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SWINE FLU (H1N1) PANDEMIC STRAIN-09 PDM: A RECENT OUTBREAK IN NORTHERN INDIA

Vinita Choudhary[1]*, Chetan Choudhary[2], Ayushi Sharma[1], Vinod Kumar Sharma[1], Pushpendra Saraswat[3]

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ABSTRACT
Objective: To Identify molecular characterization of swine flu (H1N1) pdm 09 and seasonal flu (influenza A). **Materials and methods:** NP/OP Swab specimen of 142 patients were collected aseptically in VTM. Nucleic Acid was extracted manually and was processed in Real-Time PCR for identification of swine flu (H1N1) 09 pdm and seasonal flu (inf A). **Results:** In this study, 142 patients presented with signs and symptoms of Flu like illness patients and were tested by Real-time PCR. Out of total 32 (22.53%) positive specimens, 18(56.25%) were positive swine flu(H1N1) 09 pdm and 14(43.73%) were positive seasonal flu (inf A). **Conclusion:** We report a tiny outbreak of 18 swine flu (H1N1) 09 pdm and 14 seasonal flu cases in our hospital, from Jan 2022 to June 2022. The outbreak involved persons of all age groups, but it mostly affected paediatric age group.

INTRODUCTION

Worldwide, infectious diseases continue to cause disability and death, threatening human progress and survival. There is a heavy burden of infectious diseases on the people due to the emerging epidemics that occur occasionally along with unpredictable pandemics that cause a great deal of mortality in the global population. Infections that are emerging or re-emerging are associated with higher morbidity and mortality rates because antibiotics and antiviral agents have become hard to treat. Vaccines have helped decrease the death rate from many infectious diseases, but they are still a major killer [1]. An important infectious disease of humans is influenza (also known as the flu). Acute respiratory illnesses have been caused by influenza viruses since ancient times, which are highly contagious. The epidemiology, pathogenicity, replication, and immune parameters that lead to influenza pandemics have still not been fully understood despite over 70 years of research on influenza viruses [2]**.** The influenza virus (flu) belongs to the *Orthomyxoviridae* family of viruses that cause respiratory

[1]*Department of Microbiology; MGUMST, Jaipur, Rajasthan 302022, India*
[2]*Department of Emergency Medicine, MGUMST, Jaipur, Rajasthan 302022, India*
[3]*Director of laboratory; Central Research Laboratory, MGUMST, Jaipur, 302022, India*

For Correspondence: **dr.vinita89@gmail.com**

illnesses. Influenza viruses are classified into four genera (influenza virus A, influenza virus B, influenza virus C, and influenza virus D) based on their internal glycoproteins' nucleoprotein and matrix. Humans, birds, pigs, horses, and other animals can be infected by influenza viruses, but influenza viruses B and C can only infect humans. Influenza viruses have a single-stranded negative sense RNA genome that encodes 11 proteins. There are different subtypes of influenza A virus based on the neuraminidase (NA) and hemagglutinin (HA) glycoproteins on their surface. There are 18 subtypes of influenza A viruses (HA1-H18) and 11 subtypes of influenza A viruses (NA1-N11), resulting in 144 possible combinations of the different subtypes [3–9]. The natural reservoir for these subtypes is believed to be aquatic birds, especially ducks, geese, and swans. It is an infrequent occurrence that leads to the development of an entirely new subtype when the antigenic structure shifts, causing a dramatic change in antigenic structure. Antigenic drift is a process in which antigenic composition steadily changes over time, resulting in epidemic outbreaks and regional outbreaks.

Additionally, H1N1 and H3N2 viruses have caused four major influenza pandemics: the 1918 Spanish flu, the 1958 Asian influenza, the 1968 Hong Kong flu, and the 2009 H1N1 pandemic. The highly pathogenic avian influenza (HPAI) subtype H5N1 has been reported to infect humans since 1997 [10, 11]. During 1997, HPAI outbreaks at poultry farms and markets led to 18 cases and six deaths among people with H5N1 influenza in Hong Kong. There are several symptoms associated with the H5N1 strain of the avian flu, including pneumonia, diarrhoea, and neurological symptoms. H9N2 and H7N7 subtypes of avian influenza have also been reported to infect humans [12,13]. In 2013, human-infected strains of H7N9 were detected in China [14]. In March 2009, a pandemic of influenza erupted in California. The virus spread rapidly across the globe, including India. According to the World Health Organization, a pandemic was declared on June 11, 2009. H1N1 influenza virus was formed by genetic reassortment of four strains. The mixing was performed in pigs. A disease that is transmitted from one human to another [15].

Symptoms of influenza include fever, headache, malaise, sore throat and cough, which are all symptoms of an acute respiratory tract infection. It is of concern that illnesses are spreading widely as regional outbreaks, epidemics, and pandemics and that mortality rates are high, particularly among high-risk groups [15].

Table 1: Major influenza outbreaks [15]

Years	Subtype	Extent of outbreak
1889–1890	H2N8	Severe pandemic
1900–1903	H3N8	Moderate epidemic
1918–1919	H1N1A (HswN1) (Spanish flu)	Severe pandemic
1933–1935	H1N1a (H0N1)	Mild epidemic
1946–1947	H1N1	Mild epidemic
1957–1958	H2N2 (Asian flu)	Severe pandemic
1968–1969	H3N2 (Hong Kong flu)	Moderate pandemic
1977–1978b	H1N1 (Russian flu)	Mild pandemic
2009–2010	H1N1 pdm09	Pandemic

WHO's Global Influenza Surveillance and Response System (GISRS) conducts influenza surveillance worldwide. Laboratory diagnostics, vaccines, and flu treatment are recommended based on monitoring influenza virus evolution. Alerts the world to the emergence of influenza viruses with pandemic potential. The method directly detects viral antigens; it simultaneously detects H1N1, H3N2, Influenza B via RT-PCR, Real-time RT-PCR, and ELISA Antibody detection via hemagglutination inhibition, neutralization, and ELISA [15].

MATERIALS AND METHODS

An observational study based on laboratory data is being conducted in the present study. A study was conducted at Mahatma Gandhi Medical College & Hospital, Jaipur (Rajasthan) from January 2022 to June 2022. This study processed 142 NP/OP Swabs. Demographic data (such as age, sex, in -patient, out-patient status) of the patients were recorded. In most diagnostic labs worldwide, RT-PCR is used for NAT to detect influenza viruses because it is an extremely powerful and traditional technique. For influenza diagnosis, RT-PCR involves three steps: (1) RNA extraction from clinical specimens, (2) reverse transcriptase is used to convert viral RNA into single-stranded cDNA, (3) PCR is performed by amplification followed by fluorescent detection of fluorescent PCR products.

The purpose of Real Time PCR is to have a higher sensitivity and shorter turnaround time, and can therefore be seen as a surrogate gold standard (TRUPCR® H1N1 Detection Kit) is used to detect:

i. *Type A Influenza* viruses (infA)
ii. Pandemic *Influenza A* viruses (infA pdm)
iii. Pandemic H1N1 *Influenza* virus (H1N1 pdm)
iv. Internal control (RNase P)

TaqMan® primers and probes are mixed according to the TaqMan® principle. Both forward- and reverse-reverse primers are hybridized to cDNA during PCR amplification. Fluorogenic probes are also present in the same reaction mixture with a DNA probe with a 5'-dye and a 3'-quencher.

In PCR amplification, the reporter dye and quencher are separated as the probe is cleaved. Real-time PCR can detect changes in fluorescence produced by the resulting process. The clinical specimens to be collected can include throat swabs (oropharyngeal swabs), nasal swabs, nasopharyngeal swabs, rbronchoalveolar lavages, aspirates of the trachea, nasopharyngeal, or oropharyngeal aspirates, and washes. A bronchoalveolar lavage or tracheal aspirate should be collected in patients suffering from lower respiratory tract infections (pneumonia). Viral Transport Media (VTM) and swabs with synthetic tips (such as polyester/Dacron/rayon/flocked nylon swabs) mounted on aluminium or plastic shafts. It is not recommended to use cotton swabs or wooden shafts. It is recommended to collect bronchoalveolar lavage or tracheal aspirates in sterile screw-capped containers with a quantity of 3-5 ml.

Interpretation

Case	Amplification signals in *RNaseP*	Amplification signals			Interpretation
		InfA	*InfA pdm*	*H1N1 pdm*	
1	P	P	P	P	Sample is positive for pandemic H1 influenza virus
2	P	P	P	A	Sample is positive for pandemic Influenza A viruses
3	P	P	A	A	Sample is positive for Type A Influenza viruses
4	P	A	A	A	Test sample is negative for Type A Influenza viruses, Pandemic Influenza A viruses and H1 Influenza viruses
5	A	A	A	A	PCR inhibition, retest the sample

P – Present, A – Absent

If a specimen is positive for *InfA* and only one of the subtype reactions or positive for *InfA* only, retest the sample

Laboratory-Clinical interpretation

Influenza A (InfA) positive: Detects Influenza A responsible for seasonal outbreaks.

Influenza A pdm (InfA pdm) positive: Detects Influenza A pandemic strain responsible for the 2009 influenza outbreak

H1N1 positive (H1N1 pdm): Detects swine H1N1 Influenza [16 – 19].

RESULT & DISCUSSION

An observational study based on laboratory data is being conducted in the present study. A study was conducted at Mahatma Gandhi Medical College & Hospital, Jaipur (Rajasthan) from January 2022 to June 2022. This study processed 142 NP/OP Swabs.

Table 2 Distribution of Positive and Negative samples

Total sample	Positive	Negative
142	32(22.53%)	110 (77.46%)

Table 3 Distribution of H1N1 and InfA Positive samples

Total positive samples	swine H1N1 Influenza	Inf A (Seasonal flu)
32 (100%)	18 (56.25%)	14 (43.75%)

Table 4 Distribution of H1N1 and Inf A IPD and OPD samples

Total	H1N1		Inf A	OPD
	IPD	OPD	IPD	
32(100%)	15(46.87%)	3(9.37%)	14(43.7%)	0

Table 5 Distribution of H1N1 and InfA Male & Female samples

Total	H1N1		Inf A	
	Male	Female	Male	Female
32(100%)	10(31.25%)	8(25%)	8(25%)	6(18.75%)

Table 6 Age distribution of H1N1 and Inf A Positive samples

Age groups	Total Positive	H1N1 Positive	InfA Positive
1-20	9(100%)	6(66.66%)	3(33.33%)
20-40	8(100%)	3(37.5%)	5(62.5%)
40-60	7(100%)	6(85.71%)	1(14.28%)
60-80	8(100%)	3(37.5%)	5(62.5%)

An influenza A virus' genome consists of eight segments, each encoded by a negative-sense RNA strand, which can reassort

during infection [20]. The influenza A virus exists in many subtypes and is capable of infecting a wide range of hosts. As they can transmit across species, these viruses exhibit the greatest genetic diversity, resulting in a wide variety of influenza viruses. Antigenic drift and antigenic shift are well known features of influenza viruses that allow them to escape pre-existing immunity and cause influenza outbreaks around the world [21,22]. An influenza pandemic occurs when a novel strain of swine or bird influenza acquires the HA and NA segments of the genome [23].

In the present study, 142 NP/OP swab samples from suspected influenza patients were analysed between January 2022 and June 2022. There were 142 samples tested, 18 (56.25%) were positive for 2009 influenza A(H1N1) pandemic viruses and 14 (43.75%) for seasonal influenza A viruses. There were also studies conducted in India. Almost the same frequency of positivity was found in the studies of Siddharth V et al [24].

In our hospital, we report a tiny outbreak of 18 H1N1 and influenza A cases between January 2022 and June 2022.There were cases of the outbreak in all age groups, but the majority were in the paediatric age group.

In the present study results revealed that influenza A (H1N1) pdm 09 viruses were prevalent in children 1-20 years of age and seasonal influenza A viruses were prevalent in people 21-40 years of age in their study, In their study, Siddharth V et al [24] observed similar results. The Influenza a H1N1 screening clinic at GMCH tested 4379 suspected patients. Among them, 8.3% (365) had positive results and 29.58% (108) had negative results. There were patients as young as 4 months old and as old as 80 years old, with a mean age of 27 years (median age of 24 years) among them.

H1N1 influenza primarily affected younger individuals when 81.4% of cases were reported by patients under 40 years of age, 39.8% of cases by those between 0 and 18 years, and 40.7% by those between 18 and 40 years. There were 39.3% of positive males and 42.5% of positive females in the 18-40 age group, respectively. Of the total cases, 10.18% (11 cases) were in children 0-5 years old, of which five were in children less than 1 year old. There were 17.5% of positive patients between the ages of 0-5 and >60 (10.1% and 7.4%, respectively). Accordingly, Influenza A H1N1 caused a great deal of morbidity among the younger population, i.e., those under 40 years of age, whereas the older populations were relatively spared. There were 14.6% (14) positive influenza cases among health care providers, with 85.7% (12) being doctors. There was only one case seen among nursing personnel and hospital attendants.

There were 15 (46.87%) IPD and 3 (9.37%) OPD samples that were positive for swine flu (H1N1) pdm 09 and there were 14 (43.7%) IPD samples that were positive for seasonal influenza A. Almost the same frequency of positivity was found in the studies of Siddharth V et al [24].
The distribution of H1N1 and Inf A samples among males and females. In the present study, there was a predominance of male observers. In a similar study by Siddharth V et al. [24], 56.48% of the cases (61 cases) were males and 43.51% (47 cases) were females.

CONCLUSION

An outbreak of seasonal influenza occurs due to antigenic drift, which arises from point mutations in the viral genome. The mutations lead to the development of new strains of the flu strain that are immune to previous strains. In addition to human, swine, and/or avian influenza strains having novel HA and/or NA genes, it is possible for novel influenza viruses to develop from genetic assortment among those strains. People are not immune to these strains in the majority of cases. When there are seasonal or pandemic outbreaks, detecting newly emerging viral variants quickly and accurately is crucial in starting antibiotic treatments and prophylaxis as soon as possible. Influenza virus detection by NATs has been demonstrated to be highly specific and sensitive.

We report a tiny outbreak of 18 H1N1 AND 14 Inf A cases in our hospital, from Jan 2022 to June 2022. The outbreak involved persons of all age groups, but it mostly affected paediatric age group.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Vinita Choudhary contributed in conceptualizing, data curating and formal analysis. She also contributed in writing original draft. Chetan Choudhary and Pushpendra Saraswat contributed

in investigation and supervision of whole study. Ayushi Sharma contributed in writing, reviewing and editing the manuscript. Vinod Kumar Sharma contributed in accessing resources and reviewing and editing the manuscript. All authors read and approved the final manuscript

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