. and 55°C for 30 sec).

Clinically suspected (n=5090) cases of pandemic flu Influenza A belonging to age group 0-60 years and both sexes were tested from 17 districts of Uttar Pradesh during June 2009 to February 2014. There were (947/5090) 18.60% cases found positive for pandemic flu Influenza A virus from 1975 clinically suspected cases. 400 (20.25%) cases were reported positive for swine influenza A/H1N1 infection by real time PCR detection and characterization as per the protocol of CDC. The sex distribution showed that there were 218 males and 182 female patients (Fig.1) among the 400 swine influenza A/H1N1 positive cases. The overall mortality was seen in 17 cases during this period.

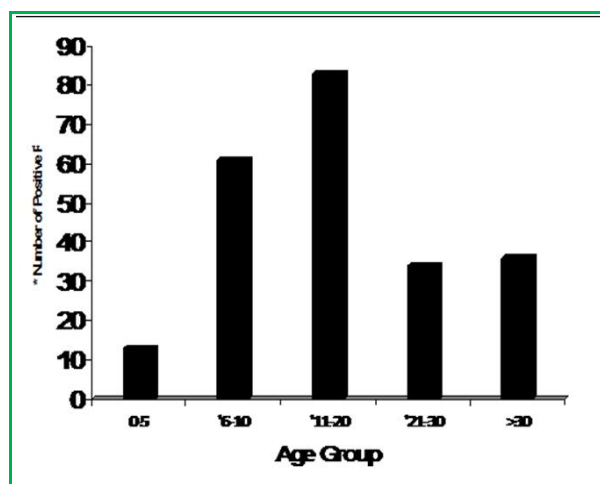
In 2010 there were 1692 clinically suspected cases of influenza and 355 (20.98%) cases were confirmed positive for swine influenza A/H1N1 infection. Among these patients there were 210 males and 145 females showing an overall male predominance. In 2011 from 551 clinically

suspected cases 50(9.07%) cases were reported positive for swine influenza A/H1N1 infection with 34 male and 25 female patients. In 2012 out of 742 clinically suspected cases there were 120(16.17%) cases which were reported positive for swine influenza A/H1N1 infection with 65 male and 55 female patients. In 2013 there were 130 clinically suspected cases only 13 (10%) cases were reported positive for swine influenza A/H1N1 infection with 8 males and 5 female patients.

There were none cases found positive for pandemic Influenza A in February 2014.



**Figure 1:** The year-wise trend of pandemic Influenza A H1N1 reported from June 2009-February 2014.



**Figure 2:** The age-wise distribution of positive cases for pandemic Influenza A H1N1 (n=947)

The infected individuals were in between the age group of 11-20 years and predominantly males were affected (Figure 2). The study provides information about recent outbreak of novel H1N1 and its circulation in northern India.

## CONCLUSION

The new reassortant A/H1N1 virus primarily infects human and has caused several deaths worldwide. It is a result of frequent gene reassortants and mutations. The clinical features of the swine influenza A/H1N1 virus infection include fever; cough sometimes pneumonia and multi-organ dysfunction syndrome. The mainstay of diagnosis is real-time PCR is still the main diagnostic method. The treatment modalities are neuraminidase inhibitors like oseltamivir, zanamivir, and peramivir which have been used successfully for clinical treatment. There is an emergent need to discover new drugs and treatment strategies. There must be strict and vigilant intensive surveillance and preparedness measures for the prevention of major outbreaks in future.

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Source of Support: Nil, Conflict of Interest: None.

