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Demographic and Clinical Characteristics of Pandemic Influenza A (H1N1) 2009 Outbreak in Kerala, Southern India

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Authors' contributions

This work was carried out in collaboration between all authors. Author VCD designed and performed the experiments. Authors VCD and PJS interpreted the data, managed the literature searches drafted the manuscript and critically revised the manuscript for intellectual content. Authors DS and PMD participated in the study design and protocol. Authors FA and GRS coordinated the data collection for the study. Author VTJ performed the statistical analysis. Author MRP gave the material and supervised all the work with the authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To study the clinical and epidemiological features in the affected individuals from different areas of Kerala, India.
Study design: Population based cross sectional study.
Place and Duration of Study: Regional Facility for Molecular Diagnostics, Rajiv Gandhi Center for Biotechnology and Directorate of Health Services, Kerala, between August 2009 and September 2010.
Methodology: We conducted active surveillance for referral hospitals with specialist in-patient care in Kerala during pandemic periods. Oropharyngeal or nasopharyngeal swabs were tested for influenza viruses by Real time reverse transcriptase PCR.

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Results: A total of 4252 samples were tested for H1N1 influenza virus, of which, 30.17% were positive for pandemic influenza A H1N1 and 10.49% were positive for Influenza A (seasonal flu). Severe disease and mortality in the pandemic influenza A (H1N1) 2009 infection predominantly affected relatively healthy adolescents and adults between the age of 10 and 50 years. Both Males (29.28%) and Females (31.15%) were equally effected even though we observed a significant difference (*P*=.02). 141 cases exhibited lower respiratory tract symptoms. Pneumonia alone accounted for 28% of complicated cases. It was observed that the majority of cases (29.28%) during the first outbreak season were imported from affected overseas regions.
Conclusion: In this study, prevalence of Influenza A H1N1 was high in the healthy younger population and there wasn't any sex related susceptibility for Influenza infection. Majority of districts showed a positivity of approximately 10-30%, few with high positivity of >30%. Our findings highlight the importance of regular influenza immunization as it is significant to understand that the H1N1 (2009) virus may still circulate for many years with similar high severity.

Keywords: Influenza A H1N1 2009; demographic; morbidity; mortality; pandemic; real-time RT-PCR.

1. INTRODUCTION

Influenza, commonly referred to as flu, is an infectious disease caused by RNA virus of the family Orthomyxoviridae, and represent major pathogens of both humans and animals with an estimated 1 million annual deaths worldwide [1]. Influenza is a seasonal disease occurring regularly both in the Northern and the Southern hemispheres each in the cold months of the year [2]. In the tropics, seasonality is less defined with high background influenza activity throughout the year [3]. In spite of marked seasonal dependency, influenza can lead to temporary outbreaks in the respective countries as new and highly pathogenic viral subtype, where immunological resistance will be few or none in the human population and hence easily transmissible between humans, and rapidly spreads worldwide. The past century was characterized by three major pandemics: The Spanish Flu of 1918 (H1N1); Asian influenza of 1957 (H2N2) and Hong Kong Flu of 1968 (H2N3) where millions of people were affected [4].

The unexpected origin of the first influenza pandemic virus of the 21st Century, an influenza A (H1N1) reassortment between North American and Eurasian–lineage swine influenza virus strains of the same subtype as circulating human seasonal influenza A (H1N1), provides another example of the immense range of possibilities of the virus [5]. In April 2009, the first case was reported from Mexico [6] and Canada [7], followed by reports from Europe, South America, Asia, New Zealand, and Israel.

Though virological surveillance for human influenza is well established globally [8] limited surveillance had been conducted in India. By 29 April 2009, an active surveillance was started in India for detection of influenza cases, after WHO had declared a phase 5 pandemic alert [9]. The first case of confirmed Pandemic Influenza A (H1N1) 2009 infection in India was documented in May, 2009 [10], and the first positive case from Kerala was reported on June 24th, 2009 [11] but after August, 2009 large number of positive cases were reported throughout the country. Billions of dollars have been spent on preparedness including antiviral therapeutics [12,13] and influenza vaccine [14,15] development. Unfortunately India does not have a vaccination policy for influenza. This is likely

compounded by misconceptions, lack of adequate knowledge regarding influenza vaccination or general ignorance to the disease severity among healthcare workers and the public. Etiology-specific diagnosis tests are not widely available in here. Therefore what we know about epidemiology and clinical features are entirely from research studies. Only few studies have been published from India, especially from Kerala. In the current report we summarize the demographic and clinical features of affected individuals from 14 districts of Kerala between August, 2009 and September, 2010 during the outbreak period which is expected to provide insights to explore its potential impact on the population.

2. MATERIALS AND METHODS

2.1 Study Subjects, Study Period and Study Design

Following emergency requests from Central government of India and Kerala State Government, Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum, Kerala provided diagnostic support as prescribed by the Centre for Disease Control and Prevention (CDC) [16] for H1N1 diagnosis. All samples sent to RGCB for screening of H1N1 were included in the analysis. Samples were received from a total of about 22 referral hospitals with specialist in-patient care that participated in the H1N1 surveillance during the outbreak. A total of 4252 subjects were included in the study from August 2009 to September 2010. A patient data record was maintained for each individual providing a reliable source of information for our study. This included demographic data, details of clinical illness such as date of onset, date of sample collection, signs and symptoms, clinical characteristics, X-ray report, treatment history, exposure data (H1N1 contact), details of travel history, etc. Data collections were also coordinated by the Directorate of Health Services, Government of Kerala.

2.2 Sample Collection and Transport

Oropharyngeal or nasopharyngeal swabs from H1N1 suspected individuals during surveillance were collected in sterile 3 ml viral transport media (VTM) clearly labeled with the patient's information and transported at cold chain to *Laboratory Medicine and Molecular Diagnosis,* formerly called as Regional Facility for Molecular Diagnostics (RFMD) at RGCB from each district's reference hospitals for routine H1N1 screening.

2.3 Sample Processing, RNA Isolation and Real-Time RT-PCR Assay

Sample processing was carried out as per WHO guidelines [17]. Viral RNA was extracted using QIAamp® Viral RNA Mini Kit (Qiagen). The *In-vitro* qualitative detection of novel H1N1 2009 virus from respiratory specimens and differentiating it from seasonal influenza viruses was performed using Real-time RT-PCR assay in accordance with the protocol from the US Centers for Disease Control and Prevention, as recommended by the WHO [16].

Real-time RT-PCR was performed using Applied Biosystem 7500 system. Four primer/probe sets were used according to the CDC protocol [16]. Inf A primer for universal detection of type A Influenza Viruses targeting the matrix (M) gene, swFlu A specifically for detecting all swine Influenza Viruses targeting the nucleoprotein (NP) gene segment, swine H1 (swH1) for the haemagglutinin (HA) gene segment (subtype H1) from S-OIV and a RNaseP (RNP) primer as an internal positive control for human nucleic acid which also validates quality of the specimen, the nucleic acid extraction procedure and reagent integrity. Negative template controls were included in each run.

We analyzed the Ct values of Influenza a matrix gene after Real Time RT-PCR -Plus or Minus Assay as quality assessment was important to establish viral load or to correlate clinical severity of infection.

Results were interpreted with respect to the following combination. The Negative template control (NTC) reactions should not exhibit fluorescence growth curves that cross the threshold line. All clinical samples should exhibit RNP reaction curves that cross the threshold line at or before 40 cycles indicating the presence of sufficient RNA from human RNase P gene showing the specimen is of acceptable quality. When the NTC and RNP reactions meet stated requirements, a specimen is considered presumptive positive for influenza a virus if the Inf A reaction growth curves cross the threshold line within 40 cycles. If the reaction for influenza A is positive, it may also be positive for Univ SW and/or SW H1. A specimen is considered presumptive positive for swine influenza A/H1 if both the Inf A and the respective subtype (SwInf A or Sw H1) reaction growth curves cross the threshold line within 40 cycles.

Data entered in Microsoft Excel 2010 software and statistically analyzed using intercooled stata 11.2 software package (STATA Corp, Texas). Prevalence of H1N1 positive was described according to demographic details, travel history and district. Nature of the analysis was mainly descriptive.

3. RESULTS

As on September, 2010 a total of 4252 cases were tested for H1N1 infections from different districts of Kerala. A total of 1725 cases (40.66%) were positive for influenza A virus, out of which 30.17% were positive for pandemic H1N1 2009 and 10.47% cases were tested as Seasonal Influenza A positive. Of the participating hospitals, maximum numbers of samples as well as positive cases were from Trivandrum district followed by Ernakulum (Table 1).

Table 1. Distribution of laboratory-confirmed seasonal influenza A and pandemic influenza virus 2009 cases in various districts of Kerala

Districts	All cases	H1N1 positives		Seasonal positive	
	N	n	%	N	%
Trivandrum	2366	639	(27.01)	289	(12.21)
Ernakulam	568	222	(39.08)	56	(9.86)
Kottayam	405	95	(23.46)	25	(6.17)
Alappuzha	251	86	(34.26)	10	(3.98)
Thrissur	191	52	(27.23)	17	(8.90)
Idukki	118	49	(41.53)	11	(9.32)
Malappuram	84	32	(38.10)	9	(10.71)
Kollam	81	38	(46.91)	8	(9.88)
Pathannamthita	81	31	(38.27)	10	(12.35)
Palakkad	35	18	(51.43)	4	(11.43)
Kannur	24	12	50.00`	3	(12.50)
Wayanad	12	3	(25.00)	0	(0.00)
Kozhikode	7	1	(14.29)	3	(42.86)
Unknown	29	5	(17.24)	0	(0.00)

Majority of districts showed a positivity of approximately 10-30%, few with high positivity of >30% for pandemic H1N1 2009. Upon analysis it was observed that seasonal flu affected males and females in a ratio of 1:1.2 while no gender specificity of infection was observed among pandemic H1N1 2009 infected cases even though we observed a significant

difference *P*=.02 (Table 2). The median age of patients with influenza a virus infection was 35 (range, below 1yr - 50+yr) years.

Table 2. Prevalence of influenza cases in Kerala

	All cases	H1N1 Positives		Seasonal Positive		Negatives		*P*-value
	N	n	%	n	%	n	%	
Sample received	4252	1283	(30.17)	445	(10.47)	2524	(59.36)	
A]Demographic Details								
age group								
0-9	701	198	(28.25)	50	(7.13)	453	(64.62)	
10-19	683	255	(37.34)	67	(9.81)	361	(52.86)	
20-29	1218	411	(33.74)	129	(10.59)	678	(55.67)	
30-39	586	159	(27.13)	81	(13.82)	346	(59.04)	
40-49	408	107	(26.23)	47	(11.52)	254	(62.25)	
50+	646	150	(23.22)	71	(10.99)	425	(65.79)	<0.001
Sex								
male	2210	647	(29.28)	210	(9.50)	1352	(61.22)	
female	2042	636	(31.15)	235	(11.50)	1171	(57.35)	0.018
B]Travel history								
Nil	3813	1133	(29.71)	403	(10.57)	2277	(59.72)	
Within India	145	42	(28.97)	15	(10.34)	88	(60.69)	
Abroad	294	108	(36.73)	27	(9.18)	159	(54.08)	0.162

A month-wise analysis of our study shows two distinct peaks of pandemic H1N1 2009 cases during North-East Monsoon months of September - November 2009, and also during summer months of May-July 2010 when there was relatively higher temperature, low rainfall and slightly humid weather (Fig. 1).

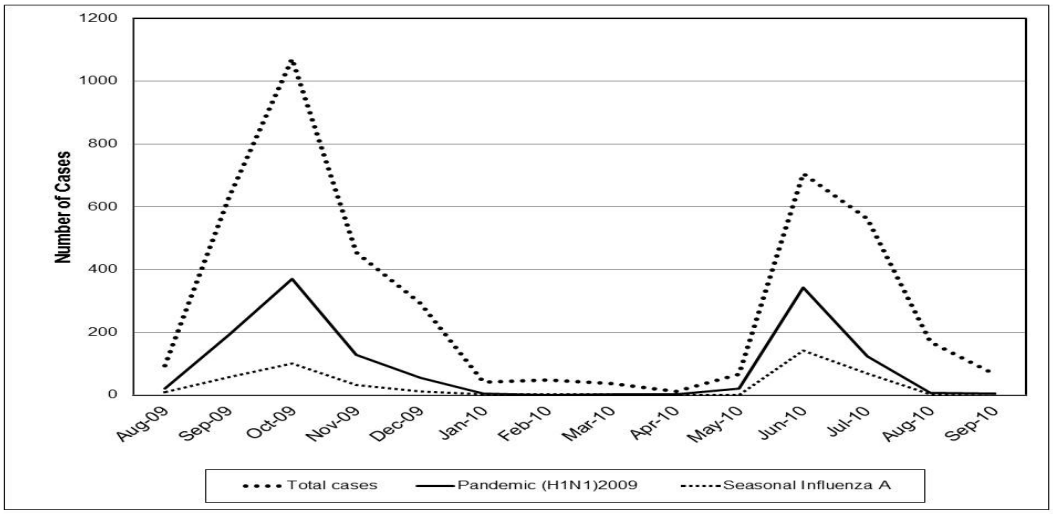

Fig. 1. Trends of pandemic influenza (H1N1) 2009 and seasonal influenza A from August 2009 till September 2010 in Kerala, Southern India

During the pandemic period (September-November'2009) we observed 35% prevalence of pandemic H1N1 2009 in Kerala, however in July'2010 virus had become endemic with almost 50% prevalence. We also observed the coexistence of pandemic H1N1 2009 along with seasonal influenza a virus (Fig. 2).

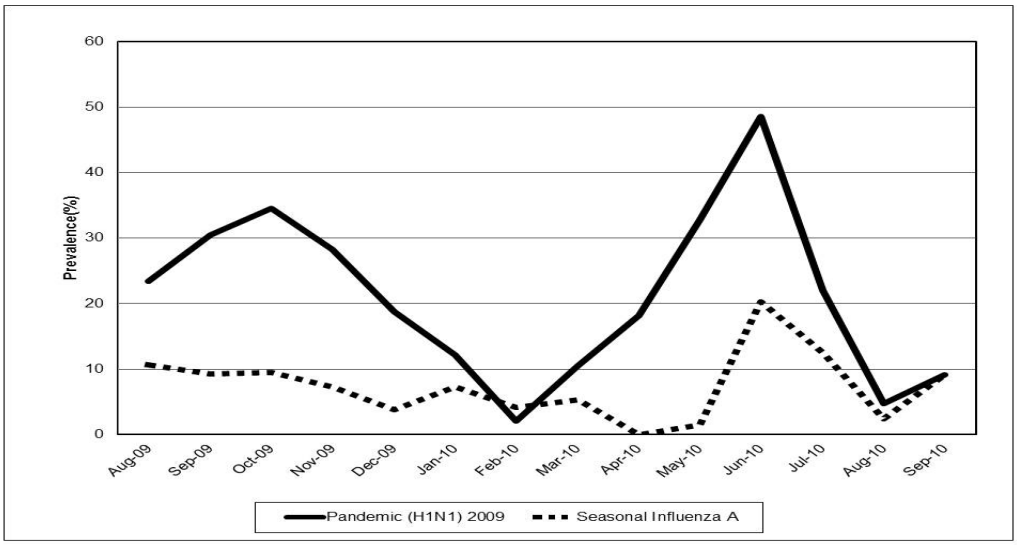

Fig. 2. Prevalence of pandemic influenza (H1N1) 2009 and seasonal influenza A overtime in Kerala, Southern India

The majority of cases during the first outbreak season were imported from affected regions with maximum travel-related cases reported from Middle-East Asia (36.73%) (Table 2) and the infection was almost community acquired during the second outbreak season of the pandemic.

Comparison of first and second outbreak season was also done in terms of parameters analyzed as given in Table 3. In our study we observed a difference in pattern of influenza A H1N1 cases during both the seasons. The infection which peaked in October'2009 showed 83.60% r RT-PCR run pattern of Inf A (+), Sw A (+) & Sw H1 (+). However infection which peaked in June'2010 showed 26.45% pattern for Sw H1 but not for Sw A subtype.

We analyzed the Ct values of Influenza a matrix gene after Real Time RT-PCR -Plus or Minus Assay as quality assessment was important to establish viral load or to correlate clinical severity of infection. Among the specimens tested; 45% had Ct values above 27 representing swabs containing very few cells/ poor quality specimen, 48% had between 20 to 27 and 7% below 20 indicating high viral titers. The mean Ct value for Influenza A matrix gene was 27.7 and for RNase P 25.3. One hundred and eight samples in our case were unsuitable for the real time assay due to negative RNase P reaction. We also attempted to study Ct values with respect to severity of symptoms and morbidity but did not observe any significant difference. The mean Ct values for Influenza A matrix gene was 27.7 for patients tested positive for H1N1, 26 for H1N1 positive death cases and 27.4 for patients who showed evidence of lower respiratory symptoms or other complications.

Table 3. Characteristics of subjects by real time reverse transcriptase PCR – A comparison of first and second outbreak seasons

Total	First wave (2009) (N=2545)		Second wave (2010) (N=1707)	
	H1N1 Positive		H1N1 Positive	
	n	(%)*	n	(%)*
	768		**515**	
Characteristics	643	(83.72)	354	(68.74)
InfA(+), SwInfA(+), SwH1(+)	53	(6.90)	142	(27.57)
InfA(+), SwInfA(-), SwH1(+)	72	(9.38)	19	(3.69)
InfA(+), SwInfA(+), SwH1(-)				

**Percentage were taken from the total H1N1 cases in each waves; Abbreviations: InfA (+), Positive for Influenza A; SwInfA (+), Positive for Classical Swine Influenza; SwH1 (+), Positive for Swine Influenza H1; (-) denotes no amplification; (+) denotes amplification*

The Infection with the pandemic H1N1 2009 virus in our study population exhibited a broad spectrum of clinical syndromes, ranging from febrile / afebrile upper respiratory illness to fulminant viral pneumonia and bronchitis. Most of the symptoms of upper respiratory tract infection were reported at disease onset. Among all influenza A positive cases, the most frequent symptoms include fever in 1428 (42.72%), cough in 1451 (42.71%), sore throat in 1089 (41.94%), nasal catarrh in 792 (41.29%), and shortness of breathing in 670 (37.79%). Mild afebrile cases were reported in 9.97% (168/1283) of all H1N1 positive cases. Among all the pandemic H1N1 2009 positive cases we studied, flu like symptoms (cough/sore-throat / nasal catarrh / shortness in breathing) was reported most frequently (1094/1283; 85.26%), followed by fever (1069/1283; 83.32%). Mean delay between days of onset of illness and sample tested was 4 days.

One hundred and forty one cases exhibited radiographic evidence of lower respiratory tract symptoms. Pneumonia alone accounted for 28% of complicated cases. The least frequent were the gastrointestinal symptoms. Chest radiographic findings commonly included bilateral pneumonia, fussy or non-homogenous opacities, bronchovascular markings, pleural effusion, and consolidation mainly in the lower lobe, bilateral patches, shadows and infiltrates. Among the pandemic H1N1 2009 positive cases, 72 patients had any one of the underlying medical condition including pregnancy, cardiac complications or diabetes.

A total of 49 laboratory confirmed death cases were reported during the study period. Of these, 31 (63.27%) were females and 18 (36.73%) were males. 44.9% of the deaths were observed in adults aged 21 to 30. The maximum cases of 19 (38.8%) were reported from Trivandrum District followed by Kollam District (30.61%). Most frequent presenting symptoms include fever in 41 (31%); cough in 35 (26%); breathlessness in 34 (25%) headache in 11 (8%); sore throat in 5 (4%). A small subset of patients also presented with myalgia, vomiting a characteristic feature which is unusual in the case of seasonal influenza [18], generalized weakness, running nose or seizures. Twenty two cases also had any one of the underlying clinical conditions: pregnancy (34.7%), cardio vascular diseases (6.12%), old age (12.24%), diabetics (4.08%), renal failure (0.02%), lung injury (0.02%) or cerebral palsy (0.02%). The most common cause of death was adult respiratory distress syndrome (48.9%), broncho-pneumonia (36.73%) or multi organ failure (2.04%). Among the 17 pregnancy associated death cases, 10 were antenatal cases, 3 were postnatal cases and 5 underwent immediate Lower Segment Caesarian Section. Fifty percent of antenatal cases belong to second trimester of pregnancy.

4. DISCUSSION

Kerala is a major state located in the south-west region of India on the Malabar Coast with 14 districts. The state experiences humid equatorial tropic climate. During the 2009 influenza outbreak, Kerala was also among the other states of India to be affected by pandemic influenza (H1N1) 2009 along with seasonal influenza.

In our study, 30.17% cases were found to be positive for pandemic H1N1 2009, which correlates well with reports from other parts of India [19] and 36% by a study from Israel [20]. Studies from Eastern India and Pune, India showed a low positivity of 9.56% and 17.8% respectively [21,22].This study also indicates 10.49% positivity for seasonal influenza A virus, which is similar to the reports from Pune, India that has also indicated a positivity of 16.3% [22],18% from South Australia,15% from Tasmania [23] and 18% positivity from Thailand [24].Our study demonstrated that severe disease and mortality in the pandemic influenza A (H1N1) 2009 infection predominantly affected relatively healthy adolescents and adults between the age of 10 and 50 years. The same has been reported by other studies from various parts of India [19,21] and from places abroad such as Singapore, California, Canada and Japan [20]. Persons older than 60 years of age were least affected, probably as a result of pre-existing immunity against antigenically similar influenza [18]. However, the study from eastern India indicates the age group>55years to be most affected [21]. Our findings also highlight the importance of influenza immunization in children of all ages and young adults with underlying medical conditions as it is significant to understand that the H1N1 (2009) virus may still circulate for many years with similar high severity [25].

Both males and females were equally affected though we observed statistically significant difference, being a large population the data won't affect the sex indicating there wasn't any sex related susceptibility for influenza infection as was also reported by studies from Eastern India [21] and Australia [26].

A month-wise analysis revealed the establishment of pandemic influenza (H1N1) 2009 since August 2009 with two successive peaks, one during the monsoon season (September-November 2009) and another during the summer months (May – July 2010). This pattern also fits into the signature features of successive peaks for a pandemic agent [27]. Studies from other parts of India also showed a similar pattern [19,21]. In our study it was observed that the majority of cases during the first outbreak season were imported from affected overseas regions even though travel was not significantly associated however the prevalence of H1N1 was 7% higher with travel history and the infection was almost community acquired during the second outbreak season. In the later months of 2010, a gradual decrease was seen in the sample size and pandemic influenza (H1N1) 2009 cases, which could be due to appeasement of population towards the initial panic-like fears of pandemic H1N1 and the practice of traditional medications for flu-like illness. Occupational history of H1N1 infection suggested that all categories of population to be equally affected. Influenza virus is known to take advantage of seasonal changes in environmental conditions. Various environmental factors especially temperature and relative humidity affect the airborne survival of influenza viruses. In our analysis, it was evident that influenza activity peaked during the monsoon season.

The vast majority of patients in our study had cough (85.26%),fever (83.32%), sore throat (63.29%), nasal catarrh (45.28%) and breathlessness (36.39%) a similar symptom presentation reported from other parts of India, from the US, Australia New Zealand and Japan [28-31]. The fatal cases reported during our study had at least one of the coexisting

conditions. 34.7% were pregnant women who were either in their antenatal or postnatal period, 12.24% were above the age of 70 years and 4.08% were diabetic. Pregnancy is a well-documented risk factor for severe infection and death in seasonal influenza and in previous pandemics [32-34].

Our report is subjected to certain limitations. The sample set originated from an arrangement with the Government Health Service Department as a part of pandemic response. Under such circumstances many ad hoc measures had to be taken and there could have been variations in training imparted, strictness of the procedures followed related to sample collection storage and transportation. These are expected to affect the detection of positive cases but it is unlikely to affect the overall conclusion. Samples for our study originated from the routine surveillance system and only data provided in the patient log book could be included in the study. We could include the demographic comparison between two waves in the study which will help us in future in terms of prevention and control. Sequence analysis was not done during the study period which could serve as a supporting evidence for what we claim in the demographic compilation.

5. CONCLUSION

To conclude, we analyzed the demographic and clinical pattern of H1N1 pandemic in the population of Kerala, southern India. The overall diversity in r RT-PCR result pattern of 2009 H1N1 influenza virus suggests that there are possibilities for co-circulation with other variants in the region. The result pattern also shows that the distribution of variants varied in both the outbreak seasons. It is possible that some mutations might have occurred owing to this co-circulation that probably leads to an increase in virulence during the second outbreak season. However detailed antigenic studies at the molecular level are required to attain any significant conclusion. Moreover ours is the first report on influenza demographics from Kerala and we don't have any knowledge of the variants circulating in our geographic area. Hence we are keen to determine the influenza variants circulated in our area during the 2009 H1N1 pandemic and also to determine the impact of co-circulation of different variants in the two outbreak seasons of pandemic on virulence. Further research is necessary to follow up this study by mutational analysis and characterization of Influenza A isolated from patient samples during the pandemic.

ETHICAL APPROVAL

The ethical clearance for the study was not required since samples were referred to us for diagnosis by Central Government and Kerala State Government as a public health response to mitigate the pandemic. The participating patient information data remained anonymous.

COMPETING INTERESTS

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of the article.

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