EPIDEMIOLOGY OF SEASONAL INFLUENZA OUTBREAK AMONG IRAQI POPULATION:2018

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ABSTRACT

This study aimed to highlight the prevalence of the seasonal influenza in the different Iraqi provinces during 2018 year to study the epidemiological aspects, and their effect on the frequency of disease and death cases caused by influenza virus. A total of 1359 throat and nasal swabs was collected from individuals suffering from influenza like illness (ILI) or severe acute respiratory infection (SARI) for testing influenza virus type A (H1N1 and H3N2) and type B. RNA extracted and amplification with specific primers and probes. Results showed the incidence rate of flu A 16.7 (227/1359) and 4.7 of flu B per 100000 people-year that included 14.9 for H1N1, 1.5 for H3N2 and the remaining value for mixed infection of H1N1 and H3N2. Regarding, the mortality rate 1.6 (21/227) with influenza A and 0.15 (2/64) with influenza B infections per 100000 person-year. The prevalence of flu A between the months showed significant differences, especially in the first two months of the year. The distribution of influenza infections in Baghdad province, which appeared the highest peak, then followed by Babyl, Waist and Salahaddin provinces. In conclusion, more surveillance studies are needed each year to provide database more important for WHO Eastern Mediterranean region (EMR) of influenza surveillance.

Keywords: Real-Time RT-PCR, Influenza type A, H1N1, Influenza type B, Iraqi provinces.

وبائية الانفلونزا الموسمية المتفشية بين المجتمع العراقي لعام 2018

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المستخلص

هدفت الدراسة إلى تسليط الضوء على مدى انتشار الأنفلونزا الموسمية في المحافظات العراقية المختلفة خلال عام 2018 لدراسة الجوانب الوبائية، وتأثيرها على تردد حالات المرض والوفاة الناجمة عن فيروس الأنفلونزا. تم جمع 1359 مسحة للحنجرة والأنف من أفراد يعانون من مرض يشبه الأنفلونزا (ILI) أو عدوى الجهاز التنفسي الحادة (SARI)لاختبار نوع فيروس الأنفلونزاA (H1N1 و H3N2) ونوعB. استخلص وضخم الحامض النووي منقوص الاوكسجين باستخدام بادئات ومجسات متخصصة. أظهرت النتائج أن معدل الإصابة بالإنفلونزا A 16. 7و 4.7 من الأنفلونزا B لكل 100000 شخص في السنة شملت 14.9 لـ H1N1 و 1.5 لـ H3N2 وما تبقى هو للإصابة المختلطة H1N1 وH3N2. أشارة الى معدل الوفيات فان 1.6 الأنفلونزا A و 0.15 مع الأنفلونزا B لكل 100000 شخص في السنة. أظهر انتشار الأنفلونزا A بين الأشهر اختلافات كبيرة ، خاصة في الشهرين الأولين من العام. توزعت الإصابة بالأنفلونزا في محافظة بغداد والتي اظهرت أعلى قمة، ثم تبعتها محافظات بابل وصلاح الدين. استنتجت الدراسة هناك حاجة إلى مزيد من دراسات المراقبة كل عام لتوفير قاعدة بيانات أكثر أهمية لمنطقة شرق المتوسط (EMR) التابعة لمنظمة الصحة العالمية لمراقبة الإنفلونزا.

الكلمات المفتاحية: تفاعل سلسلة البلمرة اللحظي، فيروس الانفلونزاA ، فيروس الانفلونزاB ، المحافظات العراقية، الانفلونزا الموسمية.

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INTRODUCTION

Newly discovered several respiratory viruses like avian influenza virus A (H5N1), avian influenza A (H9N2), swine-origin influenza A (H1N1), and the recent Middle East respiratory syndrome coronavirus (MERS-CoV) at 2012, which are usually zoonotic in origin, have emerged in the area on account that 2006 posing severe epidemic and pandemic dangers (17). There are three types of influenza viruses A, B, and C, have been recognized and well-known to infect humans. Though, the influenza C viruses are rare, they believed, to cause mild upper respiratory tract infections among children less than 2 years (18). While the influenza A and B are significant reasons of respiratory infections among all age groups, but the most severe cases arise among young children and the elderly (9). The natural hosts for influenza A viruses are ducks and waterfowl, in which all subtypes of influenza are thought to be in an evolutionary stasis, that is, in equilibrium with their hosts (14). Influenza A viruses are subtyped established on their hemagglutinin (HA) and neuraminidase (NA) superficial protein type (16). Seasonal flu is the term used to refer to the flu outbreaks that occur yearly, mainly in the late fall and winter. Seasonal Influenza Viruses: A (HINIpdm09, H3N2….etc), influenza B and C, while the non-seasonal influenza viruses: (animal origin) avian influenza: A (H5N1), (H5N6), (H7N9), (H9N2) and the swine-origin viruses: A (H3N2)v, (H1N1)v, (H1N2)v. For an archive of Iraqi health ministry, the avian influenza type H5N1 first reported in North Iraq in 2006 (2 cases) end in death. The second and last reported in Iraq (Baghdad) 2 cases, one end in death. At present, influenza A subtypes (H1N1 and H3N2) and B virus are the circulating seasonal influenza among humans. This seasonal A (H1N1) virus is the same virus that caused the 2009 influenza pandemic, as it is now circulating seasonal. In addition, there are two type B viruses that are also circulating as seasonal influenza viruses, which are named after the areas where they were first identified, Victoria lineage and Yamagata lineage. This pandemic A (H1N1) 2009 virus has been widely circulating through the world since 2009, and current traditional in human populations as a seasonal influenza virus, as mentioned above. Presently there is no extensive a pandemic virus circulating in the world (23). The World Health Organization (WHO) and the Eastern Mediterranean region (EMR) for giving its location under several migratory bird flyways, is an important region for influenza research and surveillance. Iraq is one country in the region that influenza viruses circulate with causing substantial illness and death leading to considerable economic losses. Influenza A (H1N1 and H3N2) and influenza B viruses are the current subtypes of seasonal influenza viruses circulating in humans (13). In Iraq, there are several previous papers have studied influenza viruses and its association with flu disease by two molecular and immunological methods (4, 5). Furthermore, there is a few previous Iraqi study detection and typed the seasonal influenza per annum and compared its incidence with other countries in the EMR region. So that, this study aimed to highlight the prevalence of the seasonal influenza in the different Iraqi provinces during 2018 year to study the epidemiological aspects, which involved genders, age groups, provinces and seasonality and their effect on the frequency of disease and death cases caused by influenza virus.

MATERIALS AND METHODS

Study samples

A total of 1359 respiratory samples was collected from individuals (766 male and 593 female) suffering from influenza symptoms may be positive or negative infections, between January to December 2018 selected from various public hospitals at different 17 Iraqi provinces. The samples included nasal and throat swabs, and they had been collected for routine viral diagnostic screening of

patients suffering from upper and/or lower respiratory tract diseases and the patients consented that their samples be used for testing of seasonal influenza viruses (AH1N1, AH3N2 and influenza type B). Symptoms of patients included a wide range of lung disorders ranging from mild upper respiratory tract infections (URTI) which including: (fever over 38C), common cold, cough, coryza, sore throat and shortness of breath to lower respiratory tract infections (LRTI) including: laryngitis, bronchiolitis, and pneumonia. The information about these patients and their clinical history were mentioned in the case investigation form for each influenza suspected patient. The respiratory tract samples were collected from different ages 33.2±0.65 (mean±SE from 2 months-91 years old) by using Viral Transport Media (VTM, Copan, USA) for maintaining viral viability during transportation and until its arrival to the laboratory. Samples were transported by a cool box to the department of virology, National Influenza Center at the Central Public Health Laboratory, and the samples stored at -80 Ḉ until the time of analysis.

RNA extraction

All upper respiratory tract samples (1359 nasal and throat swabs) were selected for RNA extraction to detect seasonal human influenza viruses (type A and B) by using a specific kits: QIAamp Viral RNA Mini Kit (GmbH, Hilden, Germany) as described according to manufacturer's instructions, and stored the specimen at deep freeze -80 Cº until the use (1).

Fast Real-Time Reverse transcription-Polymerase Chain Reaction (rRT-PCR)

All the clinical samples were tested for influenza A and B by rRT-PCR using CDC Influenza Virus Real-Time RT-PCR A/B Typing Panel kit (Atlanta, USA). Master mix was prepared using SuperScript III platinum one step RT-PCR kit (Ivitrogen, USA). A 25 µl master mix contained 12.5 µl reaction buffer (5x), 0.5 µl Super Script TM III RT/Platinum TM Taq mix, 0.5 µl of each primer and probe (40 µM conc.for each primer and 10 µM conc.for probe), 5.5 µl PCR water, 0.5 µl Rox dye (1/10 dilution) and 5 µl of the specimen RNA template. The amplification and detection were performed with fast 7500 Real-Time PCR system (Applied Biosystems) as follows :RT step activation at 50℃ for 5 min, Initial denaturation at 95℃ for 2 min, followed 45 cycles: 95℃ for 3sec and 55℃ for 30 sec. Samples positive for influenza A were subjected to subtyping with CDC Influenza Virus Real-Time RT-PCR Subtyping Infuenza A(H3/H1pdm09) Panel kit (Atlanta, USA). The master mix and RT-PCR thermal profile as described above.

Statistical analysis

The statistical analysis system was analyzed by IBM SPSS statistics version 25. All values, proportions and their frequencies were checked by applying the Pearson chi – square (X^2) and cross tab test to investigate significant comparison between viral infection percentages in different studying markers of population study. Also, the Kruskal-Wallis applied to the effective study of province, month and age groups along with influenza infections. A value of $P<0.05$ was considered statistically significant. Moreover, the important epidemiological frequency measures calculated according to special mathematical equations (21).

RESULTS AND DISCUSSION

Results showed the incidence rate of flu A 16.7 (227/1359) per 100000 people with flu-illness per year that included 14.9 for H1N1, 1.5 for H3N2 and the remaining value for mixed infection of H1N1 and H3N2, while the incidence rate of flu B 4.7 per 100000 person-year. The negative remaining samples of the respiratory samples may be patients have the same symptoms, but strongly associated with other viruses, bacteria and fungi, for instance the respiratory syncytial virus (RSV) (10, 11), influenza A and B; parainfluenza 1, 2, and 3 (19); adenovirus (12), human

metapneumovirus (Hmpv) (6) and bocavirus (7). It is interesting to note, the results of previous study pointed out 26.67% of coronavirus was co–infected with the most frequently Flu A (22). On the other hands, the prevalence of influenza A documented in the archives of the Iraqi ministry of public health since 2013 to 2017 which 27.03%, 0.76%, 19.76%, 15.16% and 2.71%, respectively. Regarding, the mortality rate 1.6 (21/227) in patients with influenza A and 0.15 (2/64) with influenza B infections per 100000 person-year. Furthermore, the case-fatality 9.3% (21 case) exposed patients with flu A (H1N1 alone) and 3.1% (2 cases) reported in patients infected with flu B alone. Furthermore, the case-fatality reported in the archives of the Iraqi ministry of public health at 2015 which 5.63% with H1N1 infection; in 2016 3.13% of H1N1, 6.25% for H3N2 and 1.3% in flu B; also in 2017 was 18.75% in H1N1 and 8.7% with flu B infections. Relative risk (RR) and risk differences (RD) were calculated for infections of flu A and B together compared with contact person as 8.6 RR observes the relevance of the risk factor for the disease and 84.2 RD which depends on the frequency of the disease. Among the 227 cases were detected as flu A (H1N1 and H3N2) positive, including 137 (60.4%) in male and 90 (39.6%) in female as demonstrated in the table 1. No significant differences (P>0.05) existed between the detection rates of flu B in male and female. This study pointed out the variations in infection rates that depend on gender. Males appear to suffer more frequently from most kinds of RTIs compared to females, leading to greater mortality in males (14 cases), and particularly in community-acquired pneumonia.

Table 1. Demographic appearance of influenza types A (H1N1 and H3N2) and type B infections, according to gender in population studies.

Gender (no.)	Flu A♦ (Prevalence%)	H1N1 cases	H3N2 cases	Flu B (Prevalence%)	Death cases
Male (766)	**137 * (17.9%)**	**123 ***	**12**	**34 (4.4%)**	**14**
Female (593)	**90 (15.2%)**	**79**	**8**	**30 (5.1%)**	**9**
Total (1359)	**227 (16.2%)**	**202**	**20**	**64 (5%)**	**23**
X^2 -value (p-value)	**9.7 (0.001)**	**9.5 (0.002)**	**0.8 (0.37)**	**0.3 (0.62)**	**1.1 (0.3)**

♦ There is five cases of flu A un-subtypable, *significant P<0.01. Also, there is another five cases showed mixed infection, flu A (H1N1) with flu B. Furthermore, one case of H3N2 appeared accompanying with H1N1, and the death cases observed with the flu A (H1N1) infection alone and with flu B infection alone as 21 and 2 cases, respectively.

The data were analyzed with regard to the age distribution of influenza infection. Figure 1 depicts the distribution of influenza-infected cases by age groups, all the patients were grouped into eight age groups with different positive influenza rates of infections. Generally, the frequency of positive flu A (H1N1) was highly significant values (χ^2=311.4, P<0.01) in patients aged 21–50 and less than two years which represented more than 60% of positive cases. Also the same age group achieved highly significantly (Kruskal= 39, P<0.01) with a flu B infection. Nevertheless, the results of our study showed interesting findings on H3N2 infection, which means that H3N2 may be the causal agents of respiratory diseases among patients under different clinical conditions, especially in adults (> 60 years old) with no statistical differences between groups (χ^2=3.5, P>0.05). There are no significant differences in death case occurrence between the age groups (χ^2=2.4, P>0.05). The H1N1 patients had increased headache, myalgia, and fatigue, which are common symptoms of influenza; however, there were no specific symptoms in H3N2 patients. There have been published data regarding the evolution of H3N2 since 1968 where this virus had low propensity and impact in the last 10 years (15). Low vaccine effectiveness has also been observed, however, based on the genetic mutation of the virus with

seven substitutions at key antigenic sites (8). The mutation in H3N2 virus has also been responsible for oseltamivir resistance that was observed in patients between 2009 and 2011 (20).

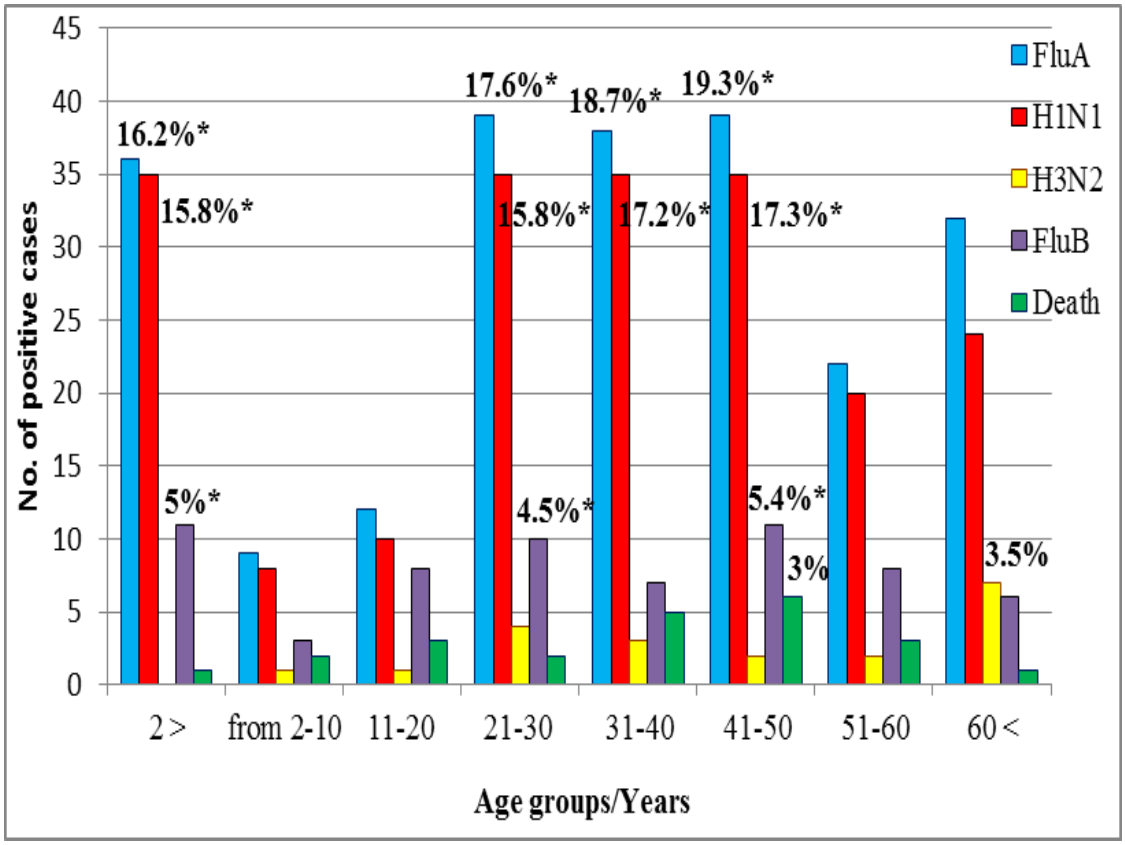

Figure 1. Distribution of influenza types A (H1N1 and H3N2) and type B infections with incidence of death cases according to eight different age groups. The percentages represent the prevalence of positive cases divided on the total samples of each group in the study population. The asterisk indicates that significant differences among them

The prevalence of flu A positive cases of respiratory tract infections during 2018 of this study period between the months showed significant differences (χ^2=21.3, P<0.01), especially in the first two months of the year. The same case, flu B appearance was limited and significant increase in Jan ((χ^2=12.4, P<0.01) (Fig. 2). Results found highly significant increasing of H1N1 and H3N2 infections observed in winter (Nov, Dec, Jan and Feb months as a seasonal infection) 196 and 20 positive cases, respectively, (χ^2=185.6, P<0.01), that a little in the remaining months of the year. Most death cases occurred in Jan followed by a Feb with slightly significant differences (χ^2=6.6, P=0.04). In figure 3 shows the distribution of influenza infections in Baghdad province, which appeared the highest peak in the study group, then followed by Babyl, Waist and Salahaddin provinces. There are highly significant differences between flu A (H1N1) infection percentages of total cases among study Iraqi province (χ^2=150.4, P<0.01). Baghdad reported the highest number of respiratory infections due to its crowded city of people may according to the increase in migration of people from the rural area to the cities so facilities transmission of these viruses among them and increase average RTI.

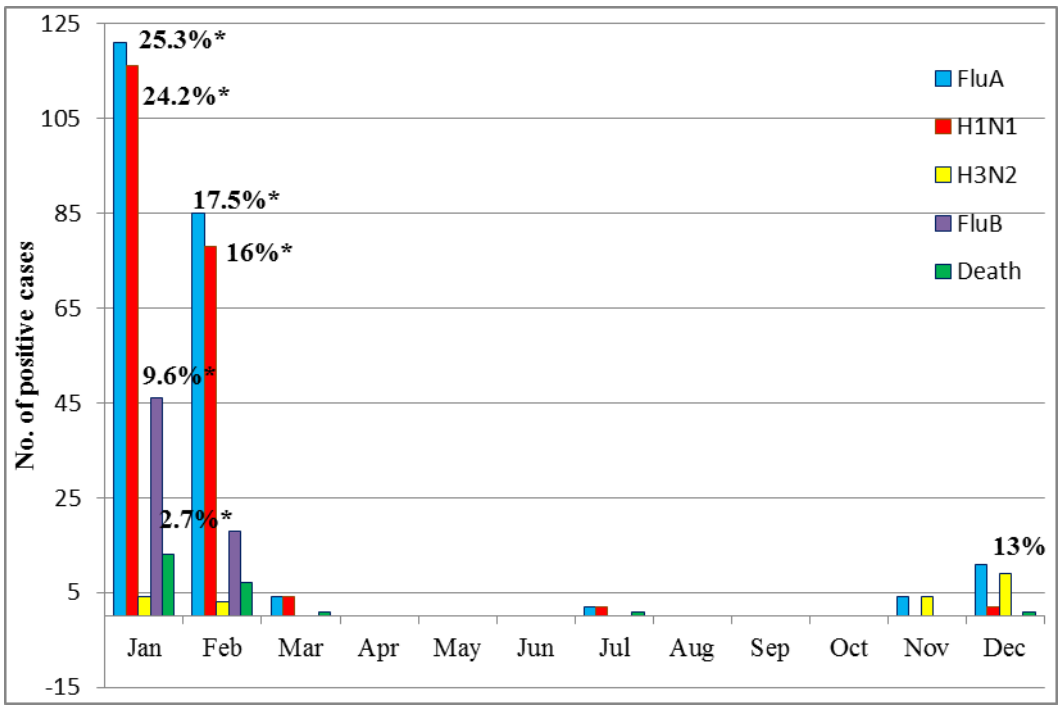

Figure 2. Distribution of influenza type A (H1N1 and H3N2) and type B infections in respiratory tract infections in months during 2018. The percentages represent the prevalence of positive cases divided on the total samples of each month in the study population. The asterisk indicates that significant differences among them

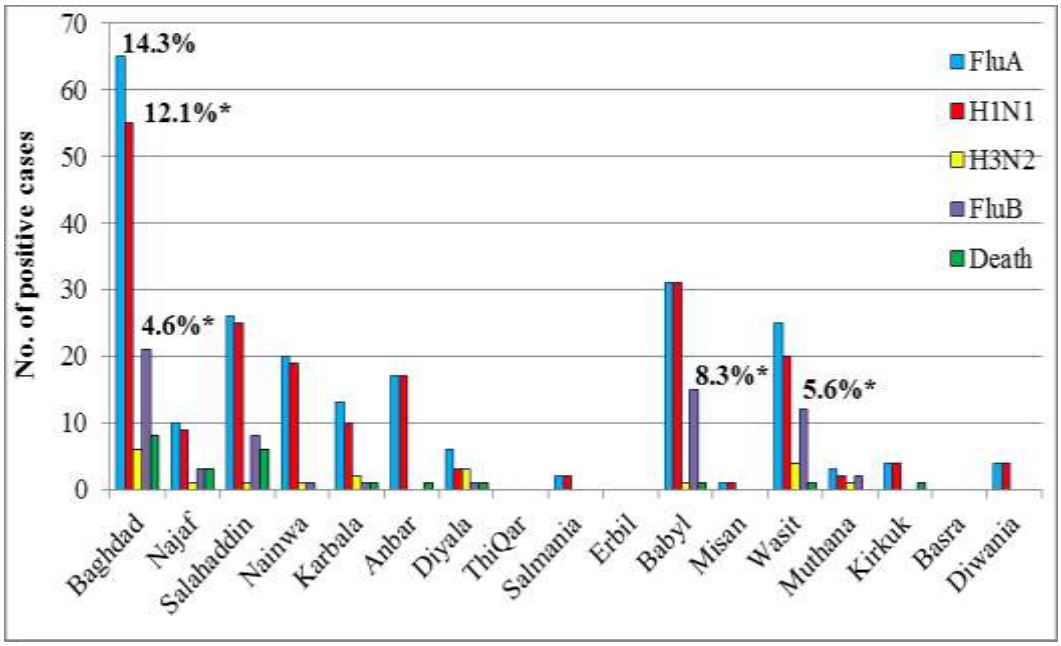

Figure 3. Prevalence of influenza type A (H1N1 and H3N2) and type B infections in respiratory tract depending on to study Iraqi province. The percentages represent the prevalence of positive cases divided on the total samples of each province in the study population. The asterisk indicates that significant differences among them

Throughout a current study, the most prevalence of flu B infections, which recorded in Baghdad province 21 (4.6%), followed by Babyl 15 (8.3), Waist 12 (5.6%) and Salahaddin 6 (12.3) with statistical significant among Iraqi provinces (χ^2=34, P<0.01), while high cases of death reported in Baghdad (8 cases) and Salahaddin (8 cases) provinces with no significant differences (χ^2=2.3, P>0.05). Furthermore, Waist province reported high rate may belong to lack of health services and less in accuracy for diagnosis most of the

viruses which causes RTI so taken incorrectly of antibiotics. As mentioned, this was the first reported about detection flu A (H1N1 and H3N2) and flu B, with death cases as outbreak infection and risk factor in Iraq included in 2018. Another Iraqi study reported from Oct to Dec- 2009 in Baghdad 42.85% cases were positive for H1N1 influenza. Of the positive cases 64.44% were males and 35.55% were females (4), while the other study in central Iraqi provinces reported 24.49% of seasonal influenza (3). Also, in Al-Najaf Iraqi province documented 64.7% as a season influenza in 2012, included 28.3% of H3N2, 14.8% of H1N1, 5.6% of H1N1 with H3N2 and 3% of influenza type A with B (2).

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