Research Article

# Swine origin influenza A H1N1 viral infection in pediatric patients at tertiary-care hospital, Ahmedabad

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**Abstract**

**Background:** In April 2009, influenza A H1N1 of swine origin was first documented in the border areas of Mexico and United States. Influenza viruses are known to cause frequent epidemics and periodic pandemics; therefore, it has been major public health problem.

**Objective:** To study the swine-origin influenza A H1N1 in pediatric patients attending civil hospital, Ahmedabad, by real-time reverse transcription–polymerase chain reaction (rRT-PCR).

**Materials and Methods:** A retrospective observational study was conducted at a tertiary-care hospital, Ahmedabad, Gujarat, India, from January to March 2015. Totally, 484 samples of pediatrics patients with suspected cases of influenza A H1N1 were received and tested by rRT-PCR.

**Result:** During January to March 2015, a total of 484 samples of pediatric patients were received for H1N1 influenza; 207 samples were positive for H1N1 influenza, of which 111 (23%) were of boys and 96 (20%) of girls. Neonates accounted for 19% cases; infants for 20%; and preschool age children for 25%; 19% were aged between 5 and 10 years and 17% between 11 and 18 years. Among these patients, the mortality was 10%.

**Conclusion:** Positivity rate of H1N1 influenza in our study was 43% for pediatric patients with peak incidence in winter in mid-February, which is a major public health problem. Regular observation is warranted for early identification of any antigenic variants to realize the seasonality and analyze the role of factors such as temperature, rainfall, and humidity in the spreading of influenza viruses.

**KEY WORDS:** H1N1 influenza A virus, real-time reverse transcriptase-polymerase chain reaction (rRT-PCR), tertiary-care hospital, pediatrics, fever

## Introduction

One of the various types of swine influenza viruses (SIV) cause swine influenza, also known as pig influenza, swine flu, hog flu, and pig flu. SIV or swine-origin influenza virus (S-OIV) is any strain that belongs to the influenza family of viruses, which causes endemic flu in pigs.[1] Until 2009, influenza C and the subtypes of influenza A—H1N1, H1N2, H2N1, H3N1, H3N2, and H2N3—are the known SIV strains.

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Globally, SIV is widespread all over the pig populations. However, acquaintance of SIV by human from pigs is rare and does not, at all times, result in human flu. Often, it leads to the generation of antibodies in the blood. The human flu that resulting rarely from such transmission is called as zoonotic swine flu. The at-risk population of swine flu infection involves people with habitual contact with pigs.

During the middle of the twentieth century, the detection of influenza subtypes was achievable, enabling precise diagnosis of spread to humans. Records of only 50 such transmissions are known since then. Moreover, human to human

transmission of these strains of swine flu is also not common. The symptoms include those of influenza and influenza-like illness (ILI) in general, such as chills, fever, sore throat, muscle pain, severe headache, coughing, weakness, and general discomfort.

Ever since the first case of S-OIV was documented in April 2009 in Mexico,[2] a highly contagious form of S-OIV (also known as the H1N1 virus) has been rapidly transmitted and is spreading among humans in many countries throughout the world. In June 2009, the WHO declared a level 6 pandemic for the S-OIV infection.[3] The first case of influenza A H1N1 in India was detected on May 16, 2009, in a man traveling from New York through Dubai and Delhi, who showed positive results in the test for the H1N1 influenza virus in Hyderabad.[4] The official declaration of the swine flu to be pandemic by WHO was in August 2010. In India, more than 31,156 swine flu-positive cases have been reported, with 1,841 deaths till March 2015.[5]

Acute respiratory tract infections (ARIs) are a leading cause of morbidity and mortality in children worldwide,[6] accounting for about 30% of all childhood deaths in the developing world.[7] Of the 2.5 million (one-quarter) total deaths seen in children aged younger than 5 years in India, approximately 20% of these are caused by ARIs. Frequent epidemics and periodic pandemics have been recorded with influenza virus, and they are exceptional in accordance with their antigenic variability, seasonality, and effect on general population. Epidemics of influenza mostly affect the children[8]; however, these viruses also cause considerable mortality in the aged and chronically ill persons.[11] ILI screening and observation program play vital roles in tracing the activity, especially of influenza viruses, across seasons. Because the significant differential diagnosis is hard, the control and management of respiratory viral disease outbreaks can be achieved via such essential programs.[13]

## Materials and Methods

We conducted a study involving pediatric patients admitted at civil hospital, Ahmedabad, Gujarat, India, during the period of January to March 2015. Eligible patients were all pediatric patients aged younger than 18 years who were admitted with diagnosis of ARI with category C according to Ministry of Health and Family Welfare pandemic influenza A (H1N1).[14]

Totally, 484 samples of pediatric patients aged < 18 years with suspicion of H1N1 infection were received. Specimens collected were nasal and throat swabs. Clinical samples collected in viral transport medium (VTM) in triple layer packing were transported in a vaccine carrier box to the virology laboratory of Microbiology Department of BJ Medical College, Ahmedabad. The samples were processed in a Biosafety Level-2 Cabinet.

The first step is aliquoting, which involves mixing of 200 µL of sample with 560 µL of buffer AVL with carrier RNA. Second step is RNA extraction done using spin column-based QAlamp® viral RNA mini kit (Qiagen) as per the manufacturer's instructions. Primers and probes were custom synthesised from Applied Biosystems (ABI) for influenza type A, swine A, and swine H1 gene sequences. Real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) was performed by using a Step One Real-Time PCR instrument (ABI). A master mix of 20.0 µL was prepared in a PCR plate, 5 µL of RNA template was added, and the plates were placed in the ABI Step One Real-time PCR instrument using cycling conditions of 50°C for 30 min of reverse transcription, followed by Taq inhibitor inactivation at 95°C for 10 min and PCR amplification (45 cycles) at 95°C for 15 s and at 55°C for 30 s. The test for H1N1 influenza A virus is regarded positive if the specimen is positive to either swFluA or swH1 probe, with a concomitant positive reaction to both InfA and RNaseP (RP) probes.

## Results

During the period of January to March 2015, totally, 484 patients aged < 18 years were admitted with suspicion of H1N1 ILI. Of them, 272 patients were boys, and 212 patients were girls. They have been tested for H1N1 influenza by RT-PCR. Of these, 207 (42.76%) patients were positive for H1N1 influenza, with 111 (23%) cases of boys and 96 (20%) cases of girls [Figure 1]. In our study, incidence of H1N1 cases in pediatric patients increased in mid-February [Figure 2]. With the onset of summer season in March, the number of cases declined.

## Discussions

In this study, the first pediatric case reported in our hospital was in the first week of February, and then, the rate of positive cases increased in late February. The rate of positive cases declined at the end of March with the onset of summer. The patient distribution was observed to be 56% boys and 44% girls, giving a male:female sex ratio of 1:0.786. It was observed that gender-wise positivity for influenza virus in boys and girls was 23% and 20%, respectively; however, statistically, there was no significant difference with respect to influenza positivity in gender-wise distribution on using two-tailed test for proportion. Neonates accounted for 19% cases; infants for 20%; and preschool age children for 25%; 19% were aged between 5 and 10 years and 17% between 11 and 18 years, which indicated that preschool age children were the most commonly affected age group in our study. Of the 484 patients, 21 (9.25%) deaths occurred [Figure 3]. In clinical presentation of our positive influenza cases, nasal discharge was the prominent clinical feature in every patient, followed by cough, fever, breathlessness, sore throat, headache, body ache, chills and rigor, fatigue, vomiting, and diarrhea.

A study was carried out in Mumbai during 2007–2009 among pediatric populations, in which of the 100 samples with ILI symptoms, it was observed that gender-wise positivity for influenza virus in male and female subjects was 12.28% (7/57) and 9.30% (4/43), respectively. Children aged between 1 and 5 years were found to be majorly affected (73%).[13]

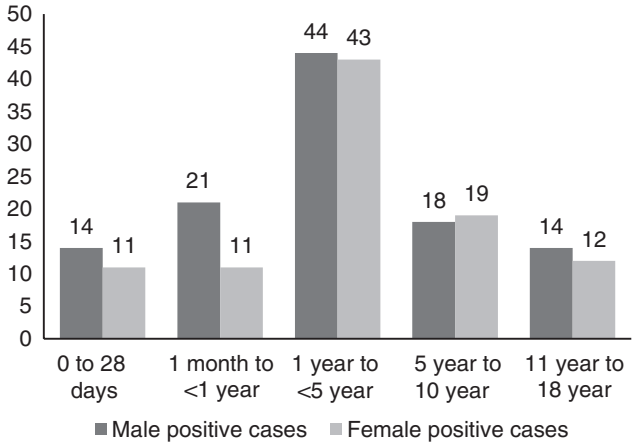


**Figure 1:** Age and sex distribution of positive H1N1 cases.

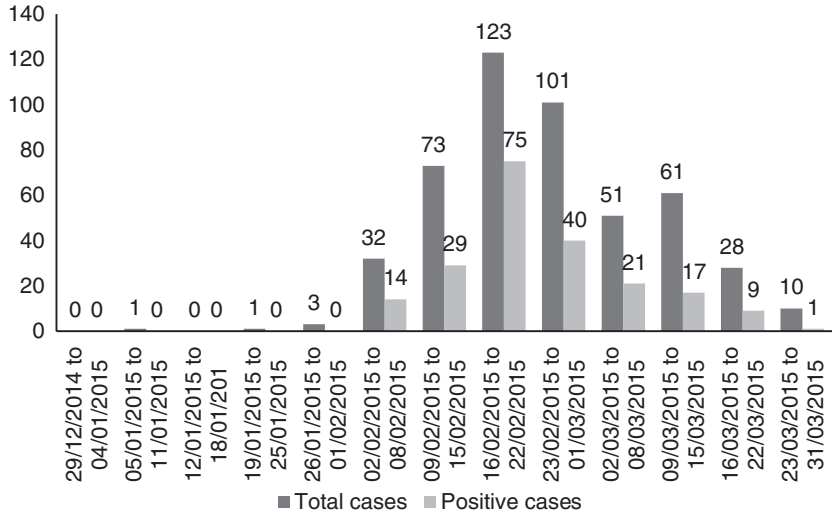


**Figure 2:** Weekly distribution of positive H1N1 cases.

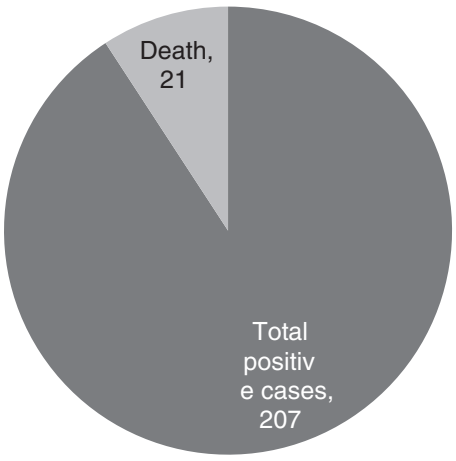


**Figure 3:** Case fatality rate (9.25%) (CFR = total no of deaths/total no of cases × 100).

A study was carried out in Chennai in the year 2002, in which, of the 240 infants and children (0–12 years) with ARIs, 12.5% (30/240) cases were positive for influenza virus, of which 10% (24/240) cases were positive for influenza A (H3N2) virus, and 1.66% (4/240) cases were positive for influenza B/Sichuan virus. In addition, 0.833% (2/240) cases were positive for influenza type A (H3N2) virus and influenza type B/Sichuan virus strain.[18]

A similar study was carried out at Delhi during 2005–2007 among pediatric patients with lower respiratory tract infection, where 301 clinical samples were processed by multiplex PCR, and it was found that 3% (9/301) cases were positive for influenza A virus.[19]

The detection of influenza occurrence in children is hard as most children with influenza show few or mild symptoms or none at all. However, schoolchildren form the center point of the influenza transmission in the community. Currently, as a preventive measure, trivalent inactivated vaccines are used. However, a more competent vaccine that is suitable

for children could be a trivalent-attenuated cold-adapted vaccine. Because of the untrustworthy of the clinical examination alone, particularly in young children, laboratory authentication is essential.[16] Several observational studies have shown that relative humidity, rainfall, and variations in temperatures affect the outbreaks of influenza. In countries with temperate climate, influenza outbreaks occur in winter.

## Conclusion

Case positivity rate in our study was 42.76%, which is quite high, and case fatality rate was also high, suggesting of major public health problem. Regular observation is warranted for early identification of any antigenic variants to realize the seasonality and analyze the role of factors such as temperature, rainfall, and humidity in the spreading of influenza viruses. We emphasise on monitoring of influenza in different geographical locations, which will be helpful in pediatric patient management who are at risk for influenza complications. In addition, healthy children with moderate to severe respiratory illness should be tested for influenza and, when infected, treated with oseltamivir or zanamivir.

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