

Full Length Research Paper

Influenza surveillance results during 2008 - 2009 season in Turkey

Nurhan Albayrak^{1*}, Meral A. Ciblak², Ayse Basak Altas¹, Melis Kanturvardar², Yavuz Odabas³, Bahadir Sucakli³, Gulay Korukluoglu¹, Selim Badur² and Mustafa Ertek¹

¹Refik Saydam National Public Health Agency, National Influenza Center, Ankara, Turkey.

²Virology Laboratory, Faculty of Medicine, Istanbul University Istanbul, Turkey.

³MoH Primary Health Care General Directory, Communicable Disease and Outbreak Control Department, Ankara, Turkey.

Accepted 15 October, 2010

National influenza surveillance was performed by two institutions in Turkey, Refik Saydam National Public Health Agency (RSNPHA) and Istanbul Faculty of Medicine (IFM), which both are National Influenza Reference Laboratories. RSNPHA received samples from 9 sentinel Provinces and IFM received samples from 5 sentinel Provinces. We report the 2008 to 2009 surveillance results from October 2008 - May 2009. As many as 1,980 clinical specimens received from 14 sentinel and non-sentinel Provinces of which 257 nasal-nasopharyngeal samples were positive for influenza viruses. The predominant virus type was influenza B (67.5% of total detections), and the dominant sub-type among Influenza A viruses was H3 (98.8%). Influenza-like illness (ILI) activity started around the 42nd week, decreased around the 23rd week and the laboratory confirmed seasonal Influenza activity started around 48th week and ended around 22nd week. The 2008 - 2009 influenza seasons in Turkey was characterized by moderate clinical activity and a dominance of influenza B. In Hemagglutination inhibition (HI) test, the majority of influenza A/H3 viruses were closely related to the vaccine virus strain of the 2008 to 2009 season, Influenza A Brisbane/10/2007 (H3N2) like virus. However, Influenza B viruses were antigenically different from the vaccine strain and more closely related to Influenza B Malaysia/2560/2004 (Victoria lineage). It is concluded that knowledge about the influenza activity in Turkey, which is at the crossroads of Europe and Asia, contributed to monitoring the movement of influenza virus epidemiology.

Key words: Influenza, surveillance, Turkey.

INTRODUCTION

Influenza viruses generally cause acute, febrile and often self-limited upper respiratory infections. Its high transmission rate by respiratory droplets they constitute it possesses a public health threat. Every year 5 to 15% of

population are affected by influenza viruses during influenza epidemics (Arkema, 2008). Influenza infections may lead to the death of young children and chronically ill patients in addition to causing work days lost and economical loss (Paget, 2007). In addition to annual epidemics, influenza viruses can also result in human pandemics with new virus subtypes arising in immunologically naive populations (Zambon, 2001).

Influenza Surveillance Networks consisting of epidemicologists and virologists have been established worldwide to monitor influenza activity globally. The information collected via surveillance networks can aid in the reduction of morbidity and mortality due to influenza, selection of vaccine virus for the following season, detection of a new virus subtype entering the circulation,

*Corresponding author. E-mail: nurhanalbayrak@yahoo.com.
Tel: +90 312 4582213. Fax: +90 312 4582437.

Abbreviations: CDC, Center for disease control; HA, hemagglutination; HI, hemagglutination inhibition; ILI, Influenza-like illness; IFM, Istanbul Faculty of Medicine; PCR, polymerase chain reaction; RSNPHA, Refik Saydam National Public Health Agency; VTM, viral transport medium; WHO, World Health Organization.

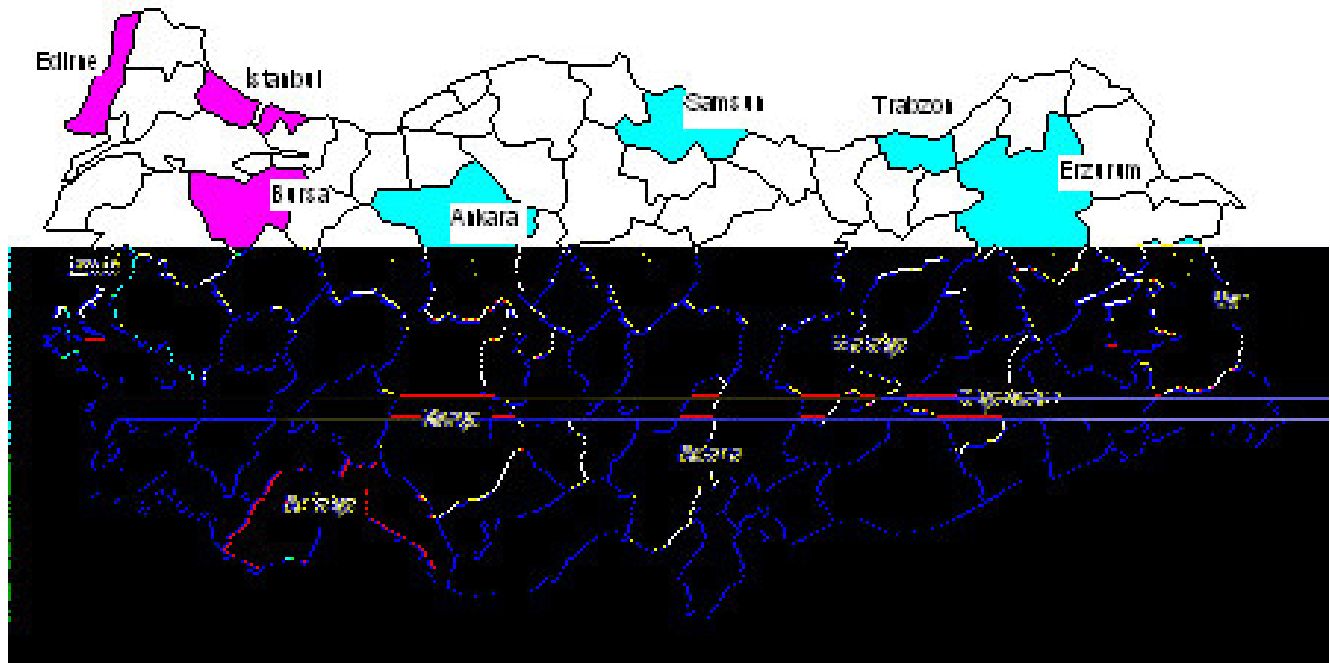


Figure 1. Sentinel centers for influenza surveillance programme. A total of 1980 nasal / nasopharyngeal samples were received from 140 centers in 14 sentinel Provinces. Purple coded Provinces (Antalya, Bursa, Edirne, Istanbul and Izmir) send samples to the laboratory in Istanbul, green coded Provinces (Adana, Ankara, Diyarbakir, Erzurum, Konya, Malatya, Samsun, Trabzon, Van) send samples to the RSNPHA laboratory in Ankara.

detection of changes in genetic background of the viruses and antiviral resistance. Surveillance data for European region were collected from Flunet and Euroflu (Arkema, 2008).

In Turkey, sentinel surveillance is conducted by Refik Saydam National Public Health Agency (RSNPHA) and Istanbul Faculty of Medicine (IFM), two National Influenza Reference Laboratories under the coordination of General Directorate of Primary Health Care of Ministry of Health. A total of 14 Provinces, representing the whole country, are included in the sentinel surveillance. Surveillance data are shared with international data bases such as EuroFlu. The surveillance results of 2008 to 2009 season starting from week 40, ending at week 22 in Turkey, a country consisting of approximately 70 million people, were reported. The 2008 to 2009 influenza season was marked with moderate activity in Turkey. In the light of this information, it is concluded that knowledge about the influenza activity in Turkey, which is at the crossroads of Europe and Asia, contributed to monitoring the movement of influenza virus epidemiology.

MATERIALS AND METHODS

National influenza surveillance system

Surveillance system of communicable diseases has been regulated in our country with the Directive of the Turkish Republic Ministry of Health Directorate General of Primary Health Care.

Influenza virus infections are notified and followed up through sentinel surveillance. Surveillance in the inter pandemic period have been conducted within the routine notification system. A rapid assessment have been done on the system, especially during the influenza season (between the 40th and 20nd weeks).

Within the National Influenza Surveillance Communicable Diseases Section of the Ministry of Health Directorate General of Primary Health Care collected weekly ILI data from a total of 140 centers in 14 Provinces and the data were shared with international networks.

Samples

A total of 1980 nasal / nasopharyngeal samples with nasal swab (Eurotubo; Rubi, Spain) were received from 140 centers in 14 sentinel Provinces as well as from non-sentinel volunteers (Figure 1). Samples were randomly selected from patients with ILI like symptoms. RSNPHA, center in Ankara, received samples from 9 sentinel Provinces (Adana, Ankara, Diyarbakir, Erzurum, Konya, Malatya, Samsun, Trabzon, Van) and IFM, center in Istanbul, received samples from 5 sentinel Provinces (Antalya, Bursa, Edirne, Istanbul and Izmir). All samples were taken based on the influenza case definition provided to the participating physicians and sent to the laboratories in viral transport medium (VTM) on ice-packs within 48 h.

Laboratory tests and algorithm

The samples were tested within 24 to 48 h of the arrival to the laboratory. After proper processing procedures, each specimen was firstly screened by in-house real-time polymerase chain reaction

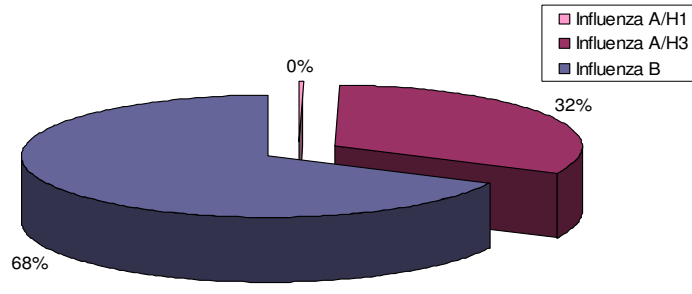


Figure 2. The frequency of Influenza viruses during 2008 - 2009 winter season in Turkey. During 2008 to 2009 Influenza season in Turkey, total of 1980 sentinel and non-sentinel samples, and 13% of the samples were positive for influenza viruses. The dominant type was Influenza B (68% all of the positive detection) and the dominant sub-type among the Influenza A viruses was A/H3.

(PCR) for rapid detection of Influenza viruses. The real-time PCR was performed with "in-house" real-time polymerase chain reaction (PCR) protocol provided by Center for Disease Control (CDC, USA), using 2x buffer and SuperScript III Platinum® One-Step Quantitative RT-PCR System (Invitrogen; CA, USA) with Stratagene Mx3005P (Stratagene; California, USA) real-time PCR machine. The 25 µl PCR mixture contained 5 µl of extracted RNA, 1 µl each of forward and reverse primers, 1 µl probe, 0.5 µl SuperScript III RT/ Platinum *Taq* mix, 12.5 µl of 2X Master mix, and 4 µl nuclease-free water. RT-PCR amplification conditions were as follows; reverse transcription at 50°C for 30 min, *Taq* inhibitor activation 95°C for 2 min and 45 cycles at 95°C for 15 s, 55°C for 30 s. Viruses were identified and sub-typed by both molecular methods and inoculation in cell culture (WHO, 2009).

All specimens were inoculated on monolayer MDCK (Madin-Darby Canine Kidney) cell cultures for virus isolation and the cells were monitored daily. After 3 days of incubation at 37°C, cell culture supernatant was used for Hemagglutination Test (HA). HA positive isolates were typed and sub-typed by Hemagglutination Inhibition Test (HI) using reference anti-sera supplied by WHO for the influenza season 2008 - 2009 (WHO, 2002, 2006).

RESULTS

As many as 1980 nasal/nasopharyngeal samples were received from 14 sentinel and non-sentinel Provinces in the 2008 - 2009 influenza season in Turkey. 1834 samples were from adult ILI suspected cases (92.6%) and 146 (7.4%) samples were from pediatric group. 1379 samples received by RSNPHA from 9 Provinces and 501 samples received by IFM from 5 Provinces. A total of 257 (13.0%) out of 1980 samples were positive for Influenza viruses. Influenza B was detected in 174 samples (67.5% of total detections) and Influenza A sub-type H1 and H3 were detected in 1 and 82 samples (0.4 and 32.1%) respectively. Among Influenza A viruses, subtype H3 was the dominant sub-type (98.8%) and subtype H1 was detected only in one sample in the 2008 to 2009 season (Figure 2).

In the 2008 to 2009 winter season, ILI activity started around the 42nd week and decreased around the 23rd

week and the laboratory confirmed that seasonal influenza activity started around the 48th week and ended around the 22nd week. The peak activity was in March and in mid-May and there was another peak of activity due to the increasing number of samples for novel H1N1 virus (Figure 3).

On an average of 5 samples per week with a total number of 140 samples for the whole season were sent from each Province to the reference laboratories from each Province. Distribution of Influenza viruses during 2008 to 2009 winter season in Turkey based on the Provinces is shown in Figure 4.

Influenza A subtype H3 positive isolates and Influenza B isolates were antigenically analyzed by HI test after propagation of the viruses in MDCK cell culture (WHO, 2002, 2006). In HI test with post-infection ferret sera, the majority of influenza A (H3) strains were closely related to the vaccine virus of 2008 to 2009 influenza A Brisbane/10/2007 (H3N2) like virus. However, Influenza B viruses were antigenically different from the vaccine strain and more closely related to influenza B Malaysia/2560/2004 (Victoria lineage).

DISCUSSION

Influenza season during 2008 to 2009 in Turkey was marked by moderate activity. A total of 1980 sentinel and non-sentinel samples were received between week 40 of 2008 and week 22 of 2009, the annual influenza surveillance period in Europe. 13% of the samples tested were positive for influenza viruses. The dominant type of a virus detected was Influenza B and the dominant sub-type among the Influenza A viruses was A/H3. A/H1 was detected only in one sample during this season (Figure 2). During 2008 to 2009 season, influenza activity started to increase on week 48 and decreased towards week 22. However, due to emergence and world-wide spread of pandemic A/H1N1 virus isolations increased again after

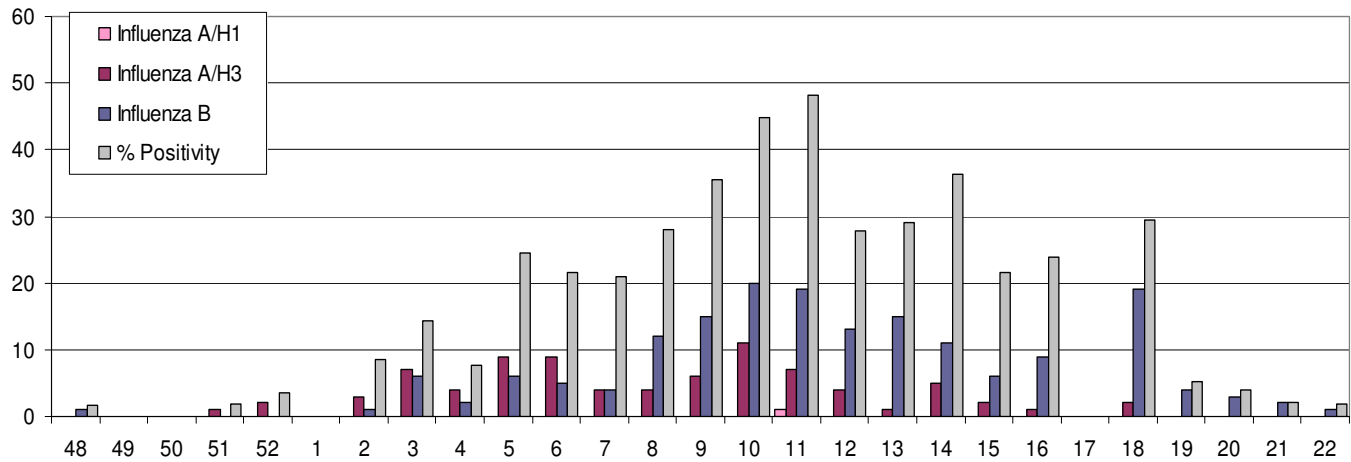


Figure 3. Weekly distribution of Influenza viruses during the 2008 to 2009 winter season in Turkey. In the 2008 to 2009 winter season, influenza activity was started to decrease around the 48th week, ended around the 22nd week, and the peak activity was at 10th week. In addition, at the 18th week there was another peak of laboratory confirmed influenza activity because of the increasing number of samples for novel H1N1 virus.

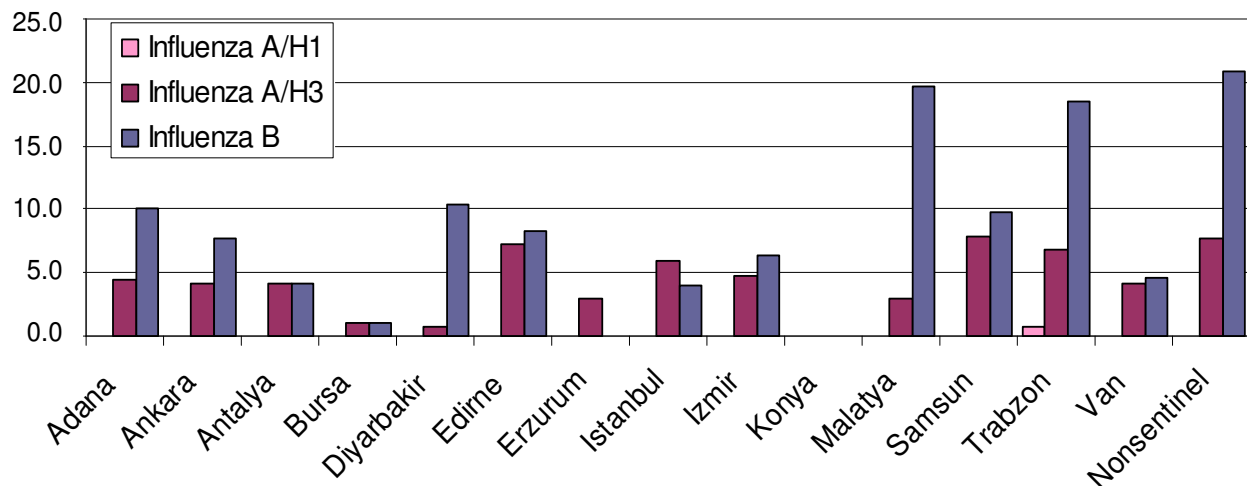


Figure 4. Distribution of Influenza viruses during 2008 to 2009 winter season in Turkey according to the Provinces (as positivity ratio). Influenza B was clearly the dominant type in the central and eastern Provinces (Adana, Ankara, Diyarbakir, Malatya, Trabzon etc.) of Turkey. However, in western Provinces (Antalya, Bursa, Istanbul) Influenza A and B were co-dominant.

week 22 (Ciblak, 2009a). The influenza season started and ended with Influenza B in Turkey. Influenza detections started increasing by February and remained high until the end of April (Figure 3). The influenza activity in Turkey, was similar to that reported from Japan and Influenza B was the dominant type for 2008 to 2009 season. In Japan, influenza activity started with influenza A/H1N1, but maintained and ended with a dominance in Influenza B (Ujike, 2010). In Turkey, the laboratory confirmed influenza activity was different from reported activity of the USA and Europe, where the season started

with oseltamivir resistant A/H1N1 and continued with A/H3N2 (Goddard, 2009; Burrell, 2009; Potter, 2009; Brammer, 2009). In Europe, the influenza season started in week 49 and characterised by influenza virus type A/H3N2 (Goddard, 2009).

As in the world, there were differences in dominant virus type between the regions of Turkey. In the Central and Eastern part of Turkey, like the Adana, Ankara, Diyarbakir, Malatya and Trabzon Provinces, Influenza B was clearly the dominant type. However, in western part of Turkey Provinces, like Antalya, Bursa, Istanbul Provinces,

Influenza A and B were co-dominant (Figure 4). This geographical difference has been reported previously (Carhan, 2009; Ciblak, 2009b).

HI test with post-infection ferret sera showed that Influenza A (H3) isolates were closely related to the vaccine virus of 2008 to 2009 Influenza A Brisbane/10/2007 (H3N2) like virus. Nevertheless, influenza B isolates from Turkey were antigenically different from the vaccine strain and more closely related to influenza B Malaysia/2560/2004 (Victoria lineage). In this context, there was a good match between the reported virus strains and the vaccine virus strain H3, but a mismatch was present between the vaccine virus type B and circulating B strains in Turkey as reported by others for 2008 to 2009 influenza season (Mossad, 2008; Goddard, 2009).

Finally, Turkey is at the crossroads of Europe and Asia, because of this important situation knowledge about the influenza activity in Turkey contributed to understanding the movement of influenza viruses.

REFERENCES

- Arkema JMS, Meijer A, Meerhoff TJ, Van der Velden J, Paget WJ (2008). European Influenza Surveillance Scheme. Epidemiological and virological assessment of influenza activity in Europe, during the 2006-2007 winter. *Eurosurveillance*, 13 (7-9).
- Brammer L, Epperson S, Blanton L (2009). Update: Influenza Activity - United States, September 28, 2008-January 31, 2009. *Morbidity and Mortality Weekly Report*. 58(05): 115-119.
- Burrell S, Roncin L, Lafon ME, Fleury H (2009). Oseltamivir susceptibility in south-western France during the 2007-2008 and 2008-9 influenza epidemics and the ongoing influenza pandemic 2009. *Eurosurveillance*, 14(38): 24.
- Carhan A, Altas AB, Albayrak N, Uyar Y (2009). Influenza surveillance results in 2007-2008 winter season in nine Provinces of Turkey. *Bull. Microbiol.*, 43(2): 235-241.
- Ciblak MA, Albayrak N, Odabas Y (2009a). Cases of influenza A(H1N1)v reported in Turkey, May - July 2009. *Eurosurveillance*, 14 (32), 13 August.
- Ciblak MA, Hasoksuz M, Escuret V (2009b). Surveillance and oseltamivir resistance of human influenza A virus in Turkey during the 2007-2008 season. *J. Med. Virol.*, 81(9): 1645-51.
- Goddard N, Zucs P, Ciancio B, Plata F, Hungnes O, Mazick A (2009). Start of the influenza season 2008-9 in Europe-increasing influenza activity moving from West to East dominated by A (H3N2). *Eurosurveillance*, 14 (3): 22.
- Mossad SB (2008). 2008 to 2009 Influenza update: A better vaccine match. *Cleveland J. Med.*, 75 (12): 865-870.
- Paget WJ, Marquet R, Meijer A, van der Velden K (2007). Influenza activity in Europe during eight seasons (1999-2007): an evaluation of the indicators used to measure activity and an assessment of the timing, length and course of peak activity (spread) across Europe. *BMC Infect. Dis.*, 7: 141-147.
- Potter R (2009). Influenza Vaccination Coverage Among Children Aged 6 Months-18 Years - Eight Immunization Information System Sentinel Sites, United States, 2008-09 Influenza Season. *Morbidity and Mortality Weekly Report*, 58(38): 1059-1062.
- Ujike M, Shimabukuro K, Mochizuki K, Obuchi M, Kageyama T, Shirakura M (2010). Oseltamivir-resistant influenza viruses A (H1N1) during 2007-2009 influenza seasons, Japan. 2010 Jun; *Emerg. Infect. Dis.*, 16(6): 926-935.
- WHO (2009). WHO information for laboratory diagnosis of pandemic (H1N1) 2009 virus in humans. Revised, 23 November 2009, World Health Organization.
- WHO (2002). Manual on Influenza Diagnosis and Surveillance-2002. 5, Department of Communicable Diseases Surveillance and Control, World Health Organization.
- WHO (2006). Reagents for Influenza Virus Diagnosis 2006, WHO collaborating Center for Reference and Research on Influenza, Melbourne, Australia.
- Zambon MC (2001). The pathogenesis of influenza in humans. *Rev. Med. Virol.*, 11: 227-241.