Virological surveillance of influenza virus in acute respiratory infection

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Abstract

Introduction: Influenza is viral fever with the main symptom of respiratory tract like cough, fever, breathlessness, nasal discharge, sore throat. The influenza affects a large segment of the world population resulting in significant mortality, morbidity and economic loss.

Objective: To monitor the trend of influenza A virus. To know the prevalence of influenza A, H1N1 and seasonal H3 subtypes of influenza virus. To study the seasonal pattern of influenza A virus.

Materials and Methods: This prospective study was carried out in the department of Microbiology, B.J. Medical College, Ahmadabad from January 2014 to December 2014. Surveillance samples were taken as per ILI (Influenza like illness) case definition for out Patient department and SARI (sever acute respiratory syndrome) for indoor patients. Total 614 samples are taken and tested with PCR method.

Result: Out of 614 samples total 201(32.73%) were positive for influenza. Out of 201 positive samples, 13 (2.11%) were positive for swine H1N1 and 11 (1.79%) for seasonal H3, 177 (28.82%) were positive for influenza A.

Conclusion: Prevalence of influenza 201(32.73%). out of this 13 (2.11%) were positive for swine H1N1 and 11 (1.79%) for seasonal H3, 177 (28.82%) were positive for influenza A. swine H1N1 was common in winter and seasonal H3 was common in late monsoon. swine H1N1 and seasonal H3 both are more common in male than female. swine H1N1 is more in >12 age and seasonal H3 is more common in <12 age.

Keywords: Influenza A, Swine H1N1, Seasonal H3, Fever, cough, Breathlessness.

Introduction

Respiratory illnesses due to influenza virus infection are a major cause of morbidity and mortality worldwide. Lower respiratory tract infections are a leading cause of deaths in developing countries and are also the third leading cause of death worldwide.[1] Globally influenza is responsible for 250,000 to 500,000 deaths annually.[1] In April 2009, the first case of influenza A H1N1 was reported from Mexico. It was a reassortant between previously circulating swine virus and a Eurasian swine virus and was also called swine origin influenza.

(S-OIV). It spread from person to person and caused a pandemic.[2] Subsequently, the infection led to spread of disease across 74 countries with 30,000 confirmed cases on June 11, 2009. Total of 214 countries were affected by the pandemic worldwide.[1]

In India, the first case of influenza A H1N1 was reported on May 16, 2009 from Hyderabad. The World Health Organization declared the post pandemic phase on August 10, 2010. The Central and State governments in India had taken the pandemic very seriously and made several unprecedented and innovative interventions including the pandemic preparedness plan.[1] Of all the viruses that cause respiratory illness, influenza virus plays a dominant role. Influenza viruses are known to cause frequent epidemics and periodic pandemics, and are unique with regard to their antigenic variability, Seasonality and impact on general

population. Though children are mainly affected during epidemics, the viruses are also responsible for substantial mortality in the aged and chronically ill persons.[4] Polymerase chain reaction (PCR) is a technique used to amplify specific regions of DNA from very low levels of starting template DNA. Reverse-transcription PCR (RT-PCR) is an extension of this technique in which template RNA is first reverse-transcribed into complementary DNA (cDNA). This cDNA then undergoes amplification by PCR.[5]

Vaccines and antiviral drugs are the two primary methods of implementing influenza prophylaxis.

Materials and Methods

This prospective study was carried out in the department of Microbiology, B.J. Medical College, Ahmadabad from January 2014 to December 2014. Surveillance samples were taken as per ILI (Influenza like illness) case definition for out Patient department and SARI (sever acute respiratory syndrome) for indoor patients.[6] Total 614 samples suspected of fever, cough, sore throat, nasal catarrh, shortness of breath were included in this study. Two samples (one nasal and one oropharyngeal/ throat swab) were collected in VTM from the suspected patients of Influenza. Samples were labeled and triple layer packing was done and sample along with request form were transported in cold chain to Virology laboratory (Microbiology department). Sample was proceeding in

BSC class II.RNA extraction was done by using

Kit Content[7]
1. QIAamp mini spin columns
2. Collection tube 2 ml
3. Buffer AVL (contains guanidine thiocyanate)
4. Buffer AW1 (concentrate)

RNA Extraction[7]

Take 1.5 ml micro centrifuged tube add 200μl sample.(1)

⇩

LYSIS: 560μl of buffer AVL + carrier RNA, Mix by pulse vortexing for 15 sec. Incubate at room temperature (15° to 25°C) for 10 minutes in (1)

⇩

Add 560μl of ethanol (96 -100%) and mix by pulse vortexing for 15 sec. in (1)

Add 630μl of the solution from (1) to QIAamp mini column (2 ml of collection tube), Centrifuged at 6000* g (8000 rpm) for 1 minute. Place the QIAamp mini column in to a clean 2 ml collection tube, and discard the tube containing the filtrate. Repeat previous step with rest of lysed solution from (1), Place the QIAamp mini column in to a clean 2 ml collection tube.

Carefully open the QIAamp mini column, and add 500 microliter of buffer AW1, Centrifuged at 6000* g (8000 rpm) for 1 minute, the QIAamp mini column in to a clean 2 ml collection tube, discard the tube containing the filtrate.

Add 500 microliter of buffer AW2, Centrifuged at 14000 rpm for 3 minute. Discard the old collection tube containing the filtrate. Place the QIAamp mini column in to a clean 1.5 ml collection tube, add 60 microliter of buffer AVE and incubate at room temperature for 1 minute. Centrifuged at 6000* g (8000 rpm) for 1 minute. Supernatant contains viral RNA.

Primers (oligonucleotides) are complementary sequence of target DNA. Total volume of master mix is 20 microliter for each gene. For each sample, 20μl of master mix and 5μl of RNA extraction is taken for each gene. For Positive control, Negative control, 20μl of

QIAamp Viral RNA Kit.
5. Buffer AW2 (concentrate)
6. Buffer AVE
7. Carrier RNA (poly A)

First RNA extraction is done using all above listed items.

master mix and 5μl of controls were taken, put the plate and process the sample in PCR machine. Thermo cycler raises and lowers the temperature to permit and regulate three steps of PCR (DNA denaturation step, primer anneling step, extension reaction step).[8] In the PCR machine following cycles are automatically done.

Holding Stage
1. Step-1 50.0°C for 30 minutes.
2. Step -2 95.0°C for 10 minutes.

Cycling Stage (45 cycles)
1. Step -1 95.0°C for 00.15 second
2. Step-2 55.0°C for 00.30 second.

The whole procedure is repeated in a programmable thermal cycler. Generally, 30-50 thermal cyclers produce millions of copies of the chosen section of the target organism DNA.[8]

After cycles are over PCR will be automatically stop, and we achieve a amplification graph on computer which is attached with machine. Ct value of each sample was detected and if the graph is raised, than consider it as positive. If not, than negative.

Result

Out of 614 total samples, Total 201 (32.73%) were positive for Influenza. Out of 201 positive samples, 13 (2.11%) were for Swine H1N1 and 11 (1.79%) for seasonal H3, 177 (28.82%) were positive for Influenza A. both Swine H1N1 and seasonal H3 are more common in male than female. swine H1N1 is more in >12 age and seasonal H3 is more common in <12 age. swine H1N1 is mostly occurs in winter and seasonal H3 is more common in late monsoon. (Fig. 1 to 3)
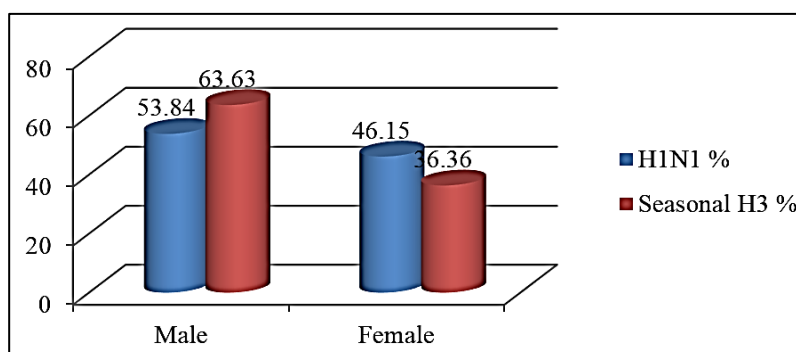
Fig. 1: Gender wise distribution of positive swine H1N1 and seasonal H3 case

The probable reason for predilection of male sex may be due to greater mobility, susceptibility and exposure to infection and also this age group consists of

economically productive mobile population, travelling more for various reasons and most susceptible to

exposure to infection, so they get exposed to virus easily and get infected easily.[11]
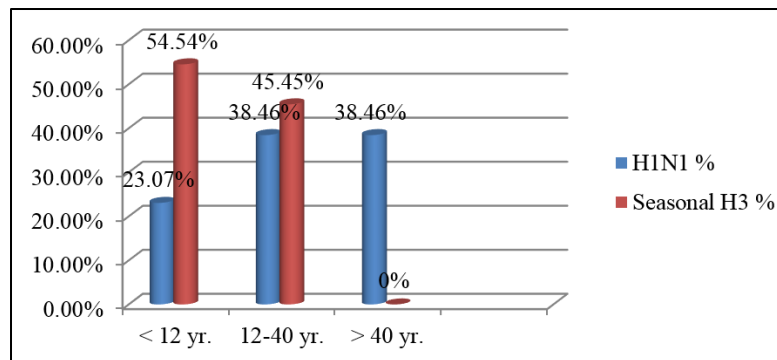

Fig. 2: Age wise distribution of positive swine H1N1 and seasonal H3 case

Swine H1N1 prevalence is high in elder people may be due to immunity is tapering at this age and so this people are more prone to infection.
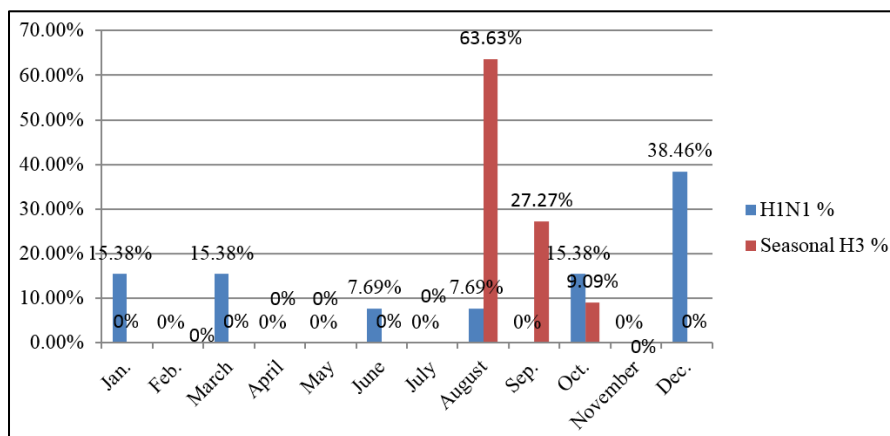

Fig. 3: Month wise distribution of positive swine H1N1 and seasonal H3 case.

Prevalence of swine H1N1 in winter mainly because of this virus can survive and multiply more in cold & dry environment. Seasonal H3 also generally occur in winter but here it may be due to some mutation, it occur in late monsoon. The reason for monsoon Predilection might be due to climatic factors such as relative humidity, rainfall and differences in temperature may cause the rapid shedding of this virus.[13]

Discussion

Table 1: Comparison of our study with other influenza surveillance studies

Study	Positivity of Swine H1N1 (%)	Positivity for seasonal H3 (%)
Our study	2.11%	1.79%
B. L. Rao, K. Banerjee et al, In Pune, India, 1978-1990.[3]	0.9%	1.16%

In our study positivity of Swine H1N1 (2.11%) is comparable with the study of B.L. Rao et al. Difference in positivity with B.L. Rao et al might be due to difference in strain and different in geographical area. Seasonal H3 positivity in our study (1.79%) is well correlated with the study of B.L. Rao et al.

Table 2: Ratio of male and female in positive Swine H1N1 patients

Study	Male%	Female%
Our study	53.84%	46.15%
Dr. Tripurari Kumar et al, Punjab.[9]	54.1%	45.9%

In our study prevalence of Swine H1N1 is more in male compared to female which is well correlated with study of Dr. Tripurari Kumar, et al.

Table 3: Ratio of male and female in seasonal H3 Positive patients

Study	Male%	Female%
Our study	63.63%	36.36%
Susanna Esposito et al.[10]	60.1%	39.9%

In our study prevalence of seasonal H3 is more in male compared to female which is well correlated with study of Susanna Esposito et al.

Our study shows that prevalence of Swine H1N1 in pediatric age group (23.07%) is lowest, followed by 12-40 years age group (38.46%), followed by aged>40 years(38.46%). The study of Arvind Chandora et al[11] also shows lowest prevalence in 0-20 years and nearly equal prevalence in middle age and > 40 years of age group. Difference in the prevalence in pediatric age group is due to selection of different age group in the study. Study done by M.P. Tambe et al[12] shows similar prevalence (23.94%) in (<10 year) pediatric patients.

In our study prevalence of seasonal H3 is highest in <12 years age group (54.54%) followed by middle age group (45.45%) and no sample positive in age group >40 years. The study done by Sam Peter et al[13] in Kerala shows relatively higher prevalence in middle age group (47.91%) than pediatric age group (43.75%) and lowest prevalence in > 45 years of age group (8.33%). This might be due to difference in geographical area and differences in sample size selected.

In our study, Fig. 3 shows prevalence of Swine H1N1 is highest in December month (38.46%). The study done by Archana Choudhry et al[1] in 2009 in Delhi (29.45%) shows highest no. of cases in December month. This was an alarming signal as we faced a big epidemic of H1N1 in 1st quarter of 2015. Seasonal H3 shows maximum number of cases during August, followed by September, followed by October month. No cases detected in other months of the year.

Conclusion

Influenza infections cause substantial morbidity and mortality every year. Influenza surveillance has focused on virological monitoring and collection of specimens to guide vaccine strain selection. Continuous monitoring would be required for early detection of any antigenic variants to understand the seasonality and analyses factors such as temperature and rainfall in the transmission of influenza viruses. Implementation of a sentinel network reinforces the importance of a national strategy for the control and prevention of Influenza.

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