Heliyon 6 (2020) e03304

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Overview of seasonal influenza and recommended vaccine during the 2016/2017 season in Nepal

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ARTICLE INFO

Keywords:
Microbiology
Genetics
Molecular biology
Health sciences
Influenza
Nepal
Prevalence
Virus
Vaccine

ABSTRACT

Background: Influenza is a highly contagious viral respiratory infection caused by influenza viruses whose epidemic and pandemic have resulted in significant morbidity and mortality. The annual epidemic of influenza results in an estimated 3–5 million cases of severe illness and about 290000–650000 deaths globally. The vaccination program has been successful to control the epidemic however, it further needs improvement. This study was aimed to investigate the types of influenza viruses prevailing in Nepal during 2016 and, to match the recommended vaccine for use during the same season.

Methods: A descriptive cross sectional study was carried out at National Public Health Laboratory, Kathmandu, Nepal for the period of one year (Jan–Dec 2016). A total of 1683 throat swab specimen was collected from patients of different age group referred to NPHL for influenza testing. The specimen was primarily stored at 4 °C and processed using ABI 7500 RT PCR system for the identification of influenza viruses.

Results: Of the total 1683 patients suspected of having influenza infection, influenza viruses were isolated from 614 (36.5%) patients with male predominance. The highest number of infection was caused by influenza A/H3 strain (51.0%) followed by influenza B (40.4%) and influenza A (H1N1) pdm09 (8.6%). Two peaks of infection were observed during the year 2016. The widely available trivalent vaccine during the season did not match the prevailing strain because of the dominance of B/Yamagata lineage over B/Victoria lineage.

Conclusion: We concluded that Nepal experiences semiannual cycle of influenza infection, firstly during the month of January–February and secondly during the month of July–August. The vaccine to be introduced in Nepal need to be decided by national authority based on prevailing influenza types to confer effective immunization.

1. Introduction

Influenza is a highly contagious viral respiratory infection caused by influenza viruses whose epidemic and pandemic have resulted in significant morbidity and mortality worldwide. The annual epidemic of influenza results in an estimated 3–5 million cases of severe illness and about 290000–650000 deaths globally [1]. Influenza virus affects population of all age-group however, younger children below 5 years, elderly population above 65 years, pregnant women and other population with certain medical conditions such as: Asthma, Diabetes, Cancer, HIV/AIDS and Heart Disease are under high risk for flu complications [2]. A study suggests that 2–7% of the death in children younger than 5 years of age in 2008 was associated with seasonal influenza, majority of which were from the developing countries [3].

Influenza virus is a member of the family orthomyxoviridae and it is classified into four genera: influenza A, B, C and D. Influenza A and B are mainly responsible for infection in human and are also the cause of seasonal epidemics [4]. Influenza C virus causes only mild illness whereas influenza D virus is not known to cause illness in human. Influenza A virus is divided into subtypes based on haemagglutin (H1 – H18) and neuraminidase (N1 – N11) transmembrane glycoproteins. Influenza B virus is divided into two lineages: B/Yamagata and B/Victoria [4]. There are 131subtypes of influenza A detected in nature among which A(H1N1) and A(H3N2) routinely circulate worldwide [4]. Influenza virus emerged as a pandemic in 1580 for the first time after which it continued to appear as an epidemic or pandemic in different time and place [5, 6]. A study on global influenza activities suggests that 171 seasonal influenza epidemics have occurred from 1997 to 2005 in different parts of the world [7, 8]. Three major pandemics have been

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https://doi.org/10.1016/j.heliyon.2020.e03304
Received 15 November 2019; Received in revised form 12 December 2019; Accepted 23 January 2020

recorded in last century: first the Spanish flu in 1918 caused by H1N1, second the Asian flu in 1957 caused by H2N2 and the third Hong Kong flu in 1968 by H3N2 [9].

Studies suggest that influenza virus has an annual or semi-annual cycle based on geography and climatic conditions. Generally, annual cycle occurs in temperate region with a peak in winter. Tropics/subtropics region may involve annual, semi-annual or year-round activity [10]. The pattern of influenza virus circulation varies or remains same throughout the year depending upon genetic re-assortment or seasonal influence. This may results in epidemic or pandemic that can alter the intervention action concerning vaccination program and other preventive measures of a country [11]. Previous records also show that pandemic in the past was either due to antigenic shift with strains from the avian reservoir or by direct introduction of an avian strain into the human population [6]. This further suggests that genetic characterization of influenza viruses circulating around the world is important and timely in order to aid in the development of vaccine and, control the spread of viruses.

The first outbreak of influenza in Nepal was detected in year 2004 at Bhutanese refugee camp in southeastern Nepal, which was caused by influenza A/H3 serotype [12]. Since then, Nepal has an increasing number of influenza positive cases with two major epidemics in year 2004 and 2009 [13,14]. The epidemic in 2009 was due to influenza A (H1N1) whose first case was seen in June 2009 among people returning from US [15, 16]. In the very preliminary phase of diagnostic services of influenza virus in Nepal, we had very limited information about influenza infection and its types. In addition, it was also difficult to conduct study at molecular level due to limitation of resources and poor setting. In this perspective we carried out this study with two central objectives: 1. to determine the seasonal activity of influenza virus in Nepal in year 2016 and, 2. to check whether the recommended vaccine matched the prevailing strain during the season or not.

2. Methods and methodology

2.1. Study design and sample collection

A descriptive cross sectional study was carried out at National Public Health Laboratory (NPHL), Kathmandu, Nepal for the period of one year (January–December 2016). Altogether 1683 samples were collected from sentinel sites of National Influenza Surveillance Network including NPHL using swabs with synthetic tip. The samples collected at sentinel sites were kept in viral transport media and transported to NPHL in cold chain box within 24 h, which were then stored at 4 °C till further processing. Patients included in the study had either influenza like illness (ILI) that includes fever (>38 °C), cough, running nose, chills and sore throat or, severe acute respiratory infection (SARI) or pneumonia which was in accordance with WHO case definition for ILI and SARI [17].

2.2. Sample processing and virus identification

All the samples collected in viral transport media were aliquoted into two micro-centrifuge tube following collection. One was used for RNA extraction and the other was stored at -70 °C. The identification of influenza virus was carried out by Applied Biosystems™ 7500 Real-Time PCR System using AgPath-ID™ One-step RT-PCR Kit (Thermo Fisher Scientific, USA). RNA of influenza viruse was extracted using QIAamp® Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany) following manufacturer's recommended procedure. The extracted RNA was first tested for the presence of influenza A and B viruses. The sample showing positive for influenza A/B was further differentiated into sub types and lineages. Influenza A positive samples were screened for Pdm A, Pdm H1, A/H3 whereas samples showing influenza B positive were tested for B/Yamagata and B/Victoria lineage. Due to limited number of PCR kit, only 106 influenza B positive samples were processed to identify their lineages. The primers and probes used in the reaction mixture for identification of different types, subtypes and lineages of influenza virus (H1N1, H3N2, H1N1pdm09, Influenza B, B/Victoria, B/Yamagata) were provided by US Center for Disease Control and Prevention (CDC) and the assays were performed following the manufacturer's protocols [18]. Samples with a cycle threshold (Ct) value <40 were considered positive.

2.3. Data analysis

The collected data was entered into and analyzed using Statistical Package for Social Sciences (SPSS) software version 20.0 [IBM Armonk, NY, USA].

2.4. Ethical consideration

Ethical approval was obtained from National Public Health Laboratory and Nepal Health Research Council (NHRC ref. no. 1673) before carrying out this study.

3. Results

3.1. Demographic characteristics of patients

During the year 2016, a total of 1683 cases were found registered at National Public Health Laboratory to test for suspected influenza infection among which influenza virus was identified in 614 cases. A relatively higher number of patients recorded were male in comparison to female. Age-group distribution of both influenza suspected cases as well as influenza positive cases showed that higher number were from adult age group, 31–65 years (31.3%) and lower number from the elderly age group (>65 years) population (Table 1). The data also revealed that higher number of suspected cases had influenza like illness (64%) followed by severe acute respiratory infection (26%) and pneumonia (10%) (Figure 1).

National Public Health Laboratory being only one diagnosis center for influenza infection in Nepal during 2016, patients from all over Nepal were referred to NPHL. We recorded total patients from 63 districts of the country. However, positive cases for influenza infection were confirmed from 44 districts only. The highest number of case was recorded from Kathmandu district which alone comprised of about near to half of the cases (40.6%). Other districts like Lalitpur, Bhaktapur, Dhading and Nuwakot also had higher number of cases (Figure 2).

3.2. Prevalence of influenza viruses

Out of total 1683 suspected cases for having influenza infection, we identified influenza viruses from 614 (36.5%) cases (Figure 3). Influenza type A accounted for 364 (59.3%) cases of infection whereas influenza type B was found in 248 (40.4%) cases. More than half number of patients 313 (51.0%) were found infected with influenza A/H3 strain alone. Among the 248 cases of influenza B infection, 106 isolates were further differentiated into lineage which revealed higher number of viruses from B/Yamagata lineage (11.1%) in comparison to B/Victoria lineage (6.2%). Interestingly, co-infection of influenza B and influenza A/H3 strain was found in two cases during the period of study (Table 2).

3.3. Seasonal distribution of influenza viruses

Figure 4 illustrates the distribution of cases of influenza in different months throughout the year 2016. It can be seen that higher number of influenza cases were reported two times during the year 2016, firstly during the month of January–February and secondly during the month of July–August. The highest number of cases suspected of having influenza infection and, influenza positive cases both were recorded in the month of August. The data also show that during those months, the number of positive cases were almost half of the total cases registered in that particular months making incidence rate 50%. Rest of the months had

Table 1. Age wise distribution of patients in year 2016.

Age-group	Influenza suspected cases			Influenza positive cases		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
0–5	192 (58.4)	137 (41.6)	329 (19.5)	45 (55.6)	36 (44.4)	81 (13.2)
6–15	136 (59.6)	92 (40.4)	228 (13.5)	70 (62.5)	42 (37.5)	112 (18.2)
16–30	195 (50.3)	193 (49.7)	388 (23.1)	97 (58.4)	69 (41.6)	166 (27.0)
31–65	263 (50.0)	263 (50.0)	526 (31.3)	111 (57.8)	81 (42.2)	192 (31.3)
>65	111 (52.4)	101 (47.6)	212 (12.6)	31 (49.2)	32 (50.8)	63 (10.3)
Total	897 (53.3)	786 (46.7)	1683 (100)	354 (57.7)	260 (42.3)	614 (100)

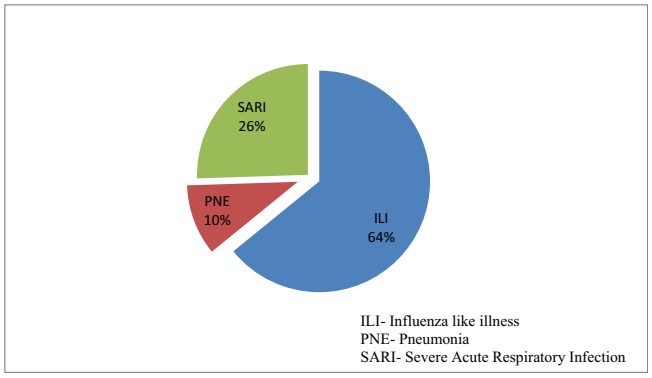

Figure 1. Clinical signs of patients suspected for influenza infection (N = 1683).

positive cases much less than half of the total cases. The least number of influenza positive cases were reported in the months of May, October and November (Figure 4).

In addition, infection with influenza A/H3 strain was found predominant in the months of January–February and July. The trend was found reversed in the month of August making influenza B predominant. Overall, influenza A/H3 was responsible for majority of infection in the month of January–February while influenza B was the major cause of infection in the peak season of July–August. Few cases of influenza A(H1N1)pdm09 were reported during beginning and end of the year but no cases were found during middle part of the year including one peak season of July–August (Figure 5).

4. Discussion

Since the first outbreak of influenza in 1580, the virus has continued to cause epidemic and pandemic in different geographical places and time scale. It has been observed that following an outbreak influenza virus tend to re-assort with different kinds of genetic modifications

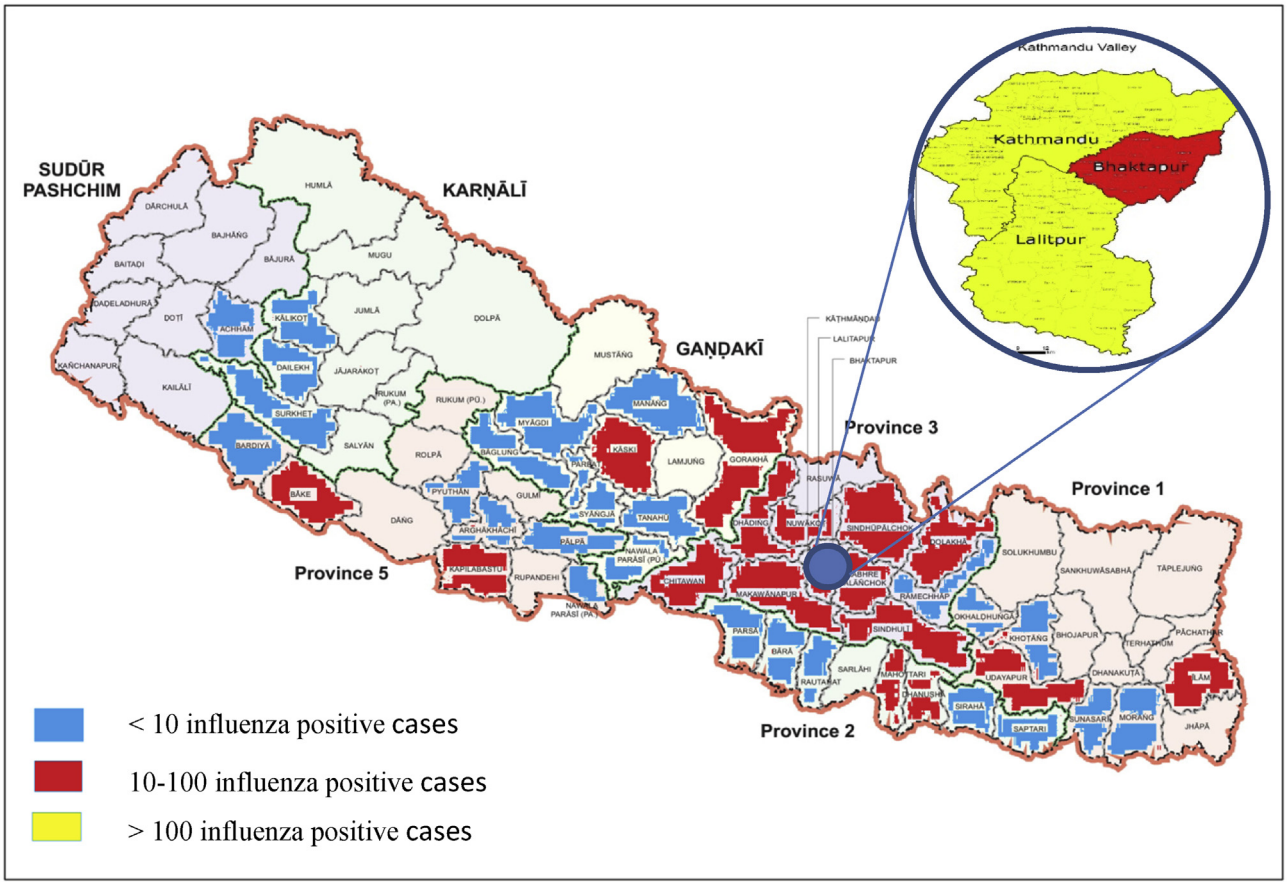

Figure 2. Map of Nepal showing districts with influenza cases in year 2016.

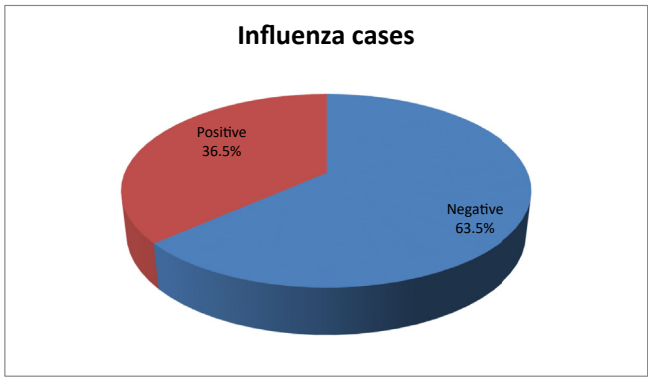

Figure 3. Incidence of influenza infection among suspected cases (N = 1683).

resulting in a new strain, which may further cause an epidemic. World Health Organization reports that in the European region, the 2018/19 influenza season is dominated by influenza A virus; co-circulation of both subtypes (H1N1pdm09 and H3N2) however, the past season (2014/15–2016/17) was dominated either by influenza A(H1N1)pdm09 or by A(H3N2) [19]. The re-emergence of influenza virus in every season with some genetic modification or, introduction of a new strain brings about novel antigenic properties in them that may decrease vaccine induced immunity due to mismatch between vaccine and circulating strains. For this reason it is necessary to identify and characterize the novel strain of virus, and modify the vaccine according to the predominating strain to confer maximum protection against influenza infection.

The findings of our study suggest that influenza A/H3 was the predominant strain circulating in Nepal in year 2016. A previous study by Upadhyay et al. also reported similar finding according to which influenza A/H3 was responsible for 60.1% of the total infection in year 2014 in Nepal [14]. A study from countries in tropical Asia also reports influenza A dominant over influenza B during 2007–2013 which is in correspondence to this study [20]. Among influenza type B virus, we noticed B/Yamagata lineage dominant over B/Victoria lineage in this study that was different from those of other countries in northern hemisphere in 2016/2017 season [21]. However, study in Asia-pacific region during 2010–2017 suggest that B/Yamagata was dominant over B/Victoria lineage which is similar to this study [22]. The difference in prevalence rate reported in various study is probably due to different time and places the study have been carried out. Furthermore, it seems likely that the geographical and climatic condition of different locations around the world is different that might be reason for high prevalence of dissimilar subtypes of influenza virus in different study.

This study illustrates that Nepal observes two peaks of influenza infection round the year; first in the month of January–February and second in the month of July–August which is similar to the pattern followed by some Asian countries in temperate zone of Northern hemisphere [22, 23]. The first peak was observed in winter season followed by the second peak coinciding with the rainy season. This is further supported by a study from India which reports maximum influenza activity during the rainy season [24]. A study carried out by Sun et al. from mainland China also reported two peak of influenza activity; first in winter and then in Spring in each monitoring year which is in correspondence to our study [25]. In contrast, mainland of japan which lies in temperate region experience only one peak of influenza activity in winter season [26]. Similarly, a study from Hong Kong also reports annual cycle of influenza A(H3N2) during the study period [27]. This suggests that seasonality of influenza appears to be country specific despite of similar type of climatic conditions. Though influenza A/H3 was the predominant circulating strain in year 2016 in Nepal, somewhat surprisingly noticed was the second peak of infection in July–August that was marked by influenza B despite of A/H3 strain. The possible explanation behind circulation of different viruses in the two peaks of the year might be the outbreak with different strain or host-related and environmental factors such as temperature and humidity which supports activity of particular strain of virus.

Table 2. Viruses identified in influenza positive cases in year 2016 (N = 614).

Influenza viruses	No. of positive cases		Percentage (%)	
Influenza A(H1N1)Pdm09	51	364	8.3	59.3
Influenza A(H3N2)	313		51.0	
Influenza B	142	248	23.1	40.4
Influenza B/Victoria lineage	38		6.2	
Influenza B/Yamagata lineage	68		11.1	
Co-infection Inf. A/H3-Inf B	2		0.3	
Total	614		100	

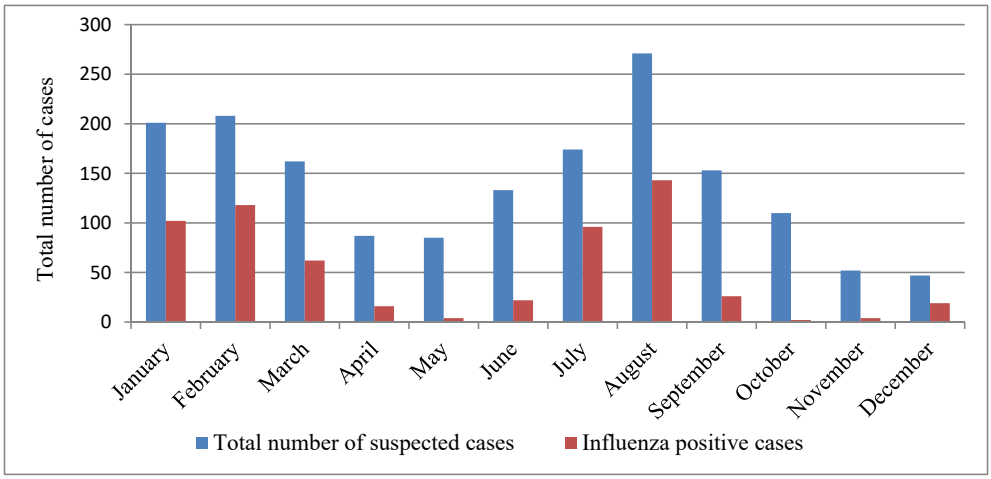

Figure 4. Month wise analysis of influenza cases in year 2016 (N = 1683).

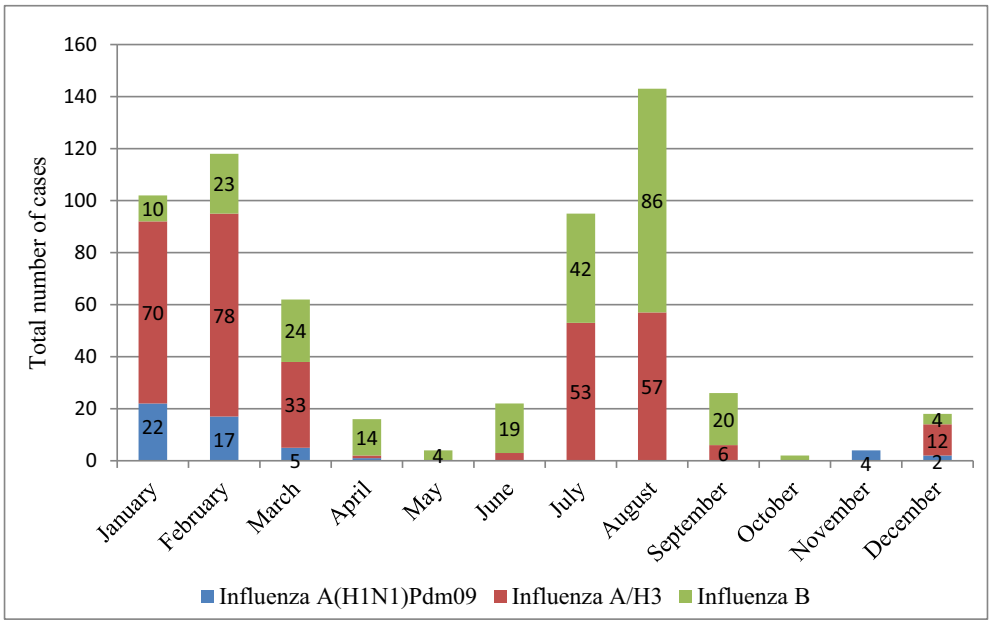

Figure 5. Influenza viruses identified in different months of the year2016.

The findings from this study also suggest that influenza prevalence was higher in Kathmandu and nearby districts. These districts are densely populated according to the report of Central Bureau of Statistics [28]. Because the transmission of influenza virus mainly occur by aerosol inhalation and spread more rapidly in crowded areas, these districts had higher prevalence of influenza in comparison to other districts of Nepal [28, 29]. Also, Kathmandu valley and nearby districts are surrounded by mountains and experience distinct climatic condition and lower temperature in comparison to other districts [30]. As higher temperature enhances defense mechanism and decreases replication of influenza virus, Kathmandu valley having lower temperature favors influenza virus replication which might also be the reason for higher prevalence rate [31].

Our study suggests that 2016 influenza season was dominated by influenza A/H3 strain. Besides that, influenza B infection was also found in higher number of cases among which B/Yamagata lineage was more prevalent than B/Victoria lineage. According to the report, WHO had recommended both trivalent and quadrivalent vaccine to be used in countries in northern hemisphere during 2016–2017 season [21]. The choice for trivalent or quadrivalent vaccine was made by national or regional authorities in accordance to their seasonability pattern of influenza virus circulation [21, 32]. In Nepal, influenza vaccine was not provided to the public at government level and also we did not find any guidelines made by national authorities in Nepal regarding influenza vaccine use. However, clinician preferred to use trivalent vaccine and, was widely available in Nepal during the season. The trivalent vaccine consisted of: A/California/7/2009 (H1N1)pdm09-like virus, A/Hong Kong/4801/2014 (H3N2)-like virus and B/Brisbane/60/2008-like virus. The antigenic and genetic characteristics of B/Brisbane/60/2008-like virus was closely related to most B/Victoria/2/87 lineage viruses.

But, since we found B/Yamagata lineage dominant in Nepal over B/Victoria lineage in year 2016, we noticed a mismatch between prevailing strain and available vaccine in 2016–2017 seasons. An implication of these findings is the potential for improvement in vaccination program in the country. The previously published researches suggest that B/Victoria lineage was dominant in most of the south Asian countries which we found different in our study [21]. For this reason the vaccine to be introduced in Nepal should be decided by national authority in Nepal based on prevailing strain to confer effective immunization. Furthermore, when we consider the timing for influenza vaccination, the vaccine needs to be introduced within 4 months prior to peak season and twice a year as this study observed two peaks in year 2016; first during winter in the month of January–February and second after rainy season in the month of July–August. Because we observe only 2016 influenza season, the recommendation for vaccination twice a year should be further conformed by a longitudinal study in the country.

5. Conclusion and recommendations

This study set out to determine that Nepal experience semiannual cycle of influenza infection firstly, during the winter month of January–February and secondly, during the month of July–August following rainy season. Despite few cases of influenza B and influenza A(H1N1) pdm09, influenza A/H3 remained predominant throughout the year 2016. This paper argued that the vaccine to be introduced in Nepal must be decided by national authority based on prevailing influenza types.

There is abundant space for further progress in recommending the timing and composition of influenza vaccine to be introduced in Nepal by the national authority to confer effective immunization and to prevent seasonal epidemic in the country. The vaccination campaign at community level also seems necessary which should be carried out by the government. In addition, a further longitudinal study with more focus on genetic characteristics of the virus need to be done to monitor evolution in circulating strain and efficacy of vaccination program.

Declarations

Author contribution statement

Bimalesh Kumar Jha: Conceived and designed the experiments; Performed the experiments.

Roshan Pandit: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Runa Jha: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Krishna Das Manandhar: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We express our deep gratitude to all the laboratory staffs of National Influenza Center of National Public Health Laboratory, Teku, Kathmandu for their kind support in completing this work. We are also thankful to the director of NPHL who provided us with the chance to carry out this study.

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