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A SINGLE — CENTER STUDY ON THE INCIDENCE OF PCR–DETECTED INFLUENZA A/B RNA IN PATIENTS WITH SEVERE ACUTE RESPIRATORY DISEASE (2015–2019)

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Influenza virus infection remains the leading cause of morbidity and mortality in the autumn-winter period. Along with insights into circulating virus subtypes and seasonal trends, reliable data on the prevalence of influenza among patients with acute respiratory illnesses is essential for guiding the selection of strains for annual vaccines and for optimizing the planning of immunization programs.

Objective. To perform a molecular diagnostic analysis of influenza in patients with severe acute respiratory illness between 2015 and 2019.

Methods. A total of 505 patients presenting with symptoms of severe acute respiratory illness and hospitalized at St. Michael's Clinical Hospital in Kyiv were included in the study. Influenza A and B virus RNA was detected using real-time PCR.

Results. During the observation period, 49% of patients tested positive for influenza. The highest positivity rate (86%) was recorded in the 2017–2018 season, with influenza B virus being the predominant strain. In contrast, influenza B virus RNA was not detected during the 2015–2016, 2016–2017, and 2018–2019 seasons. The incidence of influenza A virus during those periods was 28.1%, 50.7%, and 25.4%, respectively. No co-infections were detected.

Conclusions. Influenza A virus circulated consistently among patients hospitalized with severe respiratory illness throughout all four seasons from 2015 to 2019. The sharp increase in influenza positivity observed during the 2017–2018 season is attributed to an outbreak of influenza B.

Key words: autumn-winter epidemic period, acute respiratory diseases, influenza A, B viruses.

Influenza remains a significant global public health concern. Each year, approximately 1 billion cases of seasonal flu are reported worldwide, including 3 to 5 million cases of severe illness [1]. In Europe alone, influenza is estimated to cause around 40,000 deaths annually [2]. In Ukraine, 6 to 7 million cases are registered annually, accounting for about 75–90% of all infectious diseases in the country. The data on the annual case numbers of infectious diseases in Ukraine for the 2015–2019 period were extracted from the collection "Statistical Yearbook form of Ukraine of the State Statistics Service of Ukraine." Some of the data used are not fully available in the public domain, but data on mortality and morbidity in Ukraine for specific periods are available in WHO electronic databases [3].

Influenza is an acute respiratory illness that continues to cause seasonal epidemics and occasional pandemics, resulting in significant morbidity and mortality globally [4]. In

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temperate regions, influenza follows a distinct seasonal pattern, with large and intense outbreaks typically occurring once a year during the colder months. These are usually followed by periods of minimal activity during the warmer months when the virus becomes nearly undetectable [5]. Influenza remains one of the leading causes of winter-related morbidity and mortality [6], with activity typically beginning as early as December and lasting through April, peaking in January or February [7].

Several factors influence the incidence and spread of influenza, including socio-demographic and ethno-demographic characteristics of the population; climatic conditions such as humidity, temperature, and solar radiation; antigenic drift of the virus over time; human travel behavior and mobility patterns; spatial and temporal transmission dynamics, including recent trends in influenza wave patterns [8].

Influenza is caused primarily by influenza A and B viruses, although types C and D also exist. Influenza A and B viruses are responsible for annual seasonal epidemics and, in some cases, global pandemics. Zoonotic strains of influenza A virus can contribute to these outbreaks [9]. Influenza A and B viruses present with similar clinical and biological characteristics, including comparable levels of severity [10].

Seasonal epidemics are typically driven by influenza A or B viruses. Type A viruses have a broad host range, infecting humans, birds, pigs, horses, and other animals. Type B and C viruses primarily infect humans, although type C has occasionally been isolated in pigs and dogs [11]. Pandemics usually arise from genomic reassortment between human and animal strains, heightening the risk of widespread outbreaks during seasons with high viral activity [12].

Clinically, human influenza typically presents with a sudden onset of fever above 38.5 °C within 1–3 days of infection. Other common symptoms include headache, myalgia, fatigue, general weakness, and a dry cough. Viral shedding may begin less than 24 hours before symptom onset and usually continues for 3–5 days. Young children may shed the virus earlier and for longer durations than adults. Severe complications can include primary influenza pneumonia, encephalitis, myocarditis, and even sudden death within a few hours of symptom onset [13, 14].

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Over the past few decades, more virulent strains of influenza have emerged, affecting both humans and animals. Clinicians should be aware that while rapid diagnostic tests for influenza are convenient, they have limited sensitivity and may yield false negatives. The current gold standard for diagnosis is the PCR test. Although viral culture from nasopharyngeal samples is also a gold standard, it takes several days to produce results. Vaccination remains the most effective strategy to reduce the incidence and impact of influenza infections [16].

Nucleic acid-based tests for respiratory viruses have been in use for over two decades, with multiplex PCR technologies becoming increasingly available in the last five years. Several commercial multiplex PCR assays are now in clinical use, offering simultaneous detection of multiple pathogens [17].

This study aimed to perform a molecular diagnostic analysis of influenza in patients with severe acute respiratory illness hospitalized between 2015 and 2019.

Materials and Methods

The study was conducted during the epidemiological autumn-winter seasons, from November to March, over the period from 2015 to 2019. Nasopharyngeal swabs were collected from patients in the early stages of illness and transported to the laboratory at Oleksandrivska Clinical Hospital in Kyiv (now St. Michael's Clinical Hospital) the day after their admission to the infectious diseases department with severe symptoms of acute respiratory illness.

Molecular testing was carried out using the *PhoenixDx® Influenza A/B Virus* test system, following the manufacturer's protocols. This system enables the qualitative detection of influenza A and B virus RNA through real-time PCR. It targets one particular sequence of the influenza A virus genome and one of the influenza B virus genome. Detection of influenza A virus RNA is indicated by a FAM

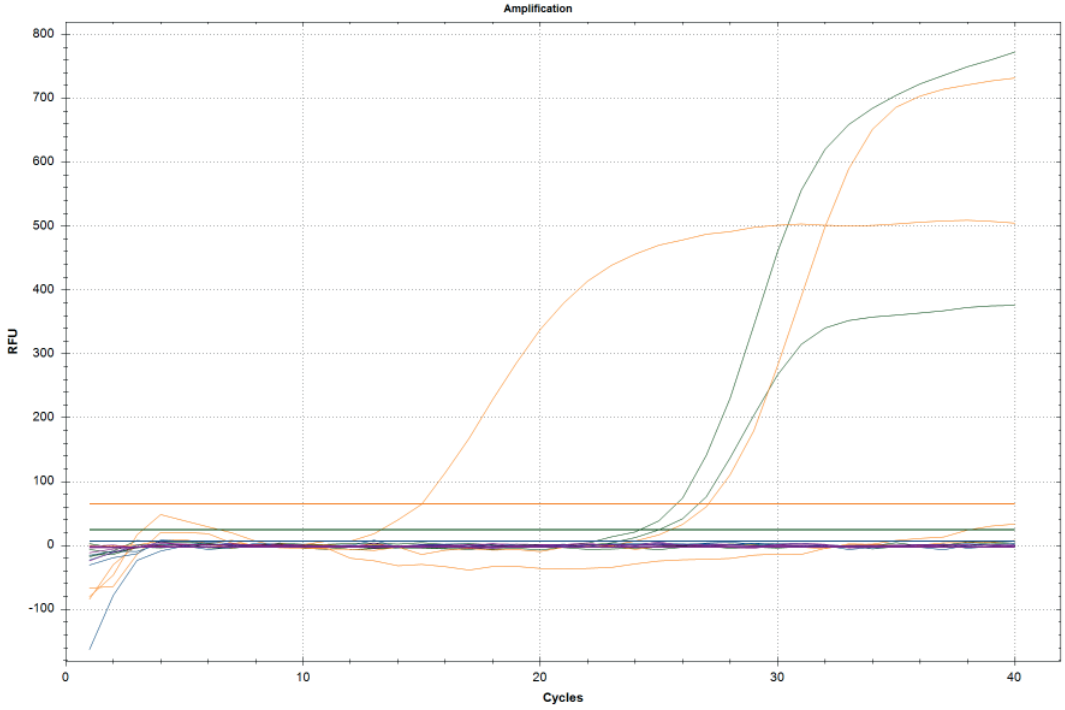
fluorescence signal at 517 nm (Fig. 1), while the influenza B virus sequence is detected via the HEX channel at 554 nm (Fig. 2). Additionally, the kit includes an internal control to detect the RNase P gene—present in human cells—, which is measured using the Cy5 signal at 670 nm.

Prior to PCR, nucleic acid extraction was performed on 180 µL of a sample using the *NucleoMag® Dx Pathogen* system (MACHEREY-NAGEL, Germany), with a final elution volume of 50 µL. Real-time PCR amplification was conducted using the *CFX96™ Real-Time PCR Detection System* (Bio-Rad, California, USA), according to the manufacturer's guidelines.

All laboratory procedures were performed in compliance with general ethical standards, ensuring the protection of life, health, dignity, and the confidentiality of patients' personal information. Appropriate protective measures were observed throughout the testing process.

Results and Discussion

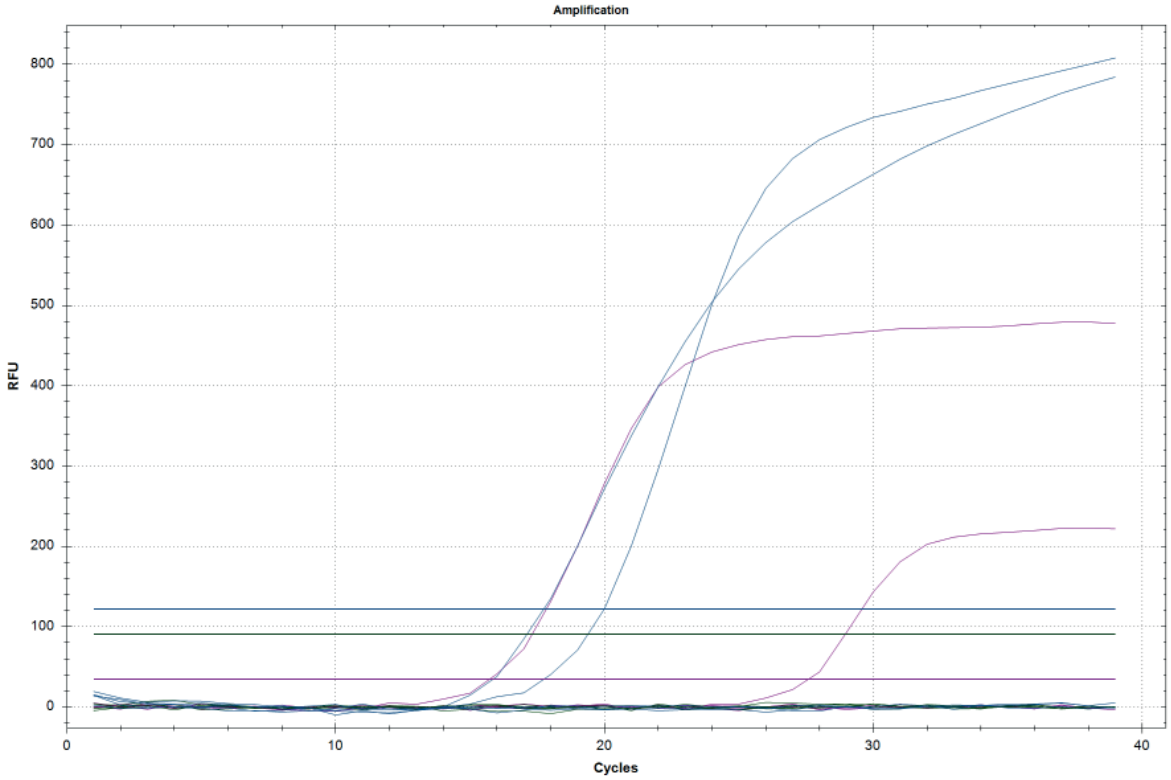
During the off-season period from 2015 to 2019, the incidence of influenza virus infection remained notably high. Throughout the study period, our laboratory received a total of 505 samples from patients with severe acute respiratory symptoms. These patients



Quantification Data

Well	Fluor	Target	Content	Sample	Cg	Cg Mean	Cg Std.Dev
A04	Cy5		Unkn	PC	26.92	26.92	0.00
B04	Cy5		Unkn	NC	0.00	0.00	0.00
C08	Cy5		Unkn	Flu A	27.29	27.29	0.00
A04	FAM		Unkn	PC	27.69	27.69	0.00
B04	FAM		Unkn	NC	0.00	0.00	0.00
C08	FAM		Unkn	Flu A	17.17	17.17	0.00

Fig. 1. **Representative amplification curves of influenza A virus RNA**



Quantification Data

Well	Fluor	Target	Content	Sample	Cg	Cg Mean	Cg Std.Dev
A04	Cy5		Unkn	PC	21.83	21.83	0.00
B04	Cy5		Unkn	NC	0.00	0.00	0.00
C08	Cy5		Unkn	Flu B	22.21	22.21	0.00
A04	HEX		Unkn	PC	23.19	23.19	0.00
B04	HEX		Unkn	NC	0.00	0.00	0.00
C08	HEX		Unkn	Flu B	27.13	27.13	0.00

Fig. 2. **Representative amplification curves of influenza B virus RNA**

had been hospitalized in the infectious disease department from various districts of Kyiv, all presenting with severe infections.

The analysis of the study data revealed that 244 (48.8%) of the 505 samples tested positive for either influenza A virus (29.1%) or influenza B virus (19.7%). Notably, no samples exhibited co-infection with both influenza A and B viruses (Fig. 3).

During the epidemiological autumn-winter period of 2015–2016, 128 patients were examined, of whom 28.1% (n = 36) tested positive for influenza A virus RNA. No influenza B virus RNA was detected in the samples (Fig. 4).

In the 2016–2017 season, the incidence of influenza A increased, with 136 patients examined. Of these, 50.7% (n = 69) tested positive for influenza A virus RNA, while no influenza B virus RNA was detected during this period.

The most challenging epidemiological situation occurred in the 2017–2018 season. Among 127 patients examined, 86% (n = 110) were diagnosed with influenza. However, the prevalence of influenza B virus was notably higher, with influenza B viral RNA detected in 76.3% (n = 97) of patients. In contrast, 10.2% (n = 13) of patients were found to have influenza A virus RNA. Our findings

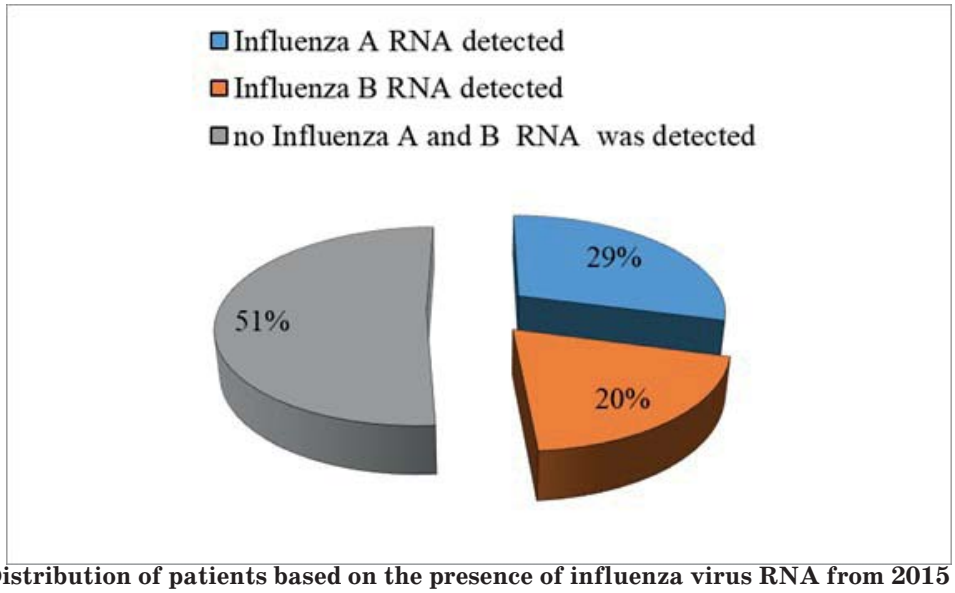


Fig. 3. **Distribution of patients based on the presence of influenza virus RNA from 2015 to 2019**

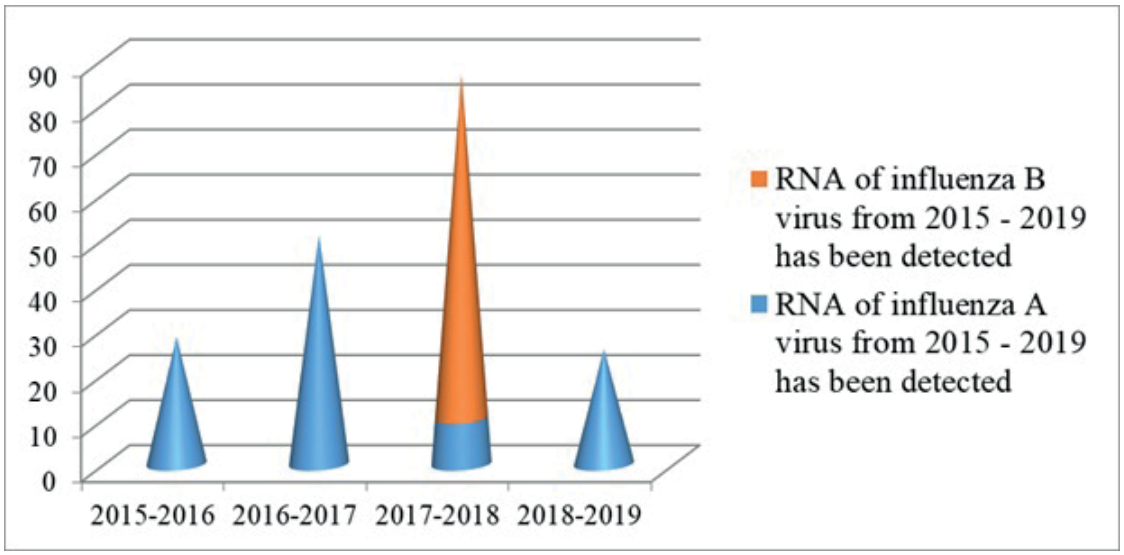


Fig. 4. **Dynamics of Influenza A and B Virus Spread Among Patients with Severe Acute Respiratory Diseases from 2015 to 2019**

differ from the data presented in the Annual Epidemiological Report on seasonal influenza for 2017–2018, as provided by the European Centre for Disease Prevention and Control. The report highlights that the majority of influenza viruses detected during this period were of type A, whereas our study found a higher prevalence of influenza B virus. Nevertheless, our data align with the findings from the European Influenza Surveillance Network, which reported a high prevalence of influenza B/Yamagata virus during the 2017–2018 season [18].

In the 2018–2019 season, there was a decrease in the incidence rate. A total of 114 patients were examined, with 25.4% (n=29) testing positive for influenza A virus RNA, while influenza B virus RNA was not detected.

Overall, the results indicate a high incidence of influenza among patients with severe respiratory disease, with 244 (48.8%) testing positive for influenza virus RNA.

Conclusions

According to the results obtained, influenza A was detected in the population of patients admitted to the hospital with severe respiratory syndrome in all four seasons (2015–2019). However, an outbreak of influenza B occurred in 2017–2018, which also showed the severity of this disease. There is a general consensus that influenza disease type B influenza is milder than type A, but data show that influenza A and B infections are not clinically different.

Source of financing

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: *O.Y. Matsas, O.V. Vorobiova;* Writing: *O.I. Mulkina,* Review and editing: *O.M. Slobodianiuk*.

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ОДНОЦЕНТРОВЕ ДОСЛІДЖЕННЯ ЩОДО ВИПАДКІВ ВИЯВЛЕННЯ РНК ВІРУСУ ГРИПУ А/В ЗА ДОПОМОГОЮ ПЛР У ПАЦІЄНТІВ ІЗ ТЯЖКИМИ ГОСТРИМИ РЕСПІРАТОРНИМИ ЗАХВОРЮВАННЯМИ (2015–2019)

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Інфекція вірусу грипу залишається основною причиною захворюваності та смертності в осінньо-зимовий період. Надійні дані щодо поширеності грипу серед пацієнтів з гострими респіраторними захворюваннями, разом з інформацією про підтипи вірусу, що циркулюють, та сезонні тенденції, є необхідними для правильного підбору штамів для щорічних вакцин та ефективного планування програм імунізації.

Мета. Провести молекулярно-діагностичний аналіз грипу у пацієнтів із тяжким гострим респіраторним захворюванням у період з 2015 по 2019 рік.

Методи. У дослідження було включено 505 пацієнтів з симптомами тяжкого гострого респіраторного захворювання, які були госпіталізовані до КНП «Свято-Михайлівська клінічна лікарня м. Києва». Виявлення РНК вірусу грипу типів А та В проводилося методом ПЛР у реальному часі.

Результати. Протягом періоду спостереження у 49% пацієнтів було виявлено позитивний результат на грип. Найвищий рівень позитивних випадків (86%) було зафіксовано у сезон 2017–2018 років, при цьому переважав вірус грипу типу В. На відміну від цього, РНК вірусу грипу В не було виявлено у сезонах 2015–2016, 2016–2017 та 2018–2019 років. Поширеність вірусу грипу типу А в ці періоди становила відповідно 28,1%, 50,7% та 25,4%. Випадків ко-інфекції не виявлено.

Висновки. Вірус грипу типу А постійно циркулював серед пацієнтів, госпіталізованих із тяжкими респіраторними захворюваннями, протягом усіх чотирьох сезонів з 2015 по 2019 рік. Різке зростання кількості позитивних випадків грипу у сезон 2017–2018 років зумовлено спалахом грипу типу В.

Ключові слова: осінньо-зимовий епідеміологічний період, гострі респіраторні захворювання, віруси грипу А, В.