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A Single-Center Study of Viral Respiratory Tract Infections in Hospitalized Children From the Kurdistan Region of Iraq

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Abstract

Viral respiratory infections are among the most common causes of disease in humans, particularly in young children, and remain a major public health problem worldwide. For many geographic regions, there is limited epidemiological information on the main causative agents of these diseases. In this article, we investigated, in a prospective study, the viral agents leading to acute respiratory disease in children younger than 15 years of age who were admitted to the pediatric emergency unit of a major teaching hospital in Erbil City, capital of the Kurdistan region, Iraq. Nasopharyngeal samples obtained from 269 hospitalized children were analyzed for viral respiratory pathogens using the xTAG Respiratory Virus Panel Fast assay, and the data were correlated with the clinical and demographic information available for these patients. One or more respiratory virus(es) were detected in 203 out of 269 (75.5%) samples. The most frequent viruses were enterovirus/rhinovirus (n = 88; 32.7%), respiratory syncytial virus (n = 55; 20.4%), and human metapneumovirus (n = 36; 13.4%). In 42 samples (15.6%), coinfections with 2 or more respiratory viruses were detected, with enterovirus/rhinovirus, respiratory syncytial virus, human metapneumovirus, and adenovirus being identified as the most common agents in viral coinfections in these patients.

Keywords

respiratory virus, acute respiratory infection, viral coinfection, children, Kurdistan, respiratory syncytial virus, coronavirus, rhinovirus, enterovirus, influenza virus, parainfluenza virus, metapneumovirus, adenovirus

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Introduction

Viral respiratory infections are among the most common causes of disease in humans, especially among children and infants, and a major public health problem due to the high prevalence, ease of transmission, and the significant morbidity and mortality associated with these infections.[1,2] Acute respiratory infections (ARIs) rank among the top 5 causes of illness and hospitalization in children,[3] with high childhood mortalities leading to about 5 million fatalities per year in developing countries in children younger than 5 years of age.[1,4]

Although ARIs can be caused by bacterial agents, they are predominantly caused by respiratory viruses.[2] Adenoviruses, coronaviruses, human enteroviruses (HEV), human rhinoviruses (HRV), influenza A and B viruses, parainfluenza viruses (PIV), and respiratory syncytial virus (RSV) are well-established causes of ARIs in both industrialized and developing countries. Also, a number of newly discovered viruses, such as human bocavirus (HBoV), human metapneumovirus (hMPV), and the human coronaviruses NL63 and HKU1 (HCoV-NL63, HCoV-HKU1) are detected in these infections, while other (zoonotic) coronaviruses, such as the severe acute respiratory syndrome coronavirus and the Middle East respiratory syndrome coronavirus, have the potential to cause local or worldwide outbreaks of

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severe lower respiratory tract infections, including acute respiratory distress syndrome, with high fatality rates.[2,5]

Polymerase chain reaction (PCR)-based molecular techniques are rapid and sensitive compared with conventional methods and are now widely used for the detection of relevant respiratory viruses, often in a multiplex-assay format suitable to detect multiple viruses in a single test system.[6] The xTAG RVP FAST assay is an example of a comprehensive multiplex assay suitable for the detection of multiple viruses including specific strains and subtypes.[6,7]

The viral etiology of ARIs in the Kurdistan of Iraq remains poorly studied. To address this gap in information, the study sought to determine the frequency and type of viral pathogens causing respiratory infections in children less than 15 years of age who were hospitalized with respiratory illness in Erbil City. To this end, a total of 269 samples obtained from an appropriate study group were analyzed using the Luminex xTAG Respiratory Virus Panel Fast assay.

Material and Methods

Patients and Sample Collection

This prospective study was conducted between February 2012 and March 2013 in the Raparin Pediatric Teaching Hospital, Erbil, the capital of the Kurdistan region, Iraq. A total of 270 hospitalized children younger than 15 years of age with respiratory infections were included in the study. All these patients presented with fever of ≥38°C on admission and with clinical signs and symptoms of an upper and/or lower respiratory tract infection that required admission to the pediatric emergency unit. The diagnosis for each patient was performed by the attending pediatrician. Exclusion criteria were premature birth, chronic disorders of the pulmonary or cardiovascular systems, diabetes mellitus, and kidney or liver dysfunction.

Nasopharyngeal samples were collected using nylon-flocked nasal swabs. The swabs were immersed in 3 mL of 1× phosphate buffered saline and kept at −70°C until further use (see below). Relevant clinical information as well as demographic data, including age, gender, and epidemiological data, were collected with a standardized questionnaire and from medical files of patients included in this study.

The study protocol was reviewed and approved by the research ethics committee of the College of Medicine, Hawler Medical University, and the collection and use of clinical samples and data from patients at the Raparin Pediatric Teaching Hospital was approved by the Erbil Director of Health. Informed consent was obtained from the parents of all the participating children.

Virus Detection Assay

Nucleic acid was extracted from clinical samples (200 µL) using the automated EZ1 Advanced XL nucleic acid extraction system (Qiagen), and EZ1 Virus Mini Kit v2.0, according to the manufacturer's instructions and subsequently used for the Luminex xTAG Respiratory Virus Panel (RVP) Fast assay (version 2) according to the manufacturer's instructions (Luminex Molecular Diagnostics Inc). This multiplex assay is suitable to detect multiple respiratory virus nucleic acids from nasopharyngeal swabs and is based on the Luminex xTAG Technology and xMAP Technology platform. The data generated by the xMAP instrument were analyzed by the xTAG Data Analysis Software RVP Fast (TDAS RVP FAST), providing a report on which viruses (if any) were present in the respective samples.

Statistical Analysis

Statistical analyses were performed using SPSS (version 18) and unpaired Student's t test, considering a $P < .05$ as significant.

Results

Demographic and Clinical Data of the Study Group

A total of 270 pediatric patients were enrolled in this study of causative viral agents of ARIs among hospitalized children. The study was conducted between February 2012 and March 2013. All patients enrolled in the study presented with typical symptoms of an ARI that required hospitalization and admission to the pediatric emergency unit. One of the patients was excluded due to insufficient material being obtained in this case. The mean age was 23.6 months (standard error of mean = 28.5, ranging from 1 month to 13 years); numbers of male and female patients were 182 (67.65%) and 87 (32.35%), respectively. Patients were assigned to 6 age groups: <1 year (n = 140; 51.8%), 1 to <2 years (n = 52; 19.2%), 2 to <3 years (n = 25; 9.2%), 3 to <4 years (n = 11; 4.1%), 4 to <5 years (n = 6; 2.2%), and >5 years (n = 30; 11.1%). For 5 patients, information on age was not available. The majority of the study population (58.4%) was urban residents. Exposure to passive smoking was recorded in 50.1% of the patients. All patients presented with fever of ≥38°C on admission; the most commonly observed respiratory symptoms were cough, difficulty in breathing, and wheezing. Few patients presented with symptoms of asthma, croup, tonsillitis, or vomiting (Table 1).

Table 1. Summary of Demographic and Clinical Data of Hospitalized Children With Acute Respiratory Infection Included in This Study.

Characteristics	Total (N = 269)	Patients With Confirmed Viral ARI (N = 203)
Age, mean ± SEM, months	23.6 ± 28.5	20.6 ± 23.9
Gender		
Male	182 (67.65%)	134 (66.0%)
Female	87 (32.35%)	69 (34.0%)
Age (years)		
≤1	140 (52.0%)	117 (57.6%)
1.1-2	52 (19.3%)	37 (18.2%)
2.1-3	25 (9.29%)	16 (7.9%)
3.1-4	11 (4.08%)	9 (4.5%)
4.1-5	6 (2.23%)	3 (1.5%)
≥5	30 (11.1%)	19 (9.4%)
Unknown age	5 (1.8%)	2 (0.9%)
Passive smoking exposure	135 (50.1%)	107 (52.7%)
Residence		
Urban	157 (58.4%)	121 (59.6%)
Rural	112 (41.6%)	82 (40.4%)
Clinical symptoms		
Wheezing	216 (80.3%)	174 (85.7%)
Difficulty in breathing	245 (91.1%)	193 (95.1%)
Apnea	106 (39.4%)	75 (36.9%)
Allergy	26 (9.6%)	20 (9.8%)
Diarrhea	20 (7.4%)	13 (6.4%)
Ear ache	65 (24.1%)	55 (27.1%)

Abbreviations: ARI, acute respiratory infection; SEM, standard error of mean.

Detection of Respiratory Viruses in the Patient Population

Nucleic acid of one (or more) respiratory virus(es) was detected using the Luminex TAG Respiratory Viral Panel assay in 203 of the 269 samples analyzed in this study (75.5%). A single viral pathogen was detected in 161 (59.9%) samples, coinfection by 2 or more viruses was detected in 42 samples (15.6%), and no viral nucleic acid was detected in 66 samples (24.5%; Table 2). The most common virus was HEV/HRV, which was detected in 88/269 (32.7%) of the samples, followed by RSV 55/269 (20.4%) and hMPV 36/269 (13.4%). In contrast, influenza B virus, adenovirus, PIV 1, and influenza A virus (H1N1pdm09 in all cases) were detected less frequently (6.3%, 6.3%, 5.9%, and 3.7%, respectively), while HBoV, PIV 3, and HCoV-NL63 were detected in just a few cases (2.2%, 1.5%, and 0.4%, respectively; Table 2). Among the 161 single-virus infections, the most common agents were found to be HEV/HRV (detected in 54 [33.5%] samples), RSV (detected in 37 [22.9%] samples), and hMPV (detected in 25 [15.5%] samples; Figure 1). Regarding age distribution, the virus positivity rate was highest in samples obtained from children younger than 1 year (117/140, 83.6%) and also very high in the combined age groups of children under 2 years of age 154/192 (80.2%), while virus-detection rates were found to decline with increasing age (Figure 2). HEV/HRV, RSV, and hMPV were identified in children of all age groups, whereas HBoV was detected only in children less than 2 years of age (Table 2).

More than one virus was detected in 42/269 (15.6%) samples. Two viruses were detected in 39/269 (14.5%) samples; coinfections with 3 viruses were confirmed for 2 samples (0.74%); and there was 1 sample in which a coinfection with 4 viruses could be confirmed (0.37%). HEV/HRV (together with at least on other virus) was detected in as many as 34/42 (80.9%) samples, thus representing the most common agent in viral coinfections in this study group. RSV was detected in 18/42 (42.8%) of the coinfection samples; adenovirus was detected in 12/42 (28.5%) coinfection samples; and hMPV was detected in 11/42 (26.1%) coinfection samples (Figure 3). Overall, 61.9% (26/42) of coinfection cases were found in children less than 12 months of age (Table 2).

The most common virus combinations in coinfections were found to be HEV/HRV + RSV (12/42, 28.5%), HEV/HRV + hMPV (9/42, 21.4%), and HEV/HRV + hAdV (human adenovirus; 7/42, 16.6%). Other combinations included RSV + hAdV (in 3 samples) and HEV/HRV + HBoV (in 2 samples). The 2 triple infections were combinations of (*a*) HEV/HRV + RSV + PIV 1 and (*b*) HEV/HRV + hMPV+ hAdV. One quadruple infection was found to be caused by HEV/HRV + RSV + hAdV + PIV1 (Figure 4).

Seasonality

The monthly distribution of virus-positive samples is shown in Figure 5. The peak incidence of confirmed virus-associated respiratory infections was in the time between late autumn and early spring (November to March), with 191/203 (94%) of the respiratory infections being positive for at least one virus in this time period.

Discussion

Viral infections are the major cause of respiratory illness in children, and they have an enormous clinical and financial impact.[3,4] Little is known about the epidemiology of viral ARIs in Kurdistan due to limited diagnostic capacity for detecting multiple respiratory viruses from single samples. To the best of our knowledge, this is the

Table 2. Mono- and Coinfections With Specific Respiratory Viruses in Different Age Groups of the Study Group[a].

	<1 Year (n = 140)	1 to <2 Years (n = 52)	2 to <3 Years (n = 25)	3 to <4 Years (n = 11)	4 to <5 Years (n = 6)	5 to <15 Years (n = 30)	Unknown Age (n = 5)	Total (N = 269)
HEV/HRV	51 (35.7)	18 (36.5)	5 (20)	4 (36.3)	1 (16.6)	8 (26.6)	1	88 (32.7)
RSV 1	18 (12.8)	2 (3.8)	2 (8)	1 (9.1)	0	2 (6.6)	0	25 (9.3)
RSV 2	26 (18.6)	3 (5.8)	0	0	1 (16.6)	0	0	30 (11.1)
hMPV	22 (15.7)	7 (13.4)	2 (8)	2 (18.2)	0	2 (6.6)	1	36 (13.4)
Inf A	2 (1.4)	4 (7.7)	2 (8)	0	0	2 (6.6)	0	10 (3.7)
Inf B	3 (2.1)	4 (7.7)	1 (4)	2 (18.2)	0	7 (23.3)	0	17 (6.3)
hAdV	12 (8.5)	4 (7.7)	0	0	0	2 (6.6)	0	18 (6.3)
PIV 1	7 (5.0)	3 (5.8)	4 (16)	1 (9.1)	0	1 (3.3)	0	16 (5.9)
PIV 3	1 (0.7)	2 (3.8)	0	1 (9.1)	0	0	0	4 (1.5)
HboV	4 (2.8)	2 (3.8)	0	0	0	0	0	6 (2.2)
HCoV-NL63	1 (0.7)	0	0	0	0	0	0	1 (0.4)
Single-virus infection	91 (65)	25 (48.1)	16 (64)	8 (72.7)	3 (50)	16 (53.3)	2	161 (59.9)
Coinfections	26 (18.6)	12 (23.1)	0	1 (9.1)	0	3 (10)	0	42 (15.6)
No virus detected	23 (16.4)	15 (28.8)	9 (36)	2 (18.2)	3 (50)	11 (36.7)	3	66 (24.5)

Abbreviations: HEV/HRV, human enterovirus/human rhinovirus; RSV1, RSV2, respiratory syncytial viruses 1 and 2; hMPV, human metapneumovirus; Inf A, influenza A virus; Inf B, influenza B virus; hAdV, human adenovirus; PIV 1, PIV 3, parainfluenza viruses 1 and 3; HBoV, human bocavirus; HCoV, human coronavirus.

[a]Shown are the total numbers of samples tested positive for a specific virus in a given age group. Numbers in parentheses indicate the proportion (in percent) of positive samples in relation to the total number of patient samples tested in this age group.

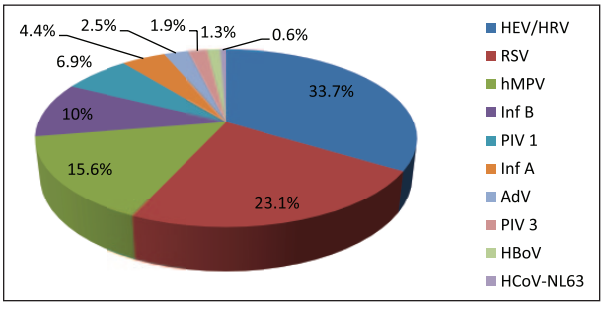

Figure 1. Proportion (in percent) of specific respiratory viruses confirmed to cause single-virus infections in the study group.

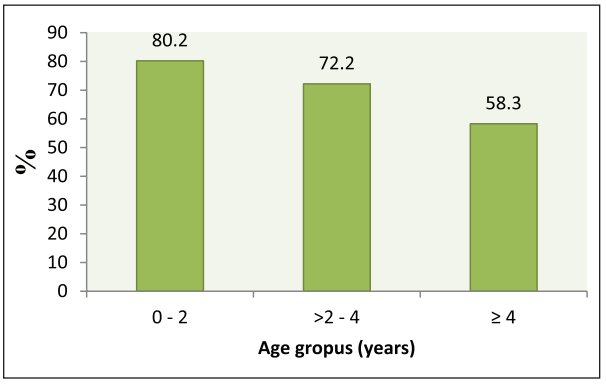

Figure 2. Respiratory virus detection rate (in percent) in different age groups of the study group.

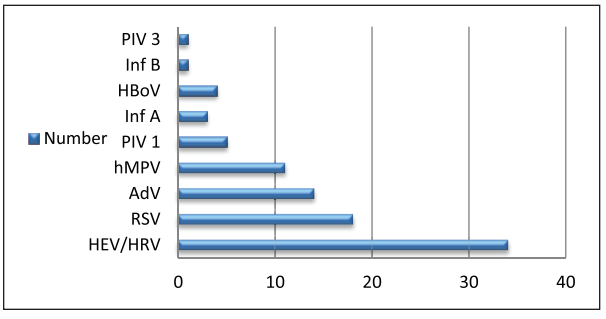

Figure 3. Detection frequency (in percent) of specific viruses in samples with more than one virus (viral coinfections).

first long-term prospective study performed to determine the involvement of 18 common respiratory viruses in ARIs among hospitalized children in Erbil, Kurdistan. In this study, the causative viruses, detection frequency, seasonality, and coinfection patterns of the most common respiratory viruses were investigated using the Luminex xTAG Respiratory Virus Panel Fast v2 assay. Our results provided a distinctive epidemiological profile of viral respiratory infections in hospitalized children with ARIs in the study area.

Overall, nucleic acid of one (or more) respiratory virus(es) was detected in as many as 75.5% of the samples obtained from the study group of 269 patients with ARIs requiring treatment in a pediatric emergency unit. These data are consistent with previous studies in which

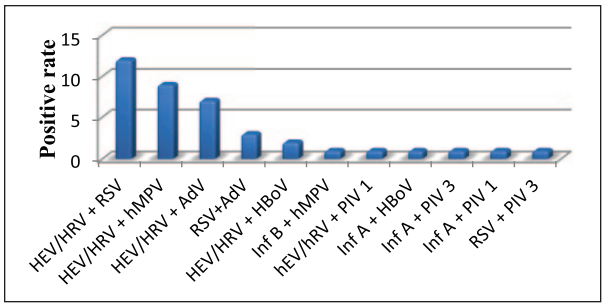

Figure 4. Frequency of confirmed coinfections with 2 viruses (as indicated) in nasopharyngeal swab samples collected from hospitalized children with acute respiratory infection.

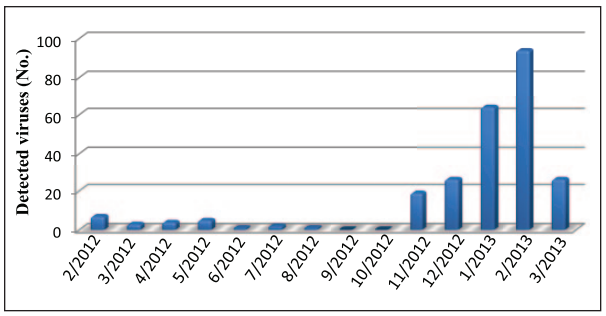

Figure 5. Seasonal distribution of confirmed virus-associated respiratory infections in the study group (n = 269) at Raparin Hospital in Erbil between February 2012 and March 2013. The columns indicate numbers of virus-positive samples collected in the respective month.

viral detection rates of 65% to 90% for at least one virus in respiratory tract samples collected from children under 15 years of age with ARIs were reported, although different test systems and molecular methods were used in those studies.[8-14]

The most frequently detected viruses causing ARIs in patients of our study group were HEV/HRV and RSV, both as single-virus infections and viral coinfections, followed by infections with hMPV and hAdV. Frequently, RSV is defined as the predominant viral etiology in hospitalized children with ARIs.[2,15] However, in our study members of the genus *Enterovirus* (HEV and HRV) was the most frequently detected virus groups. This result is in agreement with previous studies, which showed identification of *Enterovirus* genus in samples taken from children infected with ARIs.[13,14,16-19] Considering that the current study included only hospitalized children, this result highlighted the involvement and importance of *Enterovirus* genus members in the ARIs among children in study area. Also, age group, climate, geographic area, and others factors have an influence on the reported rate among different studies of each respiratory virus in hospitalized ARIs.[20-22]

RSV was the second most frequently detected virus detected in our study group (20.4%). This result is in good agreement with a previous Iraqi study conducted in Babylon City, which detected RSV in 24% of the samples from a study group of 50 children using a PCR-based assay.[23] Despite these slight differences in the detection rates for RSV, our result was in contrast to other studies indicating RSV as the dominate cause of respiratory infection in children.[8,24,25]

In several recent studies, hMPV was one of the most frequently detected viruses involved in ARIs.[26,27] Two previous studies investigated the role of hMPV infection in ARIs in Iraq using non-nucleic acid–based techniques. Al-Mola et al[28] reported a detection frequency that was similar to our result (13.3%) using a direct immunofluorescent assay for samples collected from infants of <2 years, while Aziz et al[29] determined a seroprevalence of anti-hMPV antibodies among hospitalized children of ≤5 years in Sulaimani City (immunoglobulin M [12%] and immunoglobulin G [75%]). The high seroconversion rate in young children indicates circulation of hMPV in the Kurdistan region population. The positive rate found in the current study was consistent with that reported by Albuquerque et al[30] in Brazil (12.5%) and Bicer et al[12] in Turkey (12.6%), but differ from other studies, such as those by Essa et al[31] and Mohamed et al[32] for Kuwait (5.3%) and Egypt (4%), respectively.

The overall incidence of influenza A and B viruses (9.9%) was similar to studies conducted in Iran (5.5%),[33] Malaysia (11%),[34] and Turkey (12.6%),[12] respectively. The influenza A virus isolates detected in the study were exclusively 2009 pandemic H1N1 viruses. In our study, 6.3% of the samples were tested positive for hAdV, similar to numbers reported for Kuwait (7.7%),[31] higher than those reported for Iran (3.4%),[35] and lower than those reported for Gabon (17.5%).[36]

The detection rate for PIV 1 was 4-fold higher than that of PIV 3 in our study (5.9% vs 1.5%), which differed from the report by He et al[37] in Shenzhen, China, but was similar to the data reported by Al-Ayed et al[13] for Saudi Arabia (4.6% and 0.9% for PIV 1 and PIV 3, respectively). Only very few of the patients in our study group (2.2%) were found to be infected with HBoV. He et al[37] reported 4.9% for a Chinese study group, while Bicer et al[12] and Fabbiani et al[16] reported detection rates of 0.9% for Turkey and 1.7% for Italy, respectively.

During the study period, HCoVs were detected only in a single case, in which HCoV-NL63 could be identified, indicating a minor role of these viruses in causing severe forms of ARIs in the Erbil City population. This result was similar to a study conducted in Kuwait, which

failed to detect any HCoV-NL63 infections in 735 hospitalized patients, while higher numbers were reported for other neighboring countries, such as Turkey (2.9%) and Saudi Arabia (3.7%).[12,13,31] The current study's single HCoV-NL63-positive patient was less than 8 months old, confirming that HCoV-NL63 is more frequently identified in ARIs in younger children.[38] Despite this low detection rate for HCoVs, the viruses appear to be circulating in this geographic region because a large proportion of serum samples taken from the study group was confirmed to be seropositive for HCoV-NL63 (69%) and HCoV-229E (65.5%) as determined by an enzyme-linked immune sorbent assay–based assay using recombinant N protein as antigen (data not shown). It is tempting to speculate that common HCoVs (in contrast to Middle East respiratory syndrome and severe acute respiratory syndrome coronavirus and other respiratory viruses) cause less severe ARIs that only in very few cases require hospitalization. More studies (including virological studies of ARIs in pediatric outpatient departments) are required to explore this possibility.

With the development of new molecular diagnostic methods, particularly multiplex PCR assays, significantly larger numbers of viral coinfections have been detected in recent years.[39,40] In our study, viral coinfections were confirmed in 15.6% of the patients, which was similar to other studies, such as those published by Akinloye et al[10] (16%), He et al[37] (14.9%), and Bicer et al[12] (20.4%). In our study group, dual infections involving HEV/HRV were most common, many of which occurring in combinations with RSV (12/42; 28.5%) or hMPV (9/42, 21.4%).

Regarding the seasonality of virus-associated infections in the study area of Erbil City, the study clearly shows that, in the summer (ie, the dry season in Kurdistan), the number of respiratory infections was greatly reduced. This is consistent with previous studies, showing a low-level circulation of respiratory viruses during the dry season. Seasonality of respiratory virus infections is known to vary due to temperature and humidity variations.[37,41] The study area has 4 distinct seasons, with lowest temperatures usually occurring in January (average 8°C). From February to May, the average temperature varies between 12°C and 30°C with increasing rainfall. After April, the weather turns hot (average 40°C) and remains dry during the summer months.[42] In our study, the vast majority of ARI in children occurred between November and March, demonstrating a late autumn to early spring seasonality of viral respiratory infections in children in this geographic region.

To get more insight into the epidemiology of ARIs in the Kurdistan region of Iraq, larger study groups involving more than one center should be used for monitoring respiratory pathogens. Also, (sub)typing of human enterovirus and rhinovirus isolates can be expected to provide additional insight into possible strain-specific differences in causing severe forms of ARIs requiring hospitalization. Finally, additional assays need to be included to detect viral respiratory pathogens not detected in the present study, such as cytomegalovirus, respiratory polyomaviruses, and human parechoviruses.

In conclusion, despite some limitations of the study (see above), the main findings of this prospective study provide valuable epidemiological information on the viral etiology of ARIs in Erbil, Kurdistan region, Iraq. They confirm the major role of respiratory viruses in ARIs in hospitalized children in the study area, particularly in young infants younger than 5 years of age. HEV/HRV, RSV, and hMPV were the most frequent pathogens, accounting for more than two thirds of the ARIs cases. Further studies including neighboring geographic regions, normalized and larger age groups, and extended sampling periods of several consecutive years are now being planned and will be used to establish a robust epidemiological database for respiratory virus infections in the Kurdistan region of Iraq.

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Author Contributions

DAH: Contributed to conception and design; contributed to acquisition, analysis, and interpretation; drafted manuscript; agrees to be accountable for all aspects of work ensuring integrity and accuracy.
SKR: Contributed to conception and design; contributed to analysis and interpretation; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.
JZ: Contributed to conception and design; contributed to analysis and interpretation; critically revised manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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References

1. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev*. 2010;23:74-98.
2. Mahony JB, Petrich A, Smieja M. Molecular diagnosis of respiratory virus infections. *Crit Rev Clin Lab Sci*. 2011;48:217-249.
3. Doan Q, Enarson P, Kissoon N, Klassen TP, Johnson DW. Rapid viral diagnosis for acute febrile respiratory illness in children in the emergency department. *Cochrane Database Syst Rev*. 2009;(4):CD006452.
4. Ferkol T, Schraufnagel D. The global burden of respiratory disease. *Ann Am Thorac Soc*. 2014;11:404-406.
5. Berry M, Gamieldien J, Fielding BC. Identification of new respiratory viruses in the new millennium. *Viruses*. 2015;7:996-1019. doi:10.3390/v7030996.
6. Babady NE, Mead P, Stiles J, et al. Comparison of the luminex xTAG RVP fast assay and the Idaho technology film array RP assay for detection of respiratory viruses in pediatric patients at a cancer hospital. *J Clin Microbiol*. 2012;50:2282-2288.
7. Pabbaraju K, Wong S, Tokaryk KL, Fonseca K, Drews SJ. Comparison of the luminex xTAG respiratory viral panel with xTAG respiratory viral panel fast for diagnosis of respiratory virus infections. *J Clin Microbiol*. 2011;49:1738-1744.
8. Kaplan NM, Dove W, Abd-Eldayem SA, Abu-Zeid AF, Shamoon HE, Hart CA. Molecular epidemiology and disease severity of respiratory syncytial virus in relation to other potential pathogens in children hospitalized with acute respiratory infection in Jordan. *J Med Virol*. 2008;80:168-174.
9. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol*. 2010;82:1282-1290. doi:10.1002/jmv.21790.
10. Akinloye OM, Ronkko E, Savolainen-Kopra C, et al. Specific viruses detected in Nigerian children in association with acute respiratory disease. *J Trop Med*. 2011;2011:690286. doi:10.1155/2011/690286.
11. Do HL, van Doorn HR, Nghiem MN, et al. Viral etiologies of acute respiratory infections among hospitalized Vietnamese children in Ho Chi Minh City, 2004-2008. *PLoS One*. 2011;6:e18176. doi:10.1371/journal.pone 0018176.
12. Bicer S, Giray T, Çöl D, et al. Virological and clinical characterizations of respiratory infections in hospitalized children. *Ital J Pediatr*. 2013;39:22.
13. Al-Ayed MS, Asaad AM, Qureshi MA, Ameen MS. Viral etiology of respiratory infections in children in southwestern Saudi Arabia using multiplex reverse-transcriptase polymerase chain reaction. *Saudi Med J*. 2014;35:1348-1353.
14. Stover CS, Litwin CM. The epidemiology of upper respiratory infections at tertiary care center: prevalence, seasonality, and clinical symptoms. *J Respir Med*. 2014;2014:469393.
15. Debiaggi M, Canducci F, Ceresola ER, Clementi M. The role of infections and coinfections with newly identified and emerging respiratory viruses in children. *Virol J*. 2012;9:247.
16. Fabbiani M, Terrosi C, Martoralli B, et al. Epidemiological and clinical study of viral respiratory tract infections in children from Italy. *J Med Virol*. 2009;81:750-756.
17. Tapparel C, Junier T, Gerlach D, et al. New respiratory enterovirus and recombinant rhinoviruses among circulating picrnaviruses. *Emerg Infect Dis*. 2009;15:719-726.
18. Sentilhes AC, Choumlivong K, Celhay O, et al. Respiratory virus infections in hospitalized children and adults in Lao PDR. *Influenza Other Respir Viruses*. 2013;7:1070-1078.
19. Asner SA, Petrich A, Hamid JS, Mertz D, Richardson SE, Smieja M. Clinical severity of rhinovirus/enterovirus compared to other respiratory viruses in children. *Influenza Other Respir Viruses*. 2014;8:438-442.
20. Kenmoe S, Tchendjou P, Vernet MA, et al. Viral etiology of severe acute respiratory infections in hospitalized children in Cameroon, 2011-2013. *Influenza Other Respir Viruses*. 2016;10:386-393.
21. Liu T, Li Z, Zhang S, et al. Viral etiology of acute respiratory tract infections in hospitalized children and adults in Shandong province, China. *Virol J*. 2015;12:168.
22. Shafik CF, Mohareb EW, Yassin AS, et al. Viral etiologies of lower respiratory tract infections among Egyptian children under five years of age. *BMC Infect Dis*. 2012;12:350.
23. Shameran AR, Al-Mola GA. Detection of respiratory syncytial virus (hRSV) by (PCR) technique in lower respiratory tract infection (LRTI) in in infants and children under Babylon city. *Int J Med Health Sci*. 2014;8:9. http://waset.org/pdf/books/?id=12973. Accessed June 14, 2018.
24. Malekshahi SS, Azad TM, Yavarian J, Shahmahmoodi S, Naseri M, Rezaei F. Molecular detection of respiratory viruses in clinical specimens from children with acute respiratory disease in Iran. *Pediatr Infect Dis J*. 2010;29:931-933.
25. Wang W, Cavailler P, Ren P, et al. Molecular monitoring of causative viruses in child acute respiratory infection in endemo-epidemic situations in Shanghai. *J Clin Virol*. 2010;49:211-218.
26. Kahn JS. Epidemiology of human metapneumovirus. *Clin Microbiol Rev*. 2006;19:546-557.
27. Smuts HE, Zar HJ, Workman L. Prevalence and molecular epidemiology of human metapneumovirus in infants and young children with acute lower airway obstruction. *Curr Allergy Clin Immunol*. 2008;21:193-194.
28. Al-Mola GA, Ragheb A, Abass IR. Human metapneumovirus (hMPV) associated with respiratory infection in children hospitalized with acute lower respiratory tract infection in Hilla, Iraq. *Int J Dis Disord*. 2013;1:20-23.

29. Aziz TA, Salmo N, Bayati AH. Seroprevalence of anti-human metapneumovirus antibodies in hospitalized children in Suleimani City/Iraq. *Br Microbiol Res J*. 2014;4:1325-1334.
30. Albuquerque MC, Varella RB, Santos N. Acute respiratory viral infections in children in Rio de Janeiro and Teresopolis, Brazil. *Rev Inst Med Trop Sao Paulo*. 2012;54:249-255.
31. Essa S, Owayed A, Altawalah H, Khadadah M, Behbehani N, Al-Nakib W. The prevalence of human bocavirus, human coronavirus-NL63, human metapneumovirus, human polyomavirus KI and WU in respiratory tract infections in Kuwait. *Med Princ Pract*. 2015;24:382-387. doi:10.1159/000381422.
32. Mohamed EMS, Reiche J, Jacobsen S, et al. Molecular analysis of human metapneumovirus detected in patients with lower respiratory tract infection in upper Egypt. *Int J Microbiol*. 2014;2014:290793.
33. Kahbazi M, Fahmizad A, Armin S, et al. Aetiology of upper respiratory tract infections in children in Arak city: a community based study. *Acta Microbiol Immunol Hung*. 2011;58:289-296.
34. Khor CS, Sam IC, Hooi PS, Quek KF, Chan YF. Epidemiology and seasonality of respiratory viral infections in hospitalized children in Kuala Lumpur, Malaysia: a retrospective study of 27 years. *BMC Peadiatr*. 2012;12:32.
35. Pourakbari B, Mahmoudi S, Movahedi Z, et al. Viral etiology of acute lower respiratory tract infections in hospitalized young children in a children's referral hospital in Iran. *Turk J Pediatr*. 2014;56:354-359.
36. Lekana-Douki SE, Nkoghe D, Drosten C, Ngoungou EB, Drexler JF, Leroy EM. Viral etiology and seasonality of influenza-like illness in Gabon, March 2010 to June 2011. *BMC Infect Dis*. 2014;14:373.
37. He Y, Lin GY, Wang Q, et al. A 3-year prospective study of the epidemiology of acute respiratory viral infections in hospitalized children in Shenzhen, China. *Influenza Other Respir Viruses*. 2014;8:443-451.
38. Xin Ch, Yong ZZ, Yan L, Dong ZX. Human coronavirus NL63 in hospitalized children with respiratory infection: a 2-year study from Chongqing, China. *Indian Pediatr*. 2012;49:825-828.
39. Lukšić I, Kearns PK, Scott F, Rudan I, Campbell H, Nair H. Viral etiology of hospitalized acute lower respiratory infections in children under 5 years of age—a systematic review and meta-analysis. *Croat Med J*. 2013;54:122-134.
40. Liu WK, Liu Q, Chen DH, et al. Epidemiology of acute respiratory infections in children in Guangzhou: a three-year study. *PLoS One*. 2014;9:e96674. doi:10.1371/journal.pone.0096674.
41. Lu Y, Wang S, Zhang L, et al. Epidemiology of human respiratory viruses in children with acute respiratory tract infections in Jinan, China. *Clin Dev Immunol*. 2013;2013:210490. doi:10.1155/2013/210490.
42. Shahbaz SE. *Pinales: With a Field Guide to the Trees and Shrubs of Kurdistan Region of Iraq*. Dohuk, Iraq: Khaney; 2007.