D.I. Ismagulova ⓘ , A.M. Baimukhametova ⓘ , S.B. Baiseiit ⓘ , T.I. Glebova ⓘ ,

N.G. Klivleyeva ⓘ , N.T. Saktaganov ⓘ ,

N.S. Ongarbayeva ⓘ , G.V. Lukmanova ⓘ , M.G. Shamenova ⓘ

Research and Production Center of Microbiology and Virology, Almaty, Kazakhstan
*e-mail: ismagulovadariya@gmail.com

# Study of features of influenza virus circulation in the southern region of Kazakhstan during the 2021 epidemic season

**Abstract.** Influenza viruses are among the most prevalent infectious agents, leading to massive seasonal epidemics and pandemics with high mortality rates. The ability of influenza virus to antigenic variability determines the high population susceptibility, wide spread of infection, and short intervals between epidemics. In recent years, the co-circulation of influenza viruses that belong to A/H1N1pdm09 and A/H3N2 subtypes and influenza type B virus has been observed. Here we summarize the data we explored to establish the features of the influenza virus circulation in the southern region of the Republic of Kazakhstan. To conduct virological and serological studies, 590 biosamples were collected from sick patients (419 swabs and 171 blood serums). Influenza type A virus RNA was found in 39 (9.31%) samples including that of A/H1N1pdm09 virus in 15 (3.58%), A/H3N2 virus in 23 (5.49%). It has also been noted that isolates A/Almaty/10/21 and A/Almaty/15/21 were profiled by the antigenic formula A/H3N2. The results of virological, serological, and molecular genetic studies confirm the simultaneous circulation of influenza A viruses (H1N1pdm09 and H3N2), and influenza type B virus. Some thoughts on the current scenario as well as implications for the future are presented as well.

**Key words:** biosamples, circulation, influenza virological surveillance, serological studies.

## Introduction

Being a highly contagious respiratory viral infection caused by various influenza viruses, with 290 to 650 thousand fatal cases recorded annually, seasonal influenza stays one of priority directions for the virological surveillance [1,2].

Amidst the influenza viruses that pose a grave potential threat to the national and global biological security, a special place is occupied by the genus *Alpha influenza virus* (influenza A virus) in the family Orthomyxoviridae, which includes 18 hemagglutinin subtypes (H1-H18) and 11 neuraminidase subtypes (N1-N11). Influenza A viruses containing three HA subtypes (H1, H2, and H3) and two NA subtypes (N1, N2) possess epidemic significance to humans. Type B influenza viruses also actively participate in the epidemic process [3].

As it is known, influenza A viruses periodically cause pandemics at intervals of 10-40 years. This takes place as a result of the emergence of a fundamentally novel influenza virus (subtype or variant), against which there is no immunity among the population. A unique triple-reassortant influenza A/H1N1pdm09 virus, which emerged in 2009, included a complex combination of swine, avian, and human influenza virus genes. The pandemic strain of influenza A/H1N1pdm09 virus has become the dominant agent in the etiology of subsequent epidemic seasons. It has displaced the seasonal influenza A/H1N1 virus from active circulation in the human population [4] and is currently continuing to circulate along with A/H3N2 viruses and two type B lines [5]. In this regard, one of the major tasks in solving biosecurity issues is to control the distribution of the pandemic influenza A/H1N1pdm09 virus, which has an enhanced virulence [6].

The Global Influenza Programme monitors influenza activity worldwide and publishes an update every two weeks, where information is categorized by influenza transmission zones, areas with similar influenza transmission patterns. The tireless goal of the influenza surveillance is to provide timely and high-quality epidemiological data and viral

isolates to check the duration of the influenza season as well as provide candidate viruses for vaccine production. The correct surveillance can help decision-makers prioritize resources and plan public health interventions [7-9]. The isolation of influenza virus strains from the clinical material is significant for diagnosing respiratory diseases in humans and assessing the correspondence between epidemic and vaccine strains within the limits of the surveillance over the spread of influenza infection and represents an essential component in the vaccine development and production [10-13].

Serological diagnosis is particularly important in the case of an atypical or asymptomatic course of influenza infection. The hemagglutinins inhibition assay (HAI) and enzyme-linked immunosorbent assay (ELISA) are still widely used in the diagnosis of influenza infection to determine specific antibodies in blood serums [14-17].

## Materials and methods

Kazakhstan is a major transit corridor for the passage of epidemic variants of the influenza virus, which reinforces its geopolitical importance in global influenza surveillance [15, 18, 19].

In the 2021 epidemic season, 590 biosamples (419 swabs and 171 blood serums) were obtained together with medical personnel from healthcare facilities located in the southern region of Kazakhstan, of which – 248 nasopharyngeal swabs and 171 blood serums from the Almaty region, and 171 swabs from the Kyzylorda region. Primary screening of nasopharyngeal swabs for the presence of the genetic material of influenza viruses was performed in RT-PCR. The characteristics of collected material and the results of molecular analysis in RT-PCR are presented in Table 1.

**Table 1** – Characterization of biosamples and RT-PCR-based primary screening of human nasopharyngeal swabs collected from the Almaty and Kyzylorda regions in 2021

| Sampling point | Number of biosamples | | Number of PCR-positive samples to | | | | | |
|---|---|---|---|---|---|---|---|---|
| | nasopharyngeal swab | blood serum | influenza virus | influenza type A virus | Subtype | | influenza A virus with unidentifi-ed subtype | influenza type B virus |
| | | | | | A/H1N1 pdm | A/H3N2 | | |
| Almaty region | 248 | 171 | 15 | 14 | 5 | 9 | 0 | 1 |
| Kyzylorda region | 171 | - | 27 | 25 | 10 | 14 | 1 | 2 |
| Total | 419 | 171 | 42 | 39 | 15 | 23 | 1 | 3 |
| Percentage: | 100% | 100% | 10.02% | 9.31% | 3.58% | 5.49% | 0.24% | 0.72% |

## Results and discussion

According to our data, the genetic material of influenza virus was detected in 42 swabs (10.02% of the total number of examined samples): influenza A virus in 39 samples (9.31%), influenza virus type B in 3 swabs (0.72%). While subtyping samples positive for influenza type A virus, it was found that the A/H1N1pdm09 virus RNA was detected in 3.58% of cases, that of A/H3N2 in 5.49%. In one sample (0.24%) positive for influenza type A virus, the subtype could not be identified. The results of RT-PCR-based primary screening of clinical samples showed that influenza type A and B viruses are circulating among the population in the southern region of Kazakhstan with a predominance of influenza type A viruses.

Three hemagglutinating agents were isolated in CE during virological examination of biological samples. As a result of cloning by the method of limiting dilutions in CE and MDCK cell culture, the hemagglutination titers of isolates on CE were in the range of 1:64 – 1:1024 while those in the MDCK cell culture varied within 1:8-1:32. Negative results were obtained with serums against the influenza B virus [15]. The results obtained in the HAI and NAI assays indicate that the Kazakhstan isolate A/Kyzylorda/1/21 belongs to influenza virus with the antigenic formula A/H1N1pdm09, whereas the isolates A/Almaty/10/21 and A/Almaty/15/21 to influenza A/H3N2 virus. The antigenic formulae of the isolates was confirmed by RT-PCR [20].

In order to detect specific antibodies against influenza viruses, we used 171 blood serums collected

from the Almaty region in the spring 2021. The HAI assay results are shown in Figure 1.

As shown on Figure 1, antihemagglutinins were detected against the A/H1N1 serosubtype virus in 2.92% of cases (five samples) of the total number of examined serums, against A/H1N1pdm in 12.28% (21 samples), against A/H3N2 in 4.68% (eight samples), against type influenza B virus in 15.20% (26 samples), and antibodies against simultaneously two influenza (A+B) viruses were found in 10.53% (18 serums). The antibody titers ranged in 1:80 – 1:320.

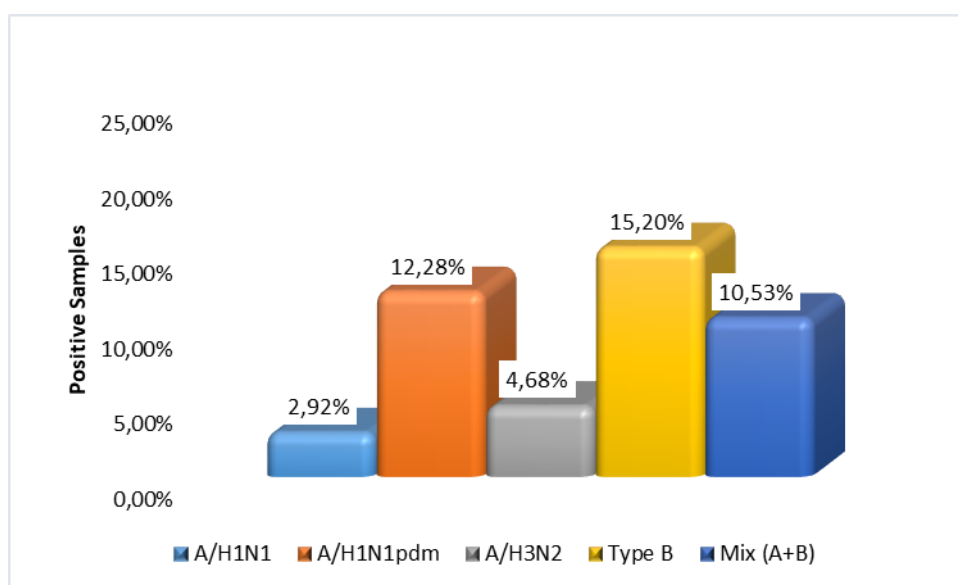Results obtained from a study of 171 blood serums in ELISA are shown in Figure 2.



**Figure 1** – Results of HAI assay on antibodies against influenza viruses in human blood serum
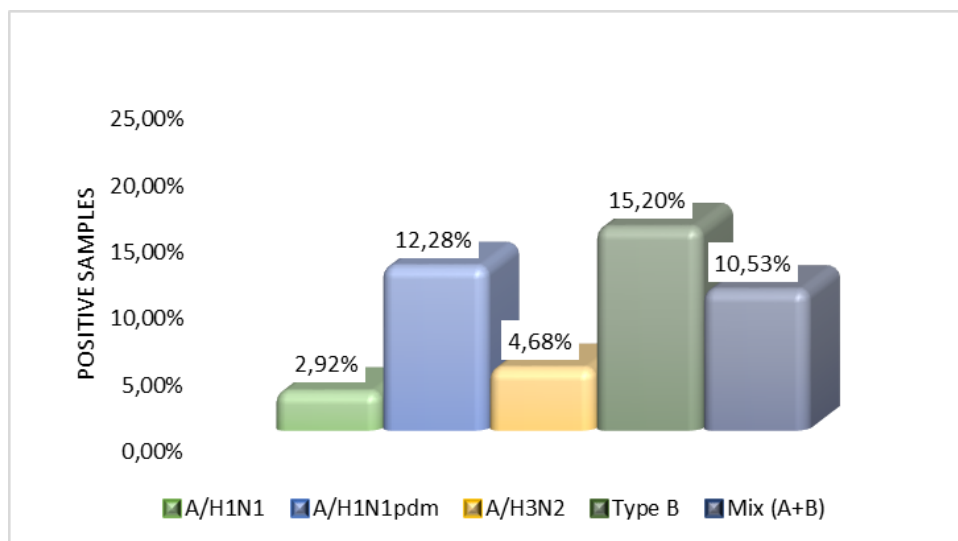


**Figure 2** – Results of ELISA on antibodies against influenza viruses in human blood serum

Isolates prepared from nasopharyngeal swabs and identified in the RT-PCR, HAI and NAI assays as influenza viruses of the A/H1N1pdm09 and A/H3N2 subtypes were characterized by high hemagglutination titers both in CE and MDCK cell culture [15, 20].

Antibodies simultaneously against two subtypes of influenza A virus were identified in 46(5.9%) of blood serums, and against influenza A and B viruses in 60(7.7%) [15].

**Additional considerations**

Another important parameter is coinfections as was shown by us for COVID-19 [22]. The timing and severity of the 2021-2022 influenza (flu) season was different than most seasons before the COVID-19 pandemic. In 702 samples (9.85%) pathogens of respiratory infections of non-influenza etiology were detected, including adenovirus, bocavirus, coronavirus, metapneumovirus, paramyxovirus types I-IV, respiratory syncytial virus, and rhinovirus. At the same time, both before and during the COVID-19 pandemic, different influenza virus variants co-circulation (A/H1N1, A/H3N2, and type B) were discovered, with a predominance of viruses with the antigenic formula A/H1N1 (Figure 3).

The results of the study indicate the need for continuous monitoring of the viral pathogens spread, which will expand the existing knowledge of the viral etiology of respiratory diseases and highlight the importance of viruses in the respiratory infections occurrence [22]. Similar results were noted for other countries [23-29]. Though relatively mild, there was more activity during the 2021-2022 flu season than during the 2020-2021 flu season, and activity remained elevated later in the spring than any flu season on record [30]. In addition to the use of everyday preventive actions, fall influenza vaccination campaigns were thought as an important component of prevention [31].
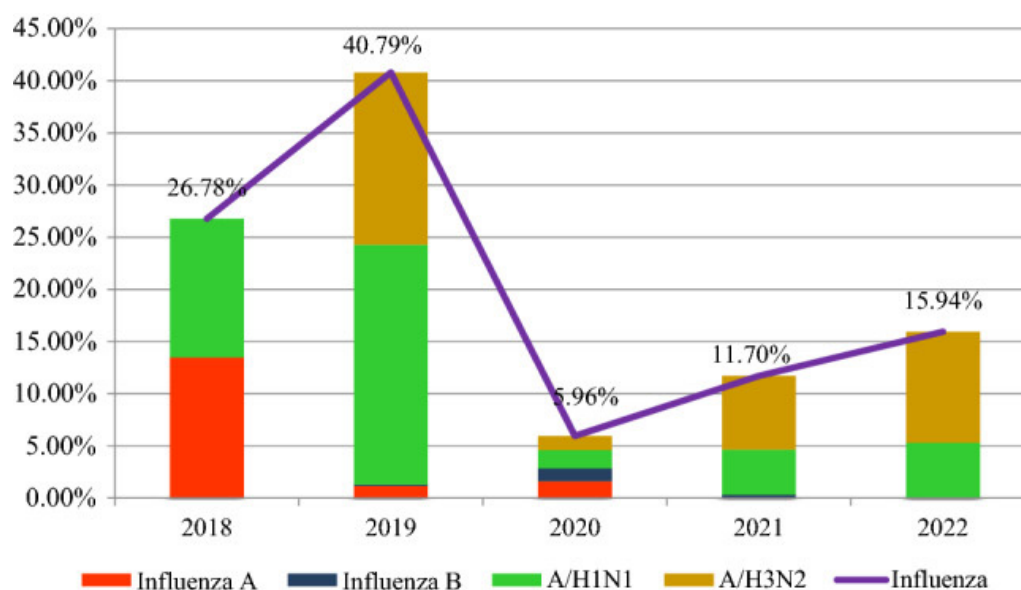


**Figure 3** – Dynamics of prevalence of different influenza types

**Conclusion**

In recent years, a rapid spread of influenza viruses of subtypes A/H1N1, A/H3N2, and type B is being observed in Kazakhstan as well as throughout the world [18, 21]. In addition, the extraordinary complexity of the epidemic situation is associated with the emergence of reassortant viruses. The epidemiology of influenza substantially depends on the antigenic variability of influenza viruses, which are able to spread throughout the world and evolve rapidly. Gradual and relatively continuous alterations in the surface glycoproteins HA and NA promotes the emergence of new antigenic variants of the influenza virus that are resistant to immunity resulting from the previous infections or vaccination. It is for this reason that seasonal epidemics reoccur every year with varying intensity and trends.

**References**

1. Krasnova E. I., Karpovich G. S., Provorova V. V., Shestakov A. E., Kazakova Yu. (2021) Influenza in COVID-19 pandemic, epidemiological characteristics, approaches to vaccination. Lechaschi Vrach., vol. 4, no 24, pp 50-56. https://doi.org/10.51793/OS.2021.98.48.009.

2. Lvov D.K., Burtseva E.I., Mukasheva E.A., Kolobukhina L.V., Bogdanova V.S., Bovin, et al. (2019) Peculiarities of the influenza viruses circulation and their properties during 2018–2019 epidemic season in Russia and countries of the Northern Hemisphere. Problems of Particularly Dangerous Infections., vol. 3, pp. 66–74. (rus.). https://doi.org/10.21055/0370-1069-2019-3-66-74.

3. Dawood F.S., Jain S., Finelli L., Shaw M.W., Lindstrom S., Garten R.J., et al. (2009) Emergence of a novel swine-origin influenza A(H1N1) virus in humans. N. Engl. J. Med., vol. 360, no 25, pp. 2605–2615. https://doi.org/10.1056/nejmoa0903810.

4. Kurskaja O. G., Susloparov I.M., Durymanov A.G., et al (2011) Izuchenie pandemicheskogo grippa A/H1N1 v Sibirskom i Dal'nevostochnom regionah (sezon 2009–2010). Vestnik NGU. (Serija: Biologija, klinicheskaja medicina)., vol 9, no 2, pp 156-161.

5. Klivleyeva N.G., Ongarbayeva N.S., Baimukhametova A.M., Saktaganov N.T., et al. (2021) Detection of influenza virus and pathogens of acute respiratory viral infections in population of Kazakhstan during 2018–2019 epidemic season. Infection and Immunity, vol. 11, no. 1, pp. 137–147. https://doi.org/10.15789/2220-7619-DOI-1348.

6. Lvov D.K., Bogdanova V.S., Kirillov I.M., Shchelkanov M.Yu., Burtseva E.I., et al. (2019) Evolution of pandemic influenza virus A(H1N1) pdm09 in 2009-2016: dynamics of receptor specificity of the first hemagglutinin subunit (HA1). Problems of Virology., vol. 64, no 2, pp. 63-72. https://doi.org/10.36233/0507-4088-2020-65-6-4.

7. World Health Organization. (2014) WHO global epidemiological surveillance standards for influenza. Geneva: World Health Organization. 84 p.

8. Global influenza strategy 2019-2030. (2019). Geneva: World Health Organization, Licence: CC BY-NC-SA 3.0 IGO. Cataloguing-in-Publication (CIP) data. CIP data available at http://apps.who.int/iris.

9. Lakdawala S.S., Jayaraman A., Halpin R.A., Lamirande E.W., Shih A.R., Stockwell T.B., et al. (2015) The soft palate is an important site of adaptation for transmissible influenza viruses. Nature., vol 526, no 7571, pp: 122–1255. https://doi.org/10.1038/nature15379.

10. Krauss S., Walker D., Webster R. G. (2012). Influenza Virus Isolation. Switzerland: Springer Nature. pp 11–24. ISSN: 1064-3745.

11. Surveillance standards for vaccine-preventable diseases, second edition. (2018) Geneva: WHO. Licence: CCBY-NC-SA3.0IGO.

12. Glebova T.I., Klivleyeva N.G., Baimukhametova A.M., Saktaganov N.T., et al. (2021) Circulation of influenza viruses in the epidemic season of 2018–2019 among people residing in Northern and Western Kazakhstan. Infectious diseases., vol 19, no 2, pp. 70–75. doi: 10.20953/1729-9225-2021-2-70-75.

13. Hoffmann E, Stech J, Guan Y.A. (2001) Universal primer set for the full-length amplification of all influenza A viruses. Arch Virol., vol 146, no 12. pp. 2275-89. https://doi.org/10.1007/s007050170002.

14. Douwdal W.A., Kendal A., Noble G.R. Influenza virus (1979) Diagnostic Procedures for Viral. Washington: Rickettsial and Chlamydial Infection, pp. 585-609.

15. Shamenova MG, Glebova TI, Klivleyeva NG, Baiseiit SB, Baimukhametova AM, Saktaganov NT, Ongarbayeva NS, Ismagulova DA. Serological studies of influenza infection among population in southern region of Kazakhstan during the 2018-2021 epidemic season. J Pak Med Assoc. 2023;73(4):804-807. doi: 10.47391/JPMA.6348.

16. Mukasheva EA, Nikolaeva LI, Makhnovsky PI, Kirillova ES, Kolobukhina LV, Merkulova LN, et al. [Diagnostic capacity of detection of specific antibodies to pandemic influenza A(H1N1)pdm09 virus]. Vopr Virusol 2017; 62: 109-14.

17. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999; 37: 937-43.

18. Klivleyeva NG, Ongarbayeva NS, Baimukhametova AM, Saktaganov NT, Lukmanova GV, Glebova TI, et al. Detection of influenza virus

and pathogens of acute respiratory viral infections in population of Kazakhstan during 2018-2019 epidemic season. Russian Journal of Infection and Immunity 2021; 11: p. 137-47. 10.15789/2220-7619-DOI-1348.

19. Glebova TI, Klivleyeva NG, Baimukhametova AM, Saktaganov NT, Lukmanova GV, Ongarbayeva NS, et al. Circulation of influenza viruses in the epidemic season of 2018–2019 among people residing in Northern and Western Kazakhstan. Infekc Bolezni 2021; 19: p. 70-75.

20. Glebova T.I., Klivleeva N.G., Ongarbaeva N.S., Bayseim S.B., et al. New strain of influenza virus H1N1A/Pig/Kostanay/06/12 used for preparation of diagnostic drugs. News of Kazakhstan Science / Novosti nauki Kazahstana . 2019, 2, p. 90-98.

21. Klivleyeva NG, Lukmanova GV, Saktaganov NT et al. (2020) Acute respiratory viral infections in Kazakhstan in 2017–2019. Bull Natl Acad Sci Republic of Kazakhstan.; 3:29–35. 10.32014/2020.2518-1467.66 (ISSN 1991–3494.

22. Klivleyeva N, Lukmanova G, Glebova T, Shamenova M, Ongarbayeva N, Saktaganov N, Baimukhametova A, Baiseiit S, Ismagulova D, Kassymova G, Rachimbayeva A, Murzagaliyeva A, Xetayeva G, Isabayeva R, Sagatova M. Spread of Pathogens Causing Respiratory Viral Diseases Before and During CoVID-19 Pandemic in Kazakhstan. Indian J Microbiol. 2023;63(1):129-138. doi: 10.1007/s12088-023-01064-x.

23. Oh DY, Buda S, Biere B, et al. (2021) Trends in respiratory virus circulation following COVID-19-targeted nonpharmaceutical interventions in Germany, January–September 2020: Analysis of national surveillance data. The Lancet Regional Health – Europe.;6:100112. 10.1016/j.lanepe.2021.100112.

24. Brestovac B, Lawrence C, Speers DJ, et al. Respiratory viral infections in Western Australians with cystic fibrosis. Respir Med. 2020;161:105854. doi: 10.1016/j.rmed.2019.105854.

25. Yum S, Hong K, Sohn S, et al. Trends in viral respiratory infections during COVID-19 Pandemic South Korea. Emerging Infectious Dis. 2021;27:1685–1688. doi: 10.3201/eid2706.210135.

26. Lagacé-Wiens P, Bullard J, Cole R, et al. (2021) Seasonality of coronaviruses and other respiratory viruses in Canada: implications for COVID-19. Canada Commun Dis Rep.;47:132–138. 10.14745/ccdr.v47i03a02

27. Biere B, Oh DY, Wolff T, et al. (2021) Surveillance of endemic human Coronaviruses in Germany, 2019/2020. The Lancet Regional Health – Europe.;11:100262. 10.1016/j.lanepe.2021.100262

28. Masse S, Capai L, Villechenaud N, et al. Epidemiology and clinical symptoms related to seasonal coronavirus identified in patients with acute respiratory infections consulting in primary care over six influenza seasons (2014–2020) in France. Viruses. 2020;12:630. doi: 10.3390/v12060630.

29. Park S, Lee Y, Michelow IC, et al. Global seasonality of human coronaviruses: a systematic review. Open Forum Infect Dis. 2020;7:ofaa44343. doi: 10.1093/ofid/ofaa443.

30. Bellizzi S, Panu Napodano CM, Pinto S, Pichierri G. COVID-19 and seasonal influenza: The potential 2021-22 "Twindemic". Vaccine. 2022;40(24):3286-3287. doi: 10.1016/j.vaccine.2022.

31. Olsen SJ, Winn AK, Budd AP, Prill MM, Steel J, Midgley CM, et al. Changes in Influenza and Other Respiratory Virus Activity During the COVID-19 Pandemic – United States, 2020-2021. MMWR Morb Mortal Wkly Rep. 2021;70(29):1013-1019. doi: 10.15585/mmwr.mm7029a1.