

## Prevalence and epidemiology of outbreaks of influenza A(H1N1)pdm09 virus in Gujarat from August 2009 to March 2016 with special reference to 2015 resurgence

Disha A Patel<sup>1</sup>, Dipa M. Kinariwala<sup>2,\*</sup>, Ami U Mistry<sup>3</sup>, Bhavikakumari C. Patel<sup>4</sup>, Mahendra M Vegad<sup>5</sup>

<sup>1</sup>Assistant Professor, <sup>2</sup>Tutor, <sup>3</sup>Resident, <sup>5</sup>Professor & HOD, Dept. of Microbiology, BJ Medical College, Ahmedabad, Gujarat

**\*Corresponding Author:**

Email: yeshujai@gmail.com

### Abstract

**Purpose:** Influenza is a widespread viral infection and a major cause of morbidity and mortality worldwide. The present study was performed to investigate the Influenza A H1N1 2009 pandemic (influenza A(H1N1)pdm09) virus outbreaks, its pattern and its clino-epidemiological correlation in and around the region of Gujarat, during the period of August 2009 to March 2016 with special reference to outbreak of 2015.

**Materials and Method:** This is a retrospective study. A total of 17206 patients attending various hospitals in Gujarat were included from August 2009 to March 2016. Nasal swabs, nasopharyngeal swabs, or throat swabs were collected and tested by real time Reverse Transcriptase PCR (rRT-PCR) for presence of influenza A(H1N1)pdm09 virus.

**Results:** Out of 17206 samples, 6078 clinical samples were positive for swine flu and 2605 were positive for seasonal Influenza A flu. Over all people in the age group between 30 years to 60 years were highest affected and females were affected more than males. Case fatality rate of the patients admitted in our hospital in 2015 outbreak was 23.07% with highest mortality in pregnant women.

**Conclusions:** Influenza A(H1N1)pdm09 is still active six years after 2009 pandemic and unable to replace seasonal influenza A virus unlike previous pandemic strains. Middle age patients and pregnant women were highly affected with high mortality in pregnant women.

**Keywords:** Influenza A(H1N1)pdm09, Seasonal influenza A, Outbreaks, rRT-PCR

### Introduction

Influenza is a widespread viral infection and a major cause of morbidity and mortality worldwide. The WHO Global Influenza Surveillance Network has greatly contributed to the knowledge about circulating influenza viruses, including emergence of novel strains.<sup>(1)</sup> In April 2009, a previously undescribed A (H1N1) influenza virus was isolated from humans in Mexico and the United States which was later declared as pandemic by World Health Organization (WHO), on June 11, 2009.<sup>(2,3)</sup> Its genome represented a quadruple re-assortment of two swine strains, one human strain and one avian strain of influenza.<sup>(4)</sup> On 10<sup>th</sup> August 2010, the pandemic was declared to have an ended with more than 18,449 deaths reported worldwide as of 1<sup>st</sup> August 2009.<sup>(5,6)</sup> In India, the first case of positive P-09-H1N1 was reported on 16<sup>th</sup> May, 2009, from a 23 year old passenger who traveled from USA arriving at Hyderabad airport.<sup>(7)</sup> After that the virus soon became endemic and spread to almost all major cities in India, and by end of the year 2010, 20604 cases with 1763 deaths were reported.<sup>(8)</sup> The country experienced three waves during the period of pandemic of 2009-2010, first one in 2009 September, followed by second wave in December 2009, and the third peak in August 2010 when the end of pandemic was declared.<sup>(9)</sup> The first case in Gujarat was seen much later when a Thai national turned out positive on July 6<sup>th</sup> 2009, Saturday in Ahmedabad after which it had rapidly covered whole region.<sup>(10)</sup>

Recently India suffered from the Influenza A(H1N1)09 pandemic strain (influenza A(H1N1)pdm09) again in 2015. Due to the pandemic threats of swine flu, its ultimate public health impact and unpredictable nature of outbreak, it has achieved a global focus.<sup>(11)</sup> The present study was performed to investigate the influenza A(H1N1)pdm09 virus outbreaks, its pattern and its clino-epidemiological correlation in Western India specifically in and around the region of Gujarat, during the period of August 2009 to March 2016 with special reference to outbreak of 2015.

### Materials and Method

This is a retrospective study performed at a tertiary care hospital in Ahmedabad, Gujarat during the period from August 2009 to March 2016. Patients from Western India including various part of Gujarat and adjoining states attend this hospital and get health care facilities. A total of 17206 patients of all age groups attending various hospitals including private hospitals in Gujarat were included in this study.

Demographic details and clinical history were obtained and recorded in laboratory request forms. Nasal swabs, nasopharyngeal swabs, or throat swabs were collected from the patients and received in viral transport media in the laboratory. All samples were checked for cold chain maintenance, leakage and quality.<sup>(12)</sup> Samples were tested by real time Reverse Transcriptase PCR (rRT-PCR) using Center for Disease Control and Prevention (CDC) validated and World

Health Organisation (WHO) approved primers and probes sequences and rRT-PCR protocols.<sup>(13)</sup> RNA extraction was performed using QIAgen Mini RNA extraction kit. Extracted RNA was subjected to one step RTPCR reaction using 4 target sequences i.e. Influenza A (InfA), swine A (SwA), swine H1 (SwH1), and ribonucleoprotein (RNP). Master Mix was prepared by using Ag-Path one step RT-PCR kit by Applied Biosystems. Amplification in RNP target sequence was used as an internal quality control. Also a set of in house positive control and negative control was put along with each run. A test was considered valid only if amplification in RNP was obtained and positive control and negative control gave proper result.

## Results

During August 2009 to March 2016, a total of 17206 clinical samples having signs and symptoms of flu were tested by rRT-PCR for the presence of the

influenza A(H1N1)pdm09 viruses. Among which 6078 clinical samples were positive for influenza A(H1N1)pdm09 viruses and 2605 were positive for seasonal Influenza A flu. Year wise presentation of the swine flu cases and seasonal influenza A cases are shown in Table 1. Table 2, 3 show the age distribution and gender distribution among the influenza A(H1N1)pdm09 viruses positive cases respectively. It shows that over all people in age group between 30 years to 60 years were highest affected and females were affected more than males. Clinical history has been described in Table 4. Month wise update of Positivity of influenza A(H1N1)pdm09 viruses and Seasonal Influenza A from outbreak cases and surveillance cases from Jan 2015 to March16 are shown in Table 5. Chart 1 shows the seasonal trend of the influenza A(H1N1)pdm09 and seasonal influenza A flu from August 2009 to March 2016.

**Table 1: Year wise cases of influenza A(H1N1)pdm09**

Year	Influenza A positive	influenza A(H1N1)pdm09 Positive	Negative	Total
From Aug to Dec 2009	366 (14.79%)	672 (27.15%)	1437	2475
2010	556 (16.97%)	1040 (31.75%)	1680	3276
2011	09 (8.26%)	007 (6.42%)	93	109
2012	12 (4.82%)	38 (15.26%)	199	249
2013	338 (18.47%)	523 (28.57%)	969	1830
2014	115 (32.58%)	41 (11.61%)	197	353
2015	973 (12.69%)	3520 (45.90%)	3176	7669
2016 (up to March)	236 (18.96%)	237 (19.04%)	772	1245
Total	2605 (15.14%)	6078 (35.32%)	8523	17206

**Table 2: Age wise distribution of influenza A(H1N1)pdm09 positive cases**

Year	≤ 5 years	>5 to ≤ 15 years	>15 to ≤30 years	>30 to ≤60 years	>60 years	Total
2009	41 (6.10%)	57 (8.48%)	320 (47.62%)	242 (36.01%)	12 (1.79%)	672
2010	100 (9.61%)	66 (6.35%)	365 (35.10%)	446 (42.88%)	63 (6.06%)	1040
2011	0	1 (14.29%)	0	6 (85.71%)	0	7
2012	0	2 (5.26%)	11 (28.95%)	24 (63.16%)	1 (2.63%)	38
2013	26 (4.97%)	14 (2.68%)	123 (23.52%)	300 (57.36%)	60 (11.47%)	523
2014	0	1(2.44%)	8 (19.51%)	27 (65.85%)	5 (12.20%)	41
2015	616 (17.5%)	212 (6.02%)	654 (18.58%)	1599 (45.43%)	439 (12.47%)	3520
2016 (up to March)	13 (5.49%)	7 (2.95%)	31(13.08%)	144 (60.76%)	42 (17.72%)	237
Total	796 (13.10%)	360 (5.92%)	1512 (24.88%)	2788 (45.87%)	622 (10.23%)	6078

**Table 3: Sex wise distribution of influenza A(H1N1)pdm09 positive cases**

Year	Male	Female	Total
2009	379 (56.40%)	293 (43.60%)	672
2010	529 (50.87%)	511 (49.13%)	1040
2011	4 (57.14%)	3 (42.86%)	07
2012	20 (52.63%)	18 (47.37%)	38
2013	250 (47.80%)	273 (52.20%)	523
2014	25 (60.98%)	16 (39.02%)	41
2015	1709 (48.55%)	1811 (51.45%)	3520

2016 (up to March)	106 (45.73%)	131 (55.27%)	237
Total	3022 (49.72%)	3056 (50.28%)	6078

**Table 4: Clinical features of 2015 outbreak**

Clinical Features	Total cases	Percentage	Positive cases	Percentage
Fever	6800/7669	88.67%	3160/3520	89.77%
Sore throat	5645/7669	73.61%	2595/3520	73.72%
Cough	6836/7669	89.14%	3184/3520	90.45%
Nasal catarrh	344/7669	4.49%	152/3520	4.32%
Breathlessness	4674/7669	60.95%	2169/3520	61.62%

**Table 5: Positivity of influenza A(H1N1)pdm09 and Seasonal Influenza A from outbreak cases and surveillance cases from Jan 2015 to March 2016**

Month & Year	Outbreak cases				Surveillance cases			
	Positive for influenza A(H1N1)pdm09	Positive for seasonal Inf A	Negative for flu	Total	Positive for influenza A(H1N1)pdm09	Positive for seasonal Inf A	Negative for flu	Total
Jan-15	119	44	64	227	8	22	26	56
Feb-15	2396	690	1080	4166	4	5	30	39
Mar-15	856	177	1291	2324	2	5	20	27
Apr-15	24	4	91	119	0	6	49	55
May-15	6	4	9	19	0	15	29	44
Jun-15	0	0	11	11	1	10	34	45
Jul-15	0	1	1	2	0	0	49	49
Aug-15	17	2	34	53	0	0	40	40
Sep-15	57	11	236	304	0	4	52	56
Oct-15	31	17	199	247	1	1	63	65
Nov-15	1	5	83	89	0	1	39	40
Dec-15	14	18	84	116	0	0	55	55
Jan-16	56	76	234	366	0	3	42	45
Feb-16	112	115	327	554	5	13	39	57
Mar-16	68	45	211	325	2	7	45	54
Total	3757	1209	3955	8921	23 (3.16%)	92 12.65%	612	727

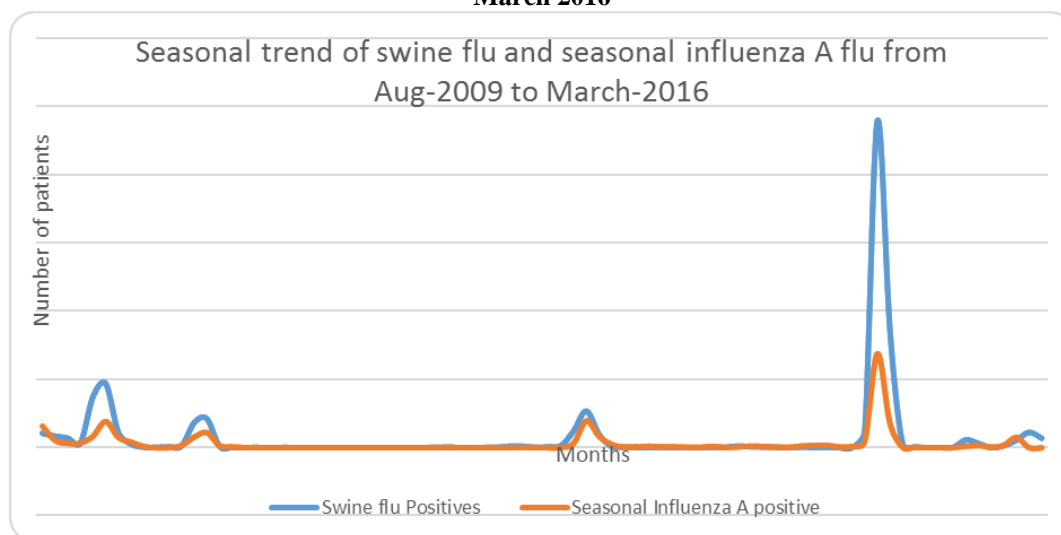
## Discussion

Influenza A H1N1 is a highly contagious pathogen which made headlines in 2009, as the so called swine flu, by causing a worldwide influenza pandemic.<sup>(14)</sup> There have been small outbreaks of Influenza A H1N1 (swine flu) since the pandemic; a recent one (2015) was in India where approximately 1900 people died out of 32000 reported cases of swine flu.<sup>(15)</sup> Gujarat was counted as worst affected state with 397 deaths out of 6226 laboratory confirmed cases of swine flu in the year 2015 itself.<sup>(16)</sup> Presence and distribution of the test positive cases were studied to generate knowledge of epidemiology of 2015 outbreak.

Three major spikes of influenza A(H1N1)pdm09 outbreaks were seen in Gujarat, from August 2009 to March 2016, viz. December 2009 to February 2010, January 2013 to May 2013 and January 2015 to May 2015 as shown in Chart 1. Out of total 17206 tested

samples, over all 6078 (35.32%) were found positive for influenza A(H1N1)pdm09 viruses and 2552 (15.56%) were positive for seasonal Influenza A. The high positivity in our study is due to counting of overall positivity from duration of 2009-16 which includes several outbreaks including recent one of 2015, which was having maximum positivity (45.90%) than previously experienced outbreaks, like 29.77% (1721/5751) in 2009-10 and 28.57% in 2013 as shown in Table 1. In the year 2015 as shown in Chart 1, the 1<sup>st</sup> wave of outbreak totally subsided in the month of May. Though over all positivity in the year 2015 was 45.90%, maximum positivity was found in the first half of the year up to the month of May and it was 49.67% (Table 5). Thus, first quarter of the 2015 had seen worst kind of infection which was also noted by Itollikar S and Nadkar MY.<sup>(17)</sup>

**Chart 1: Seasonal trend of influenza A(H1N1)pdm09 and seasonal influenza A flu from August 2009 to March 2016**



Positivity of seasonal influenza varied from 4.82% to 32.58% from August 2009 to March 2016 with overall positivity of 15.14%. It was observed that positivity of seasonal influenza A virus was 15.88%, 18.47%, 12.69% and 18.96% during outbreak years of 2009-10, 2013, 2015 and 2016 respectively. As shown in table 1, presence of seasonal influenza A along with the influenza A(H1N1)pdm09 during these outbreaks suggest that seasonal and influenza A(H1N1)pdm09, both strains co-circulated in these regions. Our findings are matching with the study done in Eastern India and Delhi by Mukharjee A *et al* and Broor S *et al* respectively.<sup>(18,1)</sup> In contrast to that during previous pandemics, the novel virus subtype had replaced previously circulating viruses. For example, H1N1 was replaced by H2N2 in 1957, subsequently H3N2 emerged in 1968 and H1N1 reemerged in 1977. The latter two have co-circulated since then, although in one season only one or the other tend to dominate.<sup>(1)</sup> Higher percentage of influenza A(H1N1)pdm09 strain than seasonal strain during outbreaks indicate higher transmissibility of pandemic strain than seasonal. Reason for low prevalence of seasonal Influenza A during outbreak is not clear but it may be attributed either to environmental or social factors. Alternatively, mild ILI systems resulting in patients not reporting to hospital could have been one of the factors.<sup>(18)</sup>

In this study period, overall people between ages of 30 years to 60 years were affected maximally (45.87%) as shown in Table 2. During 2009-10 outbreak period, young adults (15-30 years) (685/1712 - 40.01%) and middle age adults (30-60 years) (688/1712-40.18%) both age groups appeared to be affected more by influenza A(H1N1)pdm09 viruses than any other age which was a signature feature of the pandemic. The same has been reported by other studies from various parts of India.<sup>(19)</sup> During 2013 outbreak maximum age group affected was between 30yrs to 60 years (57.36%)

with significant increase in age group of above 60 years.

In the 2015 outbreak, only adults of middle age group of 30years to 60 years (45.43%) were more affected than any other age group with mean age of 34.95 years, which was also noted by Itolikar S and Nadkar MY in their article.<sup>(17)</sup> A study carried out by Patel P B *et al* also found approximately 50% positivity in age group between 20 years to 50 yrs.<sup>(20)</sup> There was significant rise observed in age group less than or equal to 5 years (from 8.24% (141/1712) in 2009-10 to 17.5% in 2015) and age group above 60 years (from 4.38% (75/1712) in 2009-10 to 12.47% in 2015) in 2015 outbreak than that of 2009-10 pandemic. Thus it suggest that there is a shifting of age group to extremes of the age from age group of 15 - 30 years gradually over a period of time (Table 2).

Table 3 shows gender distribution of the influenza A(H1N1)pdm09 cases during period from August 2009 to March 2016. Over all females (50.28%) were slightly more affected than males (49.72%). Male to female ratio in 2009-10 pandemic was 1.2:1, whereas in year 2013 and in 2015 it was 0.92:1 and 0.94:1 respectively. Though female patients outnumbered male patients during 2013 and 2015 outbreaks, there was very negligible difference between the two and both were almost equally affected. Change in male: female ratio and shifting towards female is second signature feature of 2015 outbreak which is well correlated with study carried out by Patel P B *et al* (male – 48.14%, female – 51.86%).<sup>(20)</sup>

Overall if we compare recent outbreak with pandemic of 2009-10, patients presented with the same symptoms of cough, fever, sore throat, breathlessness and nasal catarrh. Sood NS *et al* noted in their study that highest percentage of people presented with symptom of cough (94%) followed by fever (90%), breathlessness (68%), sore throat (61%) and nasal

catarrh (33%).<sup>(21)</sup> In our study we found the similar symptoms presentation in the 2015 outbreak which is shown in table 4, but differentiating point is, percentages of nasal catarrh were significantly reduced from 33 % to 4.32% and sore throat were increased from 61% to 73.72%. Vomiting and diarrhea were not the presenting symptoms in 2015 outbreak also.

Chart 1 shows seasonal trend of influenza A(H1N1)pdm09 from August 2009 to March 2016. Though the first case of influenza A(H1N1)pdm09 was reported in India in May 2009, it was reported late in July 2009 from Gujarat. Three distinctive peaks of influenza A(H1N1)pdm09 were observed during August 2009, January 2010 and September 2010 in our region during the period of August 2009 to November 2010. Influenza A(H1N1)pdm09 remained in dormant stage for two years in 2011 and 2012 except some sporadic cases and again in 2013 there was significant increase in cases in the winter and autumn season with peak in the February 2013 which declined up to the month of May and before this a slight rise of cases during monsoon (August- 2012 to October 2012) was also observed. Same way recent outbreak of 2015 (Table 5) also appeared in the winter and autumn season with highest no of cases in the month of February 2015 with cessation up to the month of May. This major outbreak was also followed by minor ones in the post monsoon season and again in current year of 2016 in winter season with highest number of cases in February. Unlike temperate regions where seasonal influenza peak synchronized during their winter months, a year around activity is observed in tropical and sub-tropical regions with annual or bi-annual peak in different countries. India being a vast country with diverse climate demonstrates two seasonal activities.<sup>(9)</sup> In our region also two seasonal peaks were observed, one during rainy and post rainy season (August-October) and other during winter and autumn season (December - April). However in eastern India only one peak of infection were observed and that was in monsoon season during 2009-10.<sup>(18)</sup> Decrease ambient temperature has frequently been associated with the influenza seasonality. Ability of viral particle for prolonged survival in colder temperature is also considered to play an important role. In addition to that overcrowding, prolonged winter, unseasonal rain, inadequate hygiene, decrease in environmental temperature increases the physiological stress and energy loss due to thermal regulation, which in turn weakens the immune system and thereby increase the susceptibility of the host to infection are also other factors.<sup>(17)</sup>

After introduction of influenza A(H1N1)pdm09 into Gujarat, laboratory based influenza surveillance from cases of Severe Acute Respiratory Illness (SARI) and Influenza Like Illness (ILI) was started from November 2011 to know the knowledge of circulating influenza virus and for early detection of next coming

outbreak including emergence of novel strains. This helped us a lot in identifying early indications of coming outbreak of 2015. As shown in Table 5, during January 2015 to March 2016, out of total 727 surveillance samples, 92 (12.65%) were positive for seasonal influenza A and 23 (3.16%) for swine flu. It was seen that in December 2015 and January 2015, surveillance samples showed 9.09% (5/55) and 14.29% (8/56) positivity for influenza A(H1N1)pdm09 respectively. This was an alarming sign for us as earlier there were hardly any such positivity found in year 2014 from surveillance samples.

A separate OPD was started, during 2009 pandemic and again in February 2015 to attend to increased number of cases and to avoid unnecessary mixing up of the cases of influenza A(H1N1)pdm09 with other cases. We tested a total of 328 outdoor patients, and 7341 indoor patients for presence of pandemic influenza A(H1N1)pdm09 viruses. These indoor patients included 1595 from our hospital and 5746 from other hospitals. From 1595 patients admitted in our hospital, 809 were females from which 39 were from Ante Natal Care (ANC) unit. A total of 819 patients were found positive from 1595 patients admitted in our hospital, from which 441 were females out of which 28 female were from ANC unit. So, positivity of female patients in all admitted female patients was 54.51% (441/809), in all admitted positive patients was 53.85% (441/819) and in ANC unit was 71.80% (28/39). A total of 189 patients expired, among which 103 were females and from these, 14 females were from ANC unit (as from data on 31<sup>st</sup> March, first three month data). The above data again suggests affection toward the female sex more than the male sex. Case fatality rate of the patients admitted in our hospital in first three months of 2015 outbreak was 189/ 790 (23.92%) which was higher than in comparison to the outbreak of 2009-10 as shown in the study carried out by Rana H *et al* (19.9%) and Patel PB *et al* (5.9%).<sup>(10,20)</sup> This indicates that general population did not benefit from cross protection of earlier flu pandemic. Mishra B also noted in his article that death toll in a span of three months during the current resurgence already crossed the number of deaths which occurred during first 6 months of the pandemic in year 2009.<sup>(9)</sup> Case fatality rate in first three months in positive females in our hospital was 24.23% (103/425) and in ANC females was 50% (14/28) which indicate severity of the disease is high in women, especially in pregnant women which shows pregnancy is the most common comorbid condition. Itolika S *et al* also noted the same finding in her note.<sup>(17)</sup> Out of 189 expired patient, 65 were on ventilator, 42 were on bi pap, and 34 were on O<sub>2</sub> mask, which shows cardio-respiratory arrest is the major cause of death.

The recent surge in the influenza deaths has prompted healthcare specialists in India to suspect a circulating H3N2 strain to be the culprit same as in US

in the same period. Genetic mutations (D222G mutation in the haemagglutination glycoprotein structure) were suspected by researchers in recent outbreak. However, officials from the National Institute of Virology had clarified that no such mutation has occurred.<sup>(17)</sup>

Finally, this study was restricted to patients visiting hospitals only. Also only category C patients were admitted in the hospitals, so many cases of category A and B which were treated only on OPD bases may have been missed. Besides many patients from the community who got infected and did not go to the hospital may have been missed. We included data regarding mortality of patients in the first three months of the outbreak, and mortality data of patients admitted in other hospitals which were tested positive in our laboratory for influenza A(H1N1)pdm09 was not available. Also except pregnancy other features related to high mortality could not be investigated due to lack of mortality data and associated history from all the outbreaks. So, these are the limitations of our study.

### Conclusion

In conclusion, influenza A(H1N1)pdm09 viruses is still active six years after 2009 pandemic and follows typical seasonal pattern in this area. We conclude that throughout the study period middle age group was more affected. Females were affected higher than males. Outbreaks occurred in monsoon and post monsoon season and winter and autumn season. Case fatality rate was pretty high in pregnant women in 2015 outbreak. In such cases regardless of the results of testing, prompt evaluation and antiviral treatment of influenza-like illness should be considered. Influenza A(H1N1)pdm09 viruses is presently circulating in almost all parts of western region. However, despite occurrence of three major peaks in the western India, it has not been able to replace seasonal influenza A completely, but there is also co-circulation of seasonal influenza A virus with influenza A(H1N1) pdm09 in our region. Transmissibility of influenza A(H1N1) pdm09 viruses is higher than seasonal influenza A. Despite of several years having passed since the 2009 pandemic the population does not seem to be better protected than the 2009 outbreak. As because of antigenic shift and drift influenza is regularly changing its antigenic structure, so to fight against next coming outbreak and to know emergence of new strain, ongoing surveillance must be continued.

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