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ORIGINAL ARTICLE

Flu seasons during the period 2018-2023 in Sarajevo Canton, Bosnia and Herzegovina: possible impact of the SARS-CoV-2 pandemic on the influenza A and B virus circulation

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ABSTRACT

Aim During the pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many countries reported a significant decrease in the prevalence of influenza virus cases. The study aimed to characterize the flu seasons from 2018 to 2023 in Sarajevo Canton, Bosnia and Herzegovina (B&H), and to assess the possible impact of the SARS-CoV-2 pandemic on the influenza A and B virus circulation.
Methods The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panels were used for the detection of influenza virus A and B, and subtyping of influenza virus A (H1pdm09 virus and H3). The data for this registry-based retrospective study were collected at the Clinical Centre of the University of Sarajevo, Unit for Clinical Microbiology (the laboratory that acts as a referral for the detection and characterization of influenza virus and SARS-CoV-2 in Federation B&H).
Results In the 2018/2019 and 2019/2020, an equal percentage of positive cases was recorded (148/410; 36%, and 182/504; 36%, respectively). The absence of the influenza virus was observed in 2020/2021. During 2021/2022, influenza virus was detected among 19/104 (18%) patients and slightly increased in 2022/2023 (45/269; 17%). The switch of the influenza B virus lineage was observed.
Conclusion The SARS-CoV-2 virus had an impact on the prevalence of influenza virus infection among the population of the Sarajevo Canton, B&H. Since the interactions between these two viruses are not entirely clear, awareness of a possible threat to public health is crucial.

Keywords: molecular epidemiology, Real-Time RT-PCR, subtyping, virus

INTRODUCTION

Since 2020 the global distribution of the influenza virus has changed during the SARS-CoV-2 (severe acute respiratory syndrome 2) pandemic (1). It was observed that the number of cases infected with influenza A and B viruses, the most common causes of seasonal flu, drastically decreased during the pandemic (2). Furthermore, unexpected disturbances in the annual distribution of influenza virus have been observed in October-May. This period is typical for seasonal flu in Europe and other parts of the Northern hemisphere (3,4).

It has been known that the symptoms of the early stage of the flu can be misdiagnosed with SARS-CoV-2 infection (5). Therefore, during the COVID-19 (coronavirus disease 2019) pandemic, combined screening for influenza viruses and SARS-CoV-2 was performed to rule out possible SARS-CoV-2 infection and prevent its further spread (5–7). While the influenza virus causes symptoms such as headache, elevated body temperature,

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muscle weakness, cough, and sneezing, the SARS-CoV-2 virus, in addition to these symptoms, can cause loss of the sense of smell and taste and more severe lung disease (pneumonia), even in early stages of the infection (8–12).

Given that both viruses appear in the same period of the year, the possibility of co-infection is high. Some new research points to the interaction of these two viruses are still challenged. *In vitro* studies showed that the influenza A virus can inhibit the replication of SARS-CoV-2 if the cell culture is infected three days before SARS-CoV-2 infection or simultaneously infected with the SARS-CoV-2 virus. However, earlier infection with the SARS-CoV-2 did not inhibit the replication of the influenza A virus (13).

On the other hand, the co-infection of these two viruses (influenza A and SARS-CoV-2) can increase the severity of clinical manifestations of infected persons. This observation was explained by a drop in the production of neutralizing antibodies against both viruses and a decline in the number of CD4+ helper T cells (14). It was also shown that vaccination against influenza A and B viruses reduced the risk of infection with SARS-CoV-2 and hospitalization due to COVID-19 (15).

The immunologic changes in the human body that may occur due to the co-infection of these two viruses (described as fluorene) have also been insufficiently investigated.

According to unpublished data (Clinical Centre of the University of Sarajevo, Unit for Clinical Microbiology) from Sarajevo Canton (SC), Bosnia and Herzegovina (B&H), during the seasons 2016/2017 and 2017/2018, 49.2% and 48.0%, respectively, of all tested flu cases were laboratory-confirmed. Influenza A/H3 was dominant during the 2016/2017 season, while influenza B virus prevailed during the 2017/2018 season.

The aim of this study was to characterize the flu seasons from 2018 to 2023 in SC (B&H) and to assess a possible impact of the SARS-CoV-2 pandemic on the influenza A and B virus circulation.

PATIENTS AND METHODS

Patients and study design

In this registry-based retrospective study, epidemiological and virological characteristics of laboratory-confirmed influenza cases during the 2018/2019, 2019/2020, 2020/2021, 2021/2022, and 2022/2023 flu seasons in SC of the Federation of B&H (FB&H) were analysed.

The patients were categorized in age groups: 0-4, 5-9, 70-79, and ≥80 years.

Patient specimens were collected at the Clinical Centre of the University of Sarajevo, Clinical Microbiology Unit, Laboratory for Molecular Diagnostic of Viral Diseases, Sarajevo, B&H. Respiratory specimens were primarily tested for diagnostic purposes (non-sentinel part of influenza surveillance) and for influenza sentinel surveillance, including SARI (severe acute respiratory illness) and ILI (influenza-like illness) cases. The case definition followed the WHO (World Health Organization) recommendation (16).

Collection and processing of nasopharyngeal swabs in a transport medium (BD Universal Viral Transport Collection Kits, Fisher Scientific, USA) of patients was performed according to the World Health Organization (WHO) recommendations (17). Viral RNA extraction was performed with 200 µl of the sample. Sample excess was stored at the temperature of -80°C.

Planning, conducting, and reporting of the data for this registry-based retrospective study were in line with the Declaration of Helsinki, as revised in 2013. Ethical approval for this study was obtained from the Clinical Centre of the University of Sarajevo (KCUS 06-04-9-46201). Data analysis was performed on an anonymized patients' identity which was protected by unique coding.

Methods

Extraction of viral RNA from respiratory samples and positive controls was performed using the commercial kit QIAamp Viral RNA Mini Kit (Qiagen GmbH, Germany), following the manufacturer's instructions.

Positive controls for the influenza virus were obtained through IRR (Influenza Reagent Resource, Influenza Division, WHO Collaborating Centre for Surveillance, Epidemiology and Control of Influenza). The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel consists of nucleic acid amplification assay for detecting influenza A and B viruses and nucleic acid amplification assay for further characterization of influenza A subtypes A/pdmH1, A/H3, and influenza B virus. Individual sets are designed to identify a part of the genome of all influenza A or influenza B viruses (M gene fragment from highly conserved regions of the M matrix gene) or detect genome regions specific for individual influenza A subtypes (HA gene fragments) or influenza B strains, respectively. All samples were initially screened for the presence of influenza virus type A and B. Samples tested positive for the M gene of influenza virus type A were further subtyped for the presence of H3 and pdmH1 specific genes.

To perform rtRT-PCR tests, the kit Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (without Rox) (Thermo Fisher SCIENTIFIC, USA) was used. A single 25 µl one-step rtRT-PCR mix consisted of 12.5 µl of 2x PCR Master Mix, 1.5 µl of virus-specific primers/probe sets (influenza A virus, influenza B virus, influenza A/H3 virus or influenza A/pdmH1 virus, respectively), 0.5 µl SuperScript™ III

RT/Platinum® Taq Mix, 5.5 µl of nuclease-free water, 5 µl of sample RNA. All probes were FAM labelled determining each reaction to be performed separately. The negative control (nuclease-free water, NC), positive controls (PCs) for each type/subtype, and human specimen control (HSC) were included in each run. The one-step rtRT-PCR thermal protocol was as follows: reverse transcription at 50°C/30 minutes; inactivation of reverse transcriptase and activation of Taq polymerase at 95°C/10 minutes and the two-step cycling consisting of the denaturation at 95°C/15 seconds and annealing at 55°C/30 seconds (45 cycles).

The results of the RT-PCR test were automatically processed on the Real-time PCR ABI 7500 (Applied Biosystems, USA) or CFX96 (Bio-Rad, USA) software, and all samples with a Ct value <37 are considered positive.

Statistical analysis

Categorical data were presented as percentages. Continuous data were expressed as mean of numbers. For several seasonal comparisons Pearson $\chi 2$ test was used. The statistical significance of the test was set at $p<0.05$ (Bonferroni corrected at p=0.0083, p value Bonferroni corrected to 0.0083, instead of 0.05 due to multiple pairwise comparisons).

RESULTS

The results of collected data for the annual season's characteristic for the spread of influenza viruses, for the period from 2018 to 2023 in Canton Sarajevo, showed deviations from the typical and expected distribution (Table 1 a-d). In the seasons 2018/2019 and 2019/2020, an equal percentage of positive cases was recorded (148/410; 36%, and 182/504; 36%, respectively), while the largest number of cases was identified in January (55/98; 56%) during the season 2018/2019, and in February (96/178; 54%) in the season 2019/2020.

The 2019/2020 season ended early in March due to the declaration of the COVID-19 pandemic (Table 1b), although the reporting of influenza season was obligatory until May (20th epidemiologic week). In the period between October 2020 and May 2021 (expected influenza season), 104 patients with characteristic and overlapped symptoms between seasonal flu and COVID-19 were tested. The influenza virus infection was not laboratory-confirmed in any of the tested patients (Table 1c).

In the season 2021/2022, the influenza virus was detected among 19/104 (18%) symptomatic patients (Table 1d) with the peak observed in April 2022 (6/15; 40%).

The number of influenza cases increased after the formal end of the pandemic in comparison to the previous season. However, the percentage of positive cases was comparable (45/269; 17%) to the season 2022/2023 (Table 1d, 1e), with the largest number of positives recorded in March 2023 (17/48; 35%). Significant disparities between seasons 2018/2019 and 2021/2022 (p<.001), seasons 2019/2020 and 2021/2022 (p<.001), seasons 2018/2019 and 2022/2023 (p<.001), and seasons 2019/2020 and 2022/2023 (p<.001) were found. Conversely, no significant difference was detected between seasons 2018/2019 and 2019/2020 (p=.947). After adjusting for multiple comparisons using the Bonferroni correction (adjusted p=0.0083), the initially observed difference between Seasons 2021/2022 and 2022/2023 (p=.033) was no longer considered statistically significant (Table 2).

During the season 2018/2019 influenza A/H3 was present in 79/148 (53%) cases, followed by influenza A/pdmH1 in 69/148 (47%), while influenza B virus was absent during the whole season (Table 1a).

Unlike the previous season, in the 2019/2020 season, the influenza A/pdmH1 virus was dominantly circulating (139/182; 76%), followed by influenza A/H3 (29/182; 16%), and influenza B/Yamagata virus (15/182; 8%) (Table 1b). During the season 2020/2021, there were zero influenza-positive cases among all 104 tested persons (Table 1c). The second influenza season during the COVID-19 pandemic (2021/2022) was characterized by the circulation of a single subtype (influenza A/H3) in 19/19 (100%) of cases (Table 1d). The influenza B virus was not detected in a single tested patient for the period of the 2021-2022 influenza seasons.

Table 1. Seasonal distribution of flu cases in Sarajevo Canton during 2018-2023

a) Monthly distribution of influenza virus during the season 2018/2019

Month/Year	No of patients tested	No (%) of positive patients	Average age of influenza positive patients (years)	Gender distribution No (%) of influenza positive		No (%) of types/subtypes of influenza virus		
				Males	Females	Inf *A/H3*	*A/pdmH1*	*B*
October/2018	14	0	0	0	0	0	0	0
November/2018	18	2 (11)	62	1 (50)	1 (50)	2 (100)	0	0
December/2018	37	7 (19)	40	3 (43)	4 (57)	7 (100)	0	0
January/2019	98	55 (56)	41	31 (56)	24 (44)	30 (54)	25 (46)	0
February/2019	158	61 (39)	49	37 (60)	24 (40)	30 (49)	21 (51)	0
March/2019	65	21 (32)	57	6 (29)	15 (71)	8 (38)	13 (62)	0
April/2019	18	2 (11)	65	1 (50)	1 (50)	2 (100)	0	0
May/2019	2	0	-	0	0	0	0	0
Total	**410**	**148 (36)**	**52**	**79 (53)**	**69 (47)**	**79 (53)**	**69 (47)**	0

b) Monthly distribution of influenza virus during the season 2019/2020

Month/Year	No of patients tested	No (%) of positive patients	Average age of influenza positive patients (years)	Gender distribution No (%) of influenza positive patients		No (%) of types/subtypes of influenza virus		
				Males	Females	Inf *A/H3*	*A/pdmH1*	*B/ Yamagata*
October/2019	12	1 (8)	37	1 (100)	0	1 (100)	0	0
November/2019	26	1 (4)	33	0	1 (100)	1 (100)	0	0
December/2019	51	9 (18)	36	7 (78)	2 (22)	9 (100)	0	0
January/2020	118	53 (45)	46	31 (58)	22 (42)	1 (2)	52 (98)	0
February/2020	178	96 (54)	46	49 (51)	47 (49)	16 (17)	70 (73)	10 (10)
March/2020	119	22 (18)	43	11 (50)	11 (50)	10 (45)	7 (32)	5 (23)
April/2020	0	0	0	0	0	0	0	0
May/2020	0	0	0	0	0	0	0	0
Total	**504**	**182 (36)**	**40**	**100 (55)**	**83 (45)**	**29 (16)**	**139 (76)**	15 (8)

c) Monthly distribution of influenza virus during the season 2020/2021

Month/Year	No of patients tested	No (%) of positive patients	Average age of influenza positive patients (years)	Gender distribution No (%) of influenza positive patients		No (%) of types/subtypes of influenza virus		
				Males	Females	Inf *A/H3*	*A/pdmH1*	*B*
October/2020	28	0	0	0	0	0	0	0
November/2020	8							
December/2020	17							
January/2021	18							
February/2021	15							
March/2021	11							
April/2021	4							
May/2021	3							
Total	**104**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

d) Monthly distribution of influenza virus during the season 2021/2022

Month/Year	No of patients tested	No (%) of positive patients	Average age of influenza positive patients (years)	Gender distribution No (%) of influenza positive patients		No (%) of types/subtypes of influenza virus		
				Males	Females	Inf *A/H3*	*A/pdmH1*	*B*
October/2021	13	0	0	0	0	0	0	0
November/2021	51	2 (4)	44	1 (50)	1 (50)	2 (100)	0	0
December/2021	39	3 (8)	9	1 (23)	2 (67)	3 (100)	0	0
January/2022	40	5 (3)	30	1 (20)	4 (80)	5 (100)	0	0
February/2022	24	1 (4)	6	0	1 (100)	1 (100)	0	0
March/2022	12	2 (17)	12	2 (100)	0	2 (100)	0	0
April/2022	15	6 (40)	34	6 (100)	0	6 (100)	0	0
May/2022	0	0	0	0	0	0	0	0
Total	**104**	**19 (18)**	**23***	**11 (58)**	**8 (42)**	**19 (100)**	**0**	**0**

e) Monthly distribution of influenza virus during the season 2022/2023

Month/Year	No of patients tested	No (%) of positive patients	Average age of influenza positive patients (years)	Gender distribution No (%) of influenza positive patients		No (%) of types/subtypes of influenza virus		
				Males	Females	Inf *A/H3*	*A/pdmH1*	*B/Victoria*
October/2022	22	0	0	0	0	0	0	0
November/2022	30	1 (3)	4	0	1 (100)	0	0	1 (100)
December/2022	56	2 (4)	8	1 (50)	1 (50)	0	0	2 (100)
January/2023	41	8 (20)	8	6 (75)	2 (25)	4 (50)	2 (25)	2 (250)
February/2023	48	13 (27)	16	6 (46)	7 (540)	4 (31)	8 (61)	1 (8)
March/2023	48	17 (35)	21	7 (41)	10 (49)	0	4 (24)	13 (76)
April/2023	21	4 (19)	10	1 (25)	3 (75)	0	0	4 (100)
May/2023	3	0	0	0	0	0	0	0
Total	**269**	**45 (17)**	**11***	**21 (47)**	**24 (53)**	**8 (18)**	**14 (31)**	**23 (51)**

In the season 2022-2023, among 45/269 (17%) influenza cases, influenza B/Victoria virus was dominant (23/45; 51%), followed by influenza A/pdmH1 (14/45; 31%), and influenza A/H3 (8/45; 18%) (Table 1e).

The highest average age of cases was observed in the season 2018/2019 (52 years), while the lowest average age was in the 2022/2023 season (11 years) (Table 1). A higher number of male infected patients was noticed in each season: 2018/2019 (79/148; 53%), 2019/2020 (100/182; 55%); 2021/2022 (11/19, 58%) except the last one, 2022/2023, when the female population dominated (24/45; 53%) (Table 1). The investigation into gender differences in test outcomes did not manifest a statistically significant association (p=0.346). Furthermore, the relationship between age groups and test outcomes yielded significant results (p=0.006). The season 2020/2021 was not considered in the context of age and gender due to the lack of influenza cases (Table 2).

Table 2. Comparison across seasons, gender and age groups

Comparison	p*	Significance
2018/2019 vs. 2021/2022	<0.001	Highly Significant
2019/2020 vs. 2021/2022	<0.001	Highly Significant
2018/2019 vs. 2019/2020	0.947	Not Significant
2021/2022 vs. 2022/2023	0.033	Not Significant
2018/2019 vs. 2022/2023	<0.001	Highly Significant
2019/2020 vs. 2022/2023	<0.001	Highly Significant
Variable	**p***	**Significance**
Gender	0.346	Not Significant
Age groups	0.006	Significant

*Bonferroni corrected at p=0.0083
Note: Season 2020/2021 was excluded from the analysis as all cases during this period were negative

DISCUSSION

The focus of our study was to characterize the flu seasons from 2018 to 2023 in SC (B&H) and to assess the possible impact of the SARS-CoV-2 pandemic on the influenza A and B virus circulation. According to our data, the years of the COVID-19 pandemic brought changes in the circulation of the influenza virus, resulting in a reduced number of cases in comparison to the seasons before the pandemic (2018-2020). The lowest percentage of positivity was recorded in the season 2022/23. Furthermore, we showed the complete absence of influenza viruses in the season 2020/2021, which was similar to the findings of a study from Great Britain (18). Unusually, on a global level, the circulation of influenza virus was particularly low in 2020 in both the Northern and Southern Hemispheres (19). The COVID-19 pandemic and the measures put in place in several countries during the winter season may have contributed to the overall low and delayed initiation of influenza virus circulation throughout the season 2021/2022, resulting in late activity when precautions were relaxed (20).

In our study, the majority of laboratory-confirmed influenza cases were detected from January to April, and peaked in earlier months in the seasons before the pandemic. Comparing the obtained data with the Annual Epidemiological Report for 2023 and the EU/EEA countries (European Union/European Economic Area), published by the European Centre for Disease Prevention and Control (ECDC), there was a notable resurgence of influenza virus activity during the 2022/2023 influenza season, approaching pre-pandemic levels. Compared to four preceding seasons, this season was distinguished by an earlier peak in positive cases (December) (21), however, in our area, the peak was observed later (in April).

The frequency of influenza virus types/subtypes in the years before the COVID-19 pandemic indicated the domination of influenza A/H3 in the season 2018/2019, and influenza A/pdmH1 during the season 2019/2020. Furthermore, influenza virus was not detected during the COVID-19 season 2020/2021, while in the next season, 2021/2022, influenza A/H3 was the most prevalent in our area. According to the ECDC Annual Report, during this season the spread of influenza type A, primarily A (H3N2), viruses was dominantly circulated as it was shown by our results (20). Interestingly, the switch of the influenza B virus lineage was observed from the season 2019/2020, when only the Yamagata lineage was identified, to the influenza B/Victoria lineage solely identified during the season 2022/2023. These data were consistent with the results of other authors who presented the global disappearance of influenza B/Yamagata lineage during the COVID-19 pandemic (22) possibly associated with age-specific patterns of infected persons. The authors showed that persons below 25 years of age are more frequently infected with influenza B/Victoria lineage (22), which is in concordance with our results considering the age of the influenza cases.

Influenza A and influenza B are known to disproportionately affect adults and older adults, who are also more susceptible to COVID-19, whereas children are more susceptible to the influenza B virus (23). Regarding these data, the average age of tested patients in our study ranged between 11 years (2022/2023 season) and 52 years in the season 2018/2019. Except for 2022/2023 season, the majority of patients belonged to adult group, and infection with influenza A was evident. However, the dominant circulation of the influenza B virus was associated with a younger population (season 2022/2023, 11 years on average).

Morbidity and mortality due to infection with influenza A is much higher in females than in males (24). Nevertheless, studies that included gender and age as covariates showed that prevalence and severity varied with

age, with more boys under the age of 15 being affected as opposed to females who were more prevalent at older ages (25). Considering our results, the male population was more frequently infected (2018-2022) whereas in the last season (2022/2023), the female population dominated.

Coinfection with two or more viruses is not uncommon, and competition between viruses has been observed (26). In the last decade, the easier availability of molecular, multiplex diagnostic tests has enabled the rapid diagnosis of a group of respiratory viruses (27).

Since many countries introduced quarantines and isolation related to traveling from the risk areas and tracking of potential contacts took place, implementation of preventive measures such as social distancing, wearing masks, and school and workplace closures, could have a possible impact on the spread of respiratory pathogens in general. Moreover, vaccination against flu is likely to be effective in reducing the incidence of influenza virus infection (28).

In conclusion, the results obtained from this study indicate the necessity of continuous monitoring of the spread of the influenza virus in parallel with SARS-CoV-2 because deviations from the expected circulation of the influenza virus were observed during the period of the COVID-19 pandemic. During the pandemic, the spread of the influenza virus was reduced, probably under the influence of restrictive measures against COVID-19. Viral circulation has been reinitiated by mitigation of measures against SARS-CoV-2.

The virus began to return after the mitigation of measures against SARS-CoV-2. Given that the interactions between these two viruses are not entirely clear, more precise information and raising awareness of a possible threat to public health is crucial.

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TRANSPARENCY DECLARATION

Conflict of interests: None to declare.

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