

AETIOLOGICAL STRUCTURE OF ACUTE RESPIRATORY TRACT INFECTIONS AMONG CHILDREN YOUNGER THAN 5 YEARS IN BULGARIA

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ABSTRACT

Acute respiratory tract infections (ARI) are a leading cause of morbidity and hospital admissions among infants and young children. This study aims to determine the viral aetiology of ARI and the clinical significance of the most common respiratory viruses in children aged <5 years in Bulgaria.

During the period October 2017-March 2019, nasopharyngeal specimens were collected from children younger than 5 years in different country regions. Real-time PCR analysis was performed for detection of influenza viruses A/B, respiratory-syncytial virus (RSV), human metapneumovirus (HMPV), parainfluenza viruses (PIV) types 1, 2 and 3, rhinoviruses (RV), adenoviruses (AdV) and bocaviruses (BoV).

Of the 953 children examined, 663 (69.6%) were positive for at least one virus. The number of detected A(H1N1)pdm09, A(H3N2), B/Yamagata, B/Victoria, RSV, HMPV, PIV-1, PIV-2, PIV-3, RV, AdV and HBoV viruses was as follows: 150 (15.7%), 51 (5.6%), 50 (5.2%), 1 (0.1%), 193 (20.3%), 38 (4%), 15 (1.6%), 5 (0.5%), 17 (1.8%), 101 (10.6%), 60 (6.3%), 77 (8.1%), respectively. Co-infections with two and three viruses were found in 95 (14.3%) of the infected children. AdV, RV, BoV and PIV-3 were the most common pathogens in co-infections. Respiratory viruses were detected

in 80%, 79.3%, 61.2% and 53.1% of children with laryngotracheitis, bronchiolitis, pneumonia and central nervous system (CNS) complications.

The results show that RSV, influenza viruses, RV and BoV were the most frequently detected viruses in children <5 years with ARI during the study period. These viruses were also leading causative agents of serious illnesses of the respiratory tract and CNS.

KEYWORDS:

viral infection, respiratory virus, bronchiolitis, pneumonia

INTRODUCTION

Acute respiratory infections (ARI) are associated with a large number of doctor visits, hospital admissions, significant mortality, serious health and social consequences. With respect to the anatomical localisation, ARIs are classified as upper and lower respiratory tract infections. Upper respiratory tract involvement usually occurs as a mild or moderate illness. The high incidence of these infections is associated with prolonged circulation of pathogens in the community. Lower respiratory tract involvement leads to severe complications as bronchitis, bronchiolitis, pneumonia, often requiring hospitalisation. In 2010, 11.9 million episodes of severe and 3 million episodes of very severe ARI have led to the admission of infants and young children to hospital worldwide (1). Bronchiolitis occurs in children <2 years of age and most often has viral aetiology, whereas pneumonia can be caused by bacteria, viruses or other pathogens. Pneumonia is the leading infectious cause of mortality in children <5 years of age (2).

A large spectrum of viruses is associated with ARI and the major causative pathogens are influenza viruses, respiratory syncytial virus (RSV), human metapneumovirus (HMPV), parainfluenza viruses (PIV) 1/2/3, rhinoviruses (RV), adenoviruses (AdV) and bocaviruses (BoV).

This study aims to determine the role of influenza viruses and 8 other respiratory viruses in the development of ARI in children younger than 5 years of age during two consecutive seasons in Bulgaria.

MATERIAL AND METHODS

A total of 953 children aged <5 years, ambulatory-

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treated or hospitalised for influenza-like illness (ILI) or acute respiratory illness (ARI) in different regions of the country were examined in the study. Of them, 444 (46%) had complications – laryngotracheitis, bronchiolitis, pneumonia or central nervous system (CNS) infections (febrile seizures, brain oedema, meningitis, encephalopathy, encephalitis).

Viral nucleic acids were automatically extracted from respiratory specimens using a commercial ExiPrep Dx Viral DNA/RNA Kit (Bioneer) according to the manufacturer's instructions. Laboratory testing was conducted at the National Laboratory “Influenza and Acute Respiratory Diseases” recognised by WHO as a National Influenza Centre. Detection and typing/subtyping of influenza viruses was carried out by real-time RT-PCR method with SuperScript III Platinum® One-Step Quantitative RT-PCR System (Invitrogen). All samples were initially tested for influenza A and B viruses using primers and probes donated by CDC Atlanta and those positive for influenza A and B were subsequently tested for A(H1N1)pdm09 and A(H3N2), B/Yamagata and B/Victoria, respectively. All samples were also examined by singleplex real-time RT-PCR assays for RSV, HMPV, PIV 1/2/3, RV, AdV and BoV using specific primers/probes and AgPath-

ID One Step RT-PCR Kit (Applied Biosystems). Primers and probes were identical to those previously described (3).

RESULTS

Respiratory virus detection

The present study included two consecutive epidemic seasons in Bulgaria – 2017/2018 and 2018/2019. The study population consisted of 953 children aged < 5 years presenting with ILI or ARI: 459 in the first and 451 in the second season, respectively and 43 outside the season in 2018. Most of the patients – 831 (87.2%) were hospitalised and 122 (12.6%) were outpatients. Age of the patients varied from 30 days to 60 months (average 21.53 ± 12.54 months). Males were 59% and females – 41%. Virus infections were laboratory-confirmed in 663 (69.6%) samples. Influenza viruses were found in 252 (26.4%) of the tested children. Influenza B/Yamagata virus was predominant during the 2017/2018 winter season, while A(H1N1)pdm09 prevailed during the next winter season. In the first season, the proportion of each influenza virus among influenza-positive samples was as follows: B/Yamagata – 57.5%, B/Victoria – 1.1%, A(H1N1)pdm09 – 35.6% and A(H3N2) – 5.7%; in the second season: A(H1N1)pdm09 – 72.1% and A(H3N2) – 27.9%.

Table 1. Distribution of respiratory viruses in outpatients and hospitalised patients.

Number of detected respiratory viruses												
	A(H1N1) pdm09	A(H3N2)	B/ Yamagata	B/ Victoria	RSV	HMPV	PIV-1	PIV-2	PIV-3	RV	AdV	BoV
2017-2018	31	5	50	1	91	27	15	2	11	72	35	48
2018-2019	119	46	-	-	101	9	-	2	4	16	20	26
Summer 2018	-	-	-	-	1	2	-	1	2	13	5	3
Outpatients	16	11	13	1	9	8	3	1	1	11	4	7
Inpatients	134	40	37	-	184	30	12	4	16	90	56	70

Among the non-influenza viruses, RSV was the most frequently detected respiratory virus found in 193 (20.3%) of the tested samples, followed by RV – 101 (10.6%), BoV – 77 (8.1%) and AdVs – 60 (6.3%). The number of detected HMPV and PIV type 1, 2 and 3 was as follows: 38 (4%), 15 (1.6%), 5 (0.5%) and 17 (1.8%), respectively (Fig. 1).

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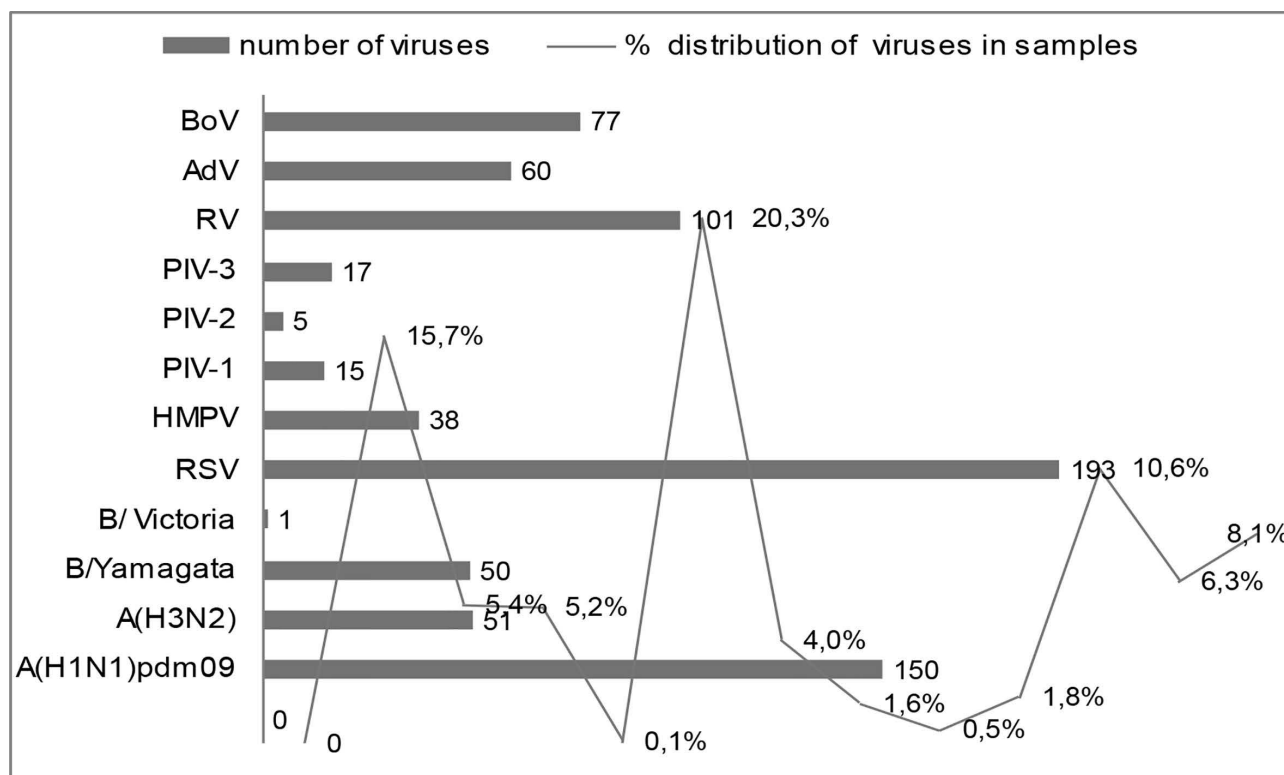


Figure 1. Distribution of respiratory viruses detected in Bulgaria during the 2017/2018 and 2018/2019 seasons and in the summer of 2018.

Single infections were detected in 568 (59.6%) patients; 88 (9.2%) children were co-infected with two and 7 (0.7%) children – with three viruses. Among the children with mono-infections, the most commonly determined pathogen was RSV, followed by influenza A(H1N1)pdm09. AdV, RV,

BoV and PIV-3 were the most frequently detected pathogens in co-infections – the proportion of mixed infections was 61.7%, 52.5%, 41.6% and 41.2%, respectively (Fig. 2). The most common combinations in mixed infections were RSV+RV, RV+BoV, RV+AdV and RSV+AdV (Table 2).

Table 2. Number of respiratory viruses, detected as a single pathogen or co-pathogen.

Viruses	A(H1N1) pdm09	A(H3N2)	Inf typeB	RSV	hMPV	PIV1	PIV2	PIV3	RV	AdV	BoV
A(H1N1)pdm09	134	-	-	4	-	-	-	2	1	5	3
A(H3N2)		44	-	4	-	-	1	-	-	-	1
Inf type B			46	2	-	-	-	-	1	1	-
RSV				147	-	-	-	2	15	10	3
hMPV					35	-	-	-	2	-	1
PIV1						11	-	-	2	-	2
PIV2							3	-	1	-	-
PIV3								10	-	1	2
RV									53	11	15
AdV										23	4
BoV											45
Co-pathogen in dual infection	15	6	4	40	3	4	2	7	48	32	31
Co-pathogen in triple infection	1	1	1	6	-	-	-	-	5	5	1

*Single pathogens are indicated in bold.

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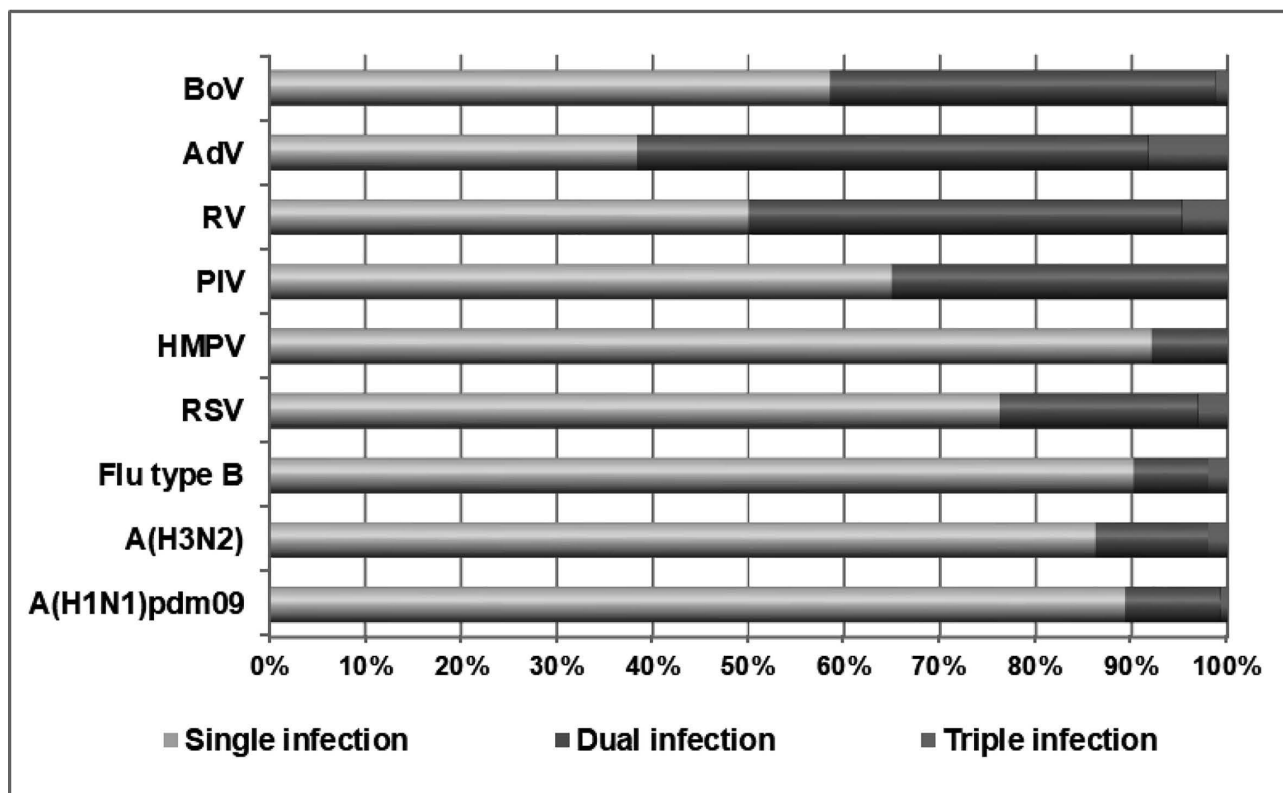


Figure 2. Proportion (%) of respiratory viruses detected in single, dual or triple infections during the 2017/2018 and 2018/2019 seasons and in the summer of 2018.

Seasonal distribution of viral agents

The greatest number of respiratory viruses was detected in specimens obtained in December 2018, February 2017 and May 2018 (Fig. 3). Influenza and RSV infections were more prevalent in winter. RV, BoV and HMPV infections occurred predominantly during fall and spring in Bulgaria.

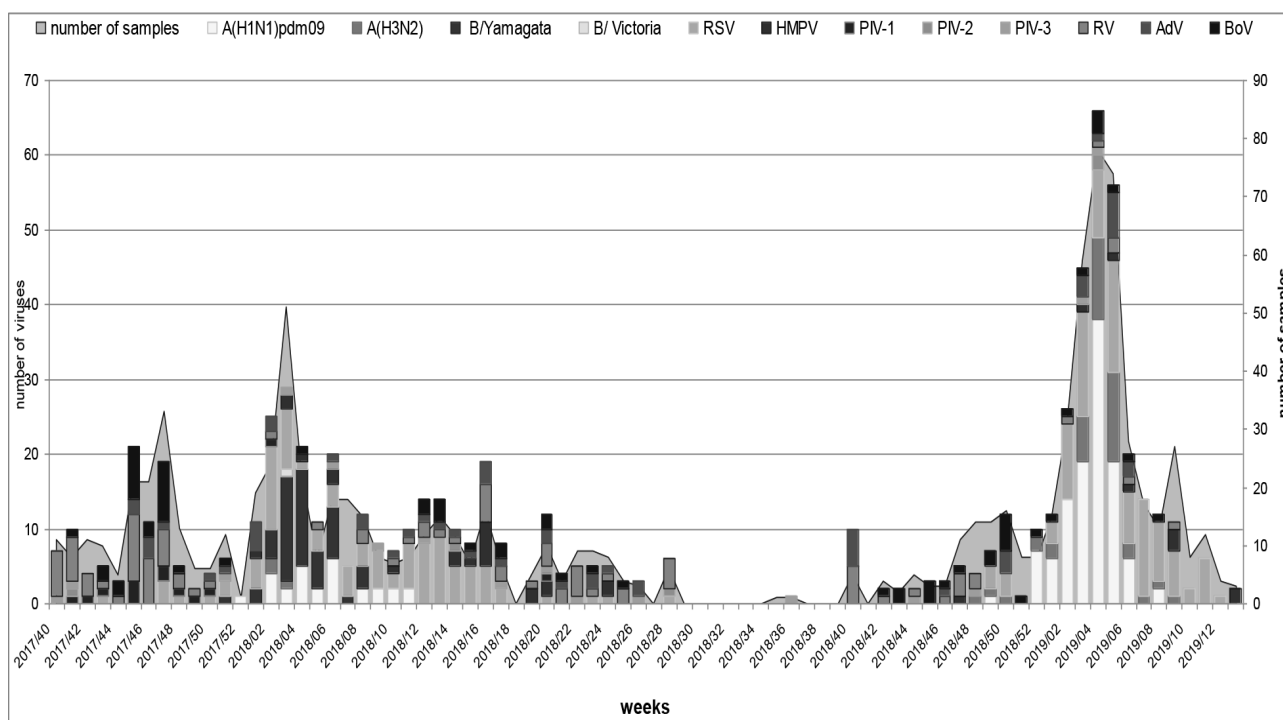


Figure 3. Weekly distribution of respiratory viruses detected during the two consecutive epidemic seasons in Bulgaria.

Clinical characteristics

During early childhood respiratory viruses may cause serious complications affecting the respiratory tract (laryngotracheitis, bronchiolitis, pneumonia) or CNS (febrile seizures, brain oedema, meningitis, encephalopathy, encephalitis). This study also analysed the involvement of influenza viruses, RSV, HMPV, PIV1/2/3, RV, AdV and BoV in development of

the complications mentioned above. A total of 85, 198, 121 and 32 cases of laryngotracheitis, bronchiolitis, pneumonia and CNS complications were investigated. At least one virus was detected in 80%, 79.3%, 61.2% and 53.1% of cases with these syndromes, respectively. Fig. 4 represents the number and proportion (%) of patients infected with respiratory viruses in the groups with different clinical diagnosis.

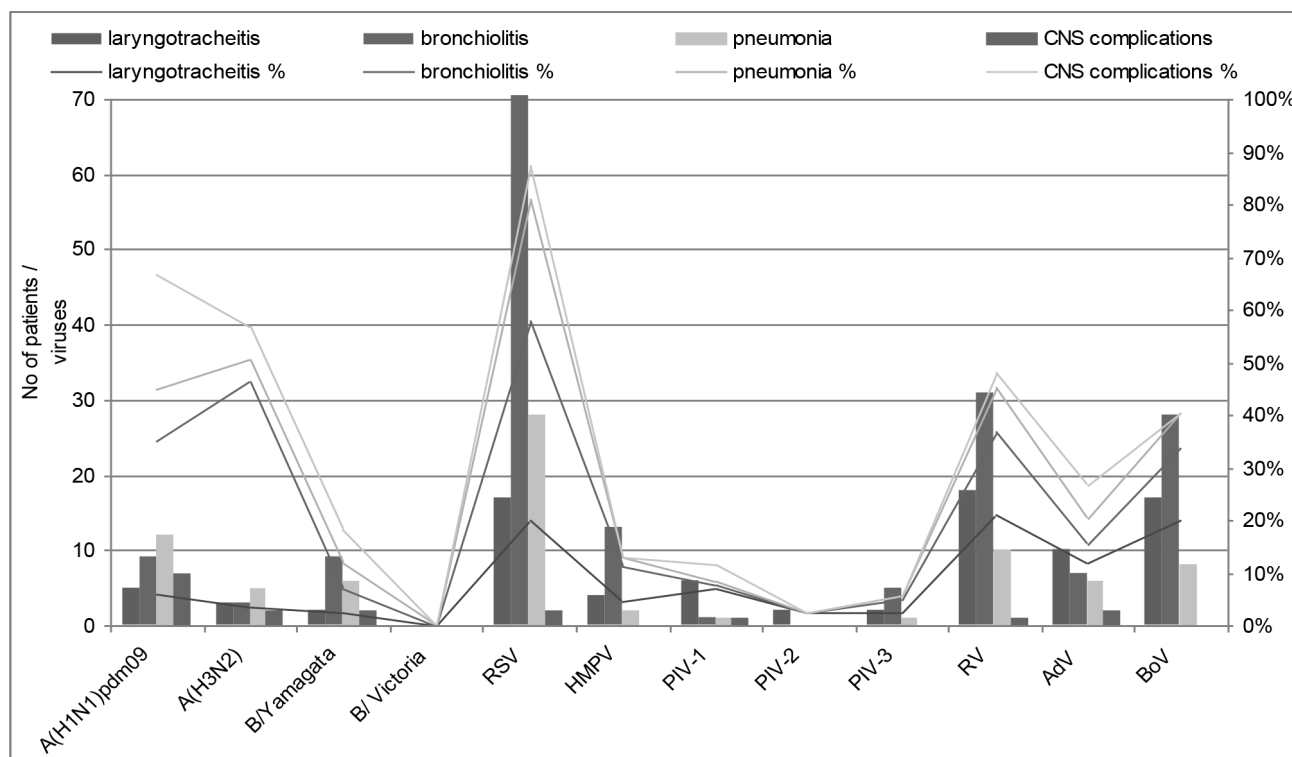


Figure 4. Number and proportion (%) of patients with different clinical diagnosis and detected respiratory viruses.

Among patients with laryngotracheitis, RVs were the most frequently identified viruses (21.2%), followed by RSV (20%) and BoV (20%). Among patients with bronchiolitis, RSV, RV and BoV were the most common pathogens – 37.9%, 15.7% and 14.1%, respectively. RSV was the most commonly identified virus in patients with pneumonia (23.1%) followed by influenza A(H1N1)pdm09 (9.9%) and RV (8.3%). In total, influenza viruses were responsible for 19% of pneumonia cases. Most (34.4%) neurological complications were associated with influenza infections.

DISCUSSION

Respiratory tract infections are a leading cause of morbidity and mortality in children <5

years around the world. This study aimed to describe the circulation of 12 respiratory viruses among infants and young children in Bulgaria during the 2017/2018 and 2018/2019 seasons. The involvement of these pathogens in the development of some serious diseases of the respiratory tract and CNS was also analysed. The high level (69.6%) of detection of respiratory viruses found in this study is comparable to other surveys (4, 5). Mixed infections were identified at a lower percentage (14.3%) compared to other studies (6, 7). In a systematic review, Goka et al. reported incidence of mixed viral infections ranging from 5% to 62% (8). A high percentage of mixed infections is likely to be observed in settings with high population density and a large number of children attending childcare

facilities. Some authors suggest that there is a link between co-infection and disease severity, especially co-infections involving RSV (9, 10, 11). The relationship between clinical severity and infection status with single vs. multiple respiratory pathogens remains inconclusive.

Influenza epidemics are characterised by variations in the types/subtypes of influenza viruses involved, their virulence and clinical manifestation. In Bulgaria, during the 2017/2018 winter season, B/Yamagata was the predominantly circulating influenza virus, while during the next 2018/2019 season A(H1N1)pdm09 was prevalent. Similar predominance of B/Yamagata and A(H1N1)pdm09 viruses was observed in most European countries (12).

RSV is the most important pathogen of ARI among infants and young children and the major causative agent of bronchiolitis and pneumonia (13, 14). In our study, RSV was the most frequently detected virus in all examined patients and a leading cause of bronchiolitis (37.9% of cases) and pneumonia (23.1% of cases).

RV infections occur early and periodically in life, mainly as a common cold. However, they are also associated with severe illnesses in infants and young children (15, 16). In this study, RVs were the 3rd most frequently identified viruses; they were also the most common pathogen in cases of laryngotracheitis (21.2% of cases) and one of the leading causative agents of bronchiolitis and pneumonia.

According to literature data, HBoVs were found as relatively frequent respiratory pathogens in children younger than 5 years of age (17, 18, 19). In this study, BoVs ranked fourth in frequency among other respiratory viruses. In agreement with other studies, they were involved in co-infections at a significant rate – 41.6% (17). Spread of BoVs was observed throughout the year, reaching peaks in the autumn and spring months.

The proportion of AdV infections found among Bulgarian children with ARI was 6.3% which was similar to the findings of other researchers (20). AdV were characterised by a significant incidence of co-infections.

HMPV is a relatively new pathogen with prevalence in the paediatric population ranging

from 5-25% (21). The results of this study showed 4% frequency of HMPV infections and 6.6% incidence in cases of bronchiolitis.

PIV type 1, 2, 3 and 4 show different clinical and epidemiological characteristics. PIV types 1 and 2 are leading causes of laryngotracheobronchitis (croup), while PIV-3 is frequently associated with bronchiolitis and pneumonia in infants and young children (22). PIVs were identified at low frequency – 3.9% of the studied children, mainly in cases of laryngotracheitis.

A limitation of this study is that human coronaviruses, which also cause ARI, were not included in the examinations.

The study highlights the role of 11 respiratory viruses in the aetiology of paediatric respiratory infections. RSV followed by influenza viruses, RVs and BoVs were found to be the most common causative agents of ARI in children younger than 5 years during two consecutive seasons (2017/2018 and 2018/2019) in Bulgaria. These pathogens were the main cause of complications such as laryngotracheitis, bronchiolitis and pneumonia that require hospital treatment. Timely and accurate diagnosis of viral respiratory infections is important in order to reduce the need for unnecessary lab tests and antibiotic use and to improve infection prevention and control measures.

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