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Title:
Severe outcomes associated with respiratory viruses in newborns and infants: a prospective viral surveillance study in Jordan

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Contributors:
NKB, NBH, SF, AS, SHV, and JVW were involved in study design, reviewing the data, and interpretation of the data. NKB, NBH, SF, LL, and AS oversaw data and clinical sample collection. LW, BP, and CF were involved in analyzing the data, and BP reviewed the data and created figures. NBH wrote the article, which was revised by SHV, NKB, and JVW and approved by all authors.

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Data sharing statement: No additional data are available.

ABSTRACT

Objective: To assess virus-specific hospitalization rates, risk factors for illness severity, and seasonal trends in children hospitalized with acute respiratory infections (ARI).

Design: Prospective cohort study.

Setting: A government hospital serving low- and middle-income population in Amman, Jordan.

Participants: Children under two years old hospitalized with fever and/or respiratory symptoms (n=3168) from 3/16/2010-3/31/2013. Children with chemotherapy-associated neutropenia and newborns who had never been discharged after birth were excluded from the study.

Outcome Measures: Hospitalization rates and markers of illness severity: admission to intensive care unit (ICU), mechanical ventilation (MV), oxygen therapy, length of stay (LOS), and death.

Results: Of the 3168 subjects, 2581 (82%) had at least one respiratory virus detected, with respiratory syncytial virus (RSV) being the most predominant pathogen isolated. During admission, 1013 (32%) received oxygen therapy, 284(9%) were admitted to ICU, 111(4%) were placed on MV, and 31(1%) children died. Oxygen therapy was higher in RSV only subjects compared to human rhinovirus only (42% vs 29%, p<0.001), adenovirus only (42% vs 21%, p<0.001), and human parainfluenza virus only (42% vs 23% p<0.001) subjects. Underlying medical condition was associated with oxygen therapy (aOR 1.95, 95% CI 1.49-2.56), ICU admission (aOR 2.51, 95%CI 1.71-3.68), MV (1.91, 95% CI 1.11-3.28), and longer LOS (aOR1.71, 95% CI 1.37-2.13). Similarly, younger age was associated with oxygen therapy (0.23, 95% CI 0.17-0.31), ICU admission (aOR 0.47, 95% CI 0.30-0.74), MV (0.28, 95% CI 0.15-0.53), and longer LOS (aOR 0.47, 95% CI 0.38-0.59). Pneumonia was strongly associated with longer LOS (aOR 2.07, 95% CI 1.65-2.60), oxygen therapy (aOR 2.94, 95% CI 2.22-3.89), ICU admission (aOR 3.12, 95% CI 2.16-4.50), and MV (aOR 3.33, 95% CI 1.85-6.00). Virus-specific hospitalization rates ranged from 0.5-10.5 per 1000 children.

Conclusion: Respiratory viruses are associated with severe illness in Jordanian children hospitalized with ARI. Prevention strategies such as extended breastfeeding, increased access to palivizumab and RSV vaccine development could help decrease hospitalization rates and illness severity, particularly in young children with underlying medical conditions.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This three-year prospective surveillance study uses real-time reverse-transcriptase polymerase chain reaction to test a cohort of over 3000 children for 11 major respiratory viruses.
- To our knowledge, this study is the first to report virus-specific hospitalization rates and illness severity in Jordanian children hospitalized with acute respiratory infections (ARI).
- We fit a multivariable logistic model to analyze the risk factors for severe illness, and used a Bayesian hierarchical model to derive the population prevalence of each respiratory virus.
- This study is limited to children from low- and middle-income population, potentially underestimating the true burden of ARI in Jordan.

INTRODUCTION

Acute respiratory infections (ARI) are the leading cause of death beyond the neonatal period in children under five years of age worldwide.[1] Historically, bacterial pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type B have been noted as predominant etiologies;[1-3] however, respiratory viruses are frequently implicated as primary causes as well. A recent study conducted in the U.S. found that respiratory viruses were much more common in children hospitalized with pneumonia than bacteria, with at least one virus detected in 65% of enrolled children.[4] A multicenter study conducted in four developing countries also noted that even though *Streptococcus pneumonia* was associated with increased risk of pneumonia deaths, respiratory viruses were frequently associated with severe, hypoxic pneumonia. [5,6]

Earlier studies have estimated that there were 66,000-199,000 RSV-related deaths in 2005 and 28,000-111,5000 influenza-related deaths in 2008 in children under five, however, these systematic reviews did not include countries in the Middle East due to paucity of population-based studies in the region.[7,8] The seasonality of respiratory viral diseases other than RSV and influenza and their overall burden in in low and middle-income countries (LMICs) also remain poorly defined.[9] Additionally, prior published viral surveillance studies from the region have been limited by their retrospective nature, the use of convenience samples over short time periods, and the lack of use of modern molecular techniques such as real-time reverse-transcriptase polymerase chain reaction (RT-PCR).[10,11] Thus, the true burden and severity of virus-driven ARIs in Middle Eastern children are less defined.

We conducted a prospective surveillance study that tracked ARI-associated hospitalizations over a three-year period among Jordanian children. The objectives of this study were to estimate virus-specific hospitalization rates, identify risk factors for illness severity, and characterize seasonal variations of respiratory viral infections in Jordan.

METHODS

Study Design and Participants

In this prospective surveillance study, we enrolled children under two years old who were hospitalized with fever and/or respiratory symptoms between March 16, 2010 and March 31, 2013 at Al-Bashir Hospital in Amman, Jordan (detailed inclusion/exclusion criteria previously reported).[12] Subjects were recruited five days a week (Sunday through Thursday) within 48 hours of hospital admission.[12] Children with chemotherapy-associated neutropenia and newborns who had never been discharged after birth were excluded from the study.

The institutional review boards at the University of Jordan, the Jordanian Ministry of Health, and Vanderbilt University approved the study. Written informed consent was obtained from parents or guardians.

Setting

Al-Bashir Hospital is one of the three major government hospitals in Amman, which is Jordan's largest city and capital.[12] It was estimated that during the study period, the hospital provided care for ≈55% of all children in Amman (author SF, unpublished data). Additionally, 17,557 children were admitted to the pediatric wards, 11,230 (64%) of whom were under two years of age.

Data and Specimen Collection

After consent, trained local research staff obtained blood either by heel stick or venipuncture, as well as nasal and throat swabs. Standardized questionnaires were used to record demographic, clinical, and socioeconomic data. Parents were queried in Arabic, and bilingual research staff transcribed the information into an English-language case report form at the time of the interview. After subjects were discharged, charts were abstracted for the following: antibiotic use and type, blood, urine, and/or cerebral spinal fluid (CSF) cultures, organisms isolated, chest radiography and results, oxygen use, intensive care unit (ICU) stay, mechanical ventilation (MV), length of stay (LOS) in the hospital, and discharge status.

All data were entered into a standardized, secure REDCap™ (Research Electronic Data Capture, Vanderbilt University, Nashville, TN, USA) database.[13] Data quality checks were performed on a minimum of 10% of the charts and data from all case report forms were verified after entry.

Variables

Admission to ICU, oxygen therapy, MV placement, LOS, and death were considered markers of severe illness. ICU admission included children who were transferred in during their hospital stay or were admitted directly. Smoke exposure included both cigarette and/or hookah pipe (nargila) smokers in household. Underlying medical conditions (UMCs) were classified as the following: diabetes, heart disease, Down syndrome, kidney disease, sickle cell disease, cystic fibrosis, cancer, genetic/metabolic, cerebral palsy, neurological, mental retardation/developmental delay, seizure disorder, chronic diarrhea (e.g. >2 weeks), gastroesophageal reflux disease, immunodeficiency, asthma/reactive airway disease, liver disease, or other. Subjects were categorized as suspected sepsis if they had the admission diagnosis of rule out sepsis or febrile neonate. Covariates included age, gestational age at enrollment, birth weight, sex (male), smoke exposure, breastfeeding status, UMC, vitamin D levels, viral detection, pneumonia, sepsis, bronchiolitis, and bronchopneumonia. Cycle threshold (Ct) values were used as a proxy for viral load, i.e. lower Ct values indicated higher viral load.

Laboratory Testing

Nasal and throat swabs were collected and combined in transport medium (M4RT®, Remel, USA), aliquoted into MagMAX™ Lysis/Binding Solution Concentrate (Life Technologies, USA), snap-frozen, and then stored at -80°C. Aliquots were shipped on dry ice to Nashville, Tennessee, USA, and were tested by RT-PCR for 11 viruses: RSV; human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A, B, and C; parainfluenza (PIV) virus 1, 2, and 3; adenovirus (AdV), and Middle East respiratory syndrome coronavirus (MERS-CoV).[14,15] Blood was placed directly onto filter paper and air dried for ≥30 minutes before storage at room temperature and kept dry through shipment to ZRT Laboratory (Beaverton, OR, USA) for 25(OH)D (vitamin D) level measurement (techniques published previously).[16]

Statistical Analysis

Descriptive statistics were presented as frequency (percentage) or median and interquartile range (IQR) where appropriate. Categorical variables were compared using Pearson Chi-square tests. Continuous variables were compared using Mann-Whitney U test. For comparisons of continuous variables for ≥3 groups, Kruskal-Wallis tests were used. We fit a multivariable logistic model to analyze the risk factors for oxygen therapy, ICU admission, MV, or longer LOS. Risk factors included breastfeeding, vitamin D level, age at enrollment, gestational age, sex, UMC, smoke exposure (both cigarette and hookah), viral detection, and four admission diagnoses (pneumonia, suspected sepsis, bronchiolitis, and bronchopneumonia) based on literature review.[17] All analyses were performed using statistical software R version 3.1.2 (http://www.R-project.org/). Bonferroni adjustments were made to account for multiple comparisons in univariate analyses.

Models for each virus were fitted using Markov chain Monte Carlo.[18,19] Models were run for 100,000 iterations, with the first 90,000 iterations conservatively discarded as burn-in. Models were checked for convergence using the Gelman-Rubin diagnostic.[20,21]

Rate Calculation

Al-Bashir Hospital admissions data were used to estimate the population prevalence of each respiratory virus. These data were filtered to exclude admissions of individuals not residing in greater Amman. We used a Bayesian hierarchical model to derive estimates for each of the three years of the study. [20] Estimates of the under 2-year-old Jordanian population were obtained from the World Bank online database, and the proportion of the population residing in Amman (35%) was taken from the 2012 national census. These values were used in a binomial model to estimate the population of children <2 years of age in greater Amman in 2010-2012. A binomial data likelihood was specified:

$$y_{a,t}^{v} \sim Binomial\left(n_{a,t}, p_{a,t}^{v}\right)$$

where $y_{a,t}^{v}$ is the number of children of age *a* in year *t* at Al-Bashir observed with virus *v*, while $n_{a,t}$ is the population of children age *a* in year *t* eligible for sampling, and $p_{a_t}^{v}$ the prevalence of virus *v* among children age

a in year *t*. The sampled population $n_{a,t}$ was similarly estimated from a binomial model that sampled from $N_{a,t}$ the total Amman population of age *a* in year *t*:

$$n_{a,t} \sim Binomial(N_{a,t}, \pi)$$

Here, π is the proportion of the Amman population that is eligible to be sampled, which accounts for the market share m and the sampling intensity (5 days out of 7):

$$\pi = m(5/7)$$

The market share for Al-Bashir Hospital was modeled as a random variable, and given a uniform prior distribution between 50% and 60%, based on prior information. Prevalence was given diffuse beta(1,5) priors for all models. Note that the model structure implies all individuals carrying the virus in question seek hospital care.

Data sharing statement: Technical appendix, statistical code, and dataset available upon request with proper ethical approval.

RESULTS

Study Population

From March 16, 2010 through March 31, 2013, we screened and confirmed eligibility for 3793 patients. Of the 3793 patients, 618 had parent/guardian refusal, three were determined to be ineligible after enrollment due to age, and four were diagnosed with meningitis. Our final sample consisted of 3168 subjects.

Demographics and Clinical Characteristics

The median age was 3.5 months, (range 0.07-23.96 months), 60% were male, 12% had UMCs, and 14% were premature (<37 weeks gestation) (table 1). Of the 375 subjects with UMCs, heart disease was the most common, 146/357 (39%). Nearly 90% of the children's parents self-identified as Jordanian, and 7% reported Palestinian as their nationality. Primary and secondary education was the highest attainment in 41% and 44% of the mothers respectively. The median birth weight was 3.0 kg, and 28% were born by cesarean section. The median number of siblings was two, 1.6% attended daycare, and 77% were exposed to smoke (73% and 18% to cigarette and hookah, respectively).

Prior to hospitalization, 41% of the children had received antibiotics, and 92% were administered an antibiotic during their hospital stay (table 1). The seven main admission diagnoses included: bronchopneumonia (32%), suspected sepsis (28%), bronchiolitis (17%), pneumonia (12%), pertussis-like cough (7%), asthma/reactive airway disease (5%), and febrile seizure (3%). The median length of stay was 5 days, with 9% admitted to the ICU, 4% on MV, and 32% receiving oxygen therapy (table 1). Of the 2688 (85%) subjects who were tested for vitamin D levels, 49% had vitamin D deficiency (<20 ng/mL). The median level was 16.5 ng/mL (IQR 5.2, 26)(table 1). During the study period, 31 (1%) children died (table 1). Among the 31 subjects, 21 (68%) had at least one respiratory virus detected (8 HRV-positive; 5 RSV-positive; 1 AdV-positive; 1 Flu-positive; and 6 co-infection cases).

Viral Detection

At least one virus was detected in 2581 (81.5%) of the 3168 children. RSV (1397, 44%) was the most common virus detected, followed by HRV (1238, 39%), AdV (475, 15%), HMPV (273, 9%), PIV1-3 (175, 6%), and Flu A-C (119, 4%) (figure 1A). MERS-CoV was not detected in any sample. Viral co-detection was common, with 944/2581 (37%) having at least one other virus detected, ranging from 48% with RSV to 78% with AdV (figure 1A). Single virus detection (i.e., excluding the children with viral co-detection) was 728 for RSV only, 541 for HRV only, 106 for AdV only, 128 for HMPV only, 84 for PIV1-3 only, and 50 for Flu A, B and C only. Comparison of Ct values for single virus detection with co-detection revealed a slightly higher viral load for single detection for RSV (25.2 vs. 26.4, p<0.001); HRV (30.1 vs. 31.5, p<0.001); HMPV (29.0 vs. 32.0, p<0.001); and PIV (28.8 vs 32.4), p<0.01) cases. No significant differences in Ct values were seen for Flu and

AdV cases. Figure 1B shows the viral distribution by age, with viral co-detection common in all age groups. The percentage of viral detection by admission diagnoses are displayed in Figure 1C. The frequency and distribution of all viruses over the three-year period is displayed in Figure 2, with >95% viral detection in the winter months, predominately RSV.

Hospitalization Rates

Of our 3168 subjects, 3048 (96.2%) resided in Amman. The highest rates of hospitalization were due to RSV in years one (7.8 per 1000 children) and two (8.4 per 1,000 children) and HRV in year 3 (9.6 per 1,000). Hospitalization rates were higher in children under six months old for all viruses compared to the older age groups, and those who were 6-11 months had higher rates compared to 12-23 months, based on non-overlapping credible intervals (figure 3).

Clinical Outcomes

Virus-positive children were significantly more likely to require oxygen therapy (34% vs 23%, p<0.001) and receive an antibiotic prior to admission (43% vs 32%, p<0.001) (table 1) compared to virus-negative subjects. They were also more likely to present with cough (82% vs. 42%, p<0.001) and shortness of breath (63% vs. 34%, p<0.001); have wheezing (60% vs. 37%, p<0.001), flaring (45% vs. 24%, p<0.001), and cyanosis (21% vs. 14%, p<0.001) on physical examination; and have the diagnoses of bronchopneumonia (34% vs. 23%, p<0.001), bronchiolitis (19% vs. 7%, p<0.001), and pneumonia (14% vs. 7%, p<0.001) compared to virus-negative children. In contrast, virus-negative children were significantly more likely to be younger (table 1); present with fever (64% vs. 54%, p<0.001), vomiting (23% vs. 15%, p<0.001), diarrhea (15% vs. 9%, p<0.001), poor appetite (29% vs. 20%, p<0.001), and seizures (7% vs. 3%, p<0.001); have a diagnosis of suspected sepsis (50% vs. 24%, p<0.001) and febrile seizure (5% vs. 2%, p<0.001)..

When comparing RSV only with HRV only, AdV only, HMPV only, PIV only and Flu only, RSV-positive children were significantly younger than HMPV-positive children (3.4 months vs 6.1 months, p<0.001) (table 1). RSV-positive subjects were significantly more likely to require oxygen therapy compared to HRV (42% vs 29%, p<0.001), AdV (42% vs 21%, p<0.001), and PIV-positive children (42% vs 23%, p<0.001) (table 1). Additionally, RSV-positive children were significantly more likely to have lower vitamin D levels (15.3 ng/mL vs 18.5 ng/mL, p<0.001), receive antibiotics prior to hospitalization (47% vs 32%, p<0.001), and less likely to have UMCs than HRV-positive children (9% vs 15%, p<0.001) (table 1). RSV-positive subjects significantly were less likely to present with fever and more likely to present with cough, shortness of breath, and have flaring on exam compared to HRV, AdV, PIV, and Flu-positive children *(supplemental figure 1)*. RSV-positive subjects significantly were less likely to have seizures/convulsions but more likely to have wheezing on exam compared to HRV and AdV-positive children *(supplemental figure 1)*. RSV-positive subjects significantly were less likely to present with diarrhea but more likely to have cyanosis on exam compared to HRV, Flu, and AdV-positive children *(supplemental figure 1)*.

Risk Factors for Illness Severity

Longer LOS was associated with younger age (aOR 0.47, 95% CI 0.38-0.59), lower gestational age (aOR 0.93, 95% CI 0.86-0.99), lower birth weight (aOR 0.85, 95% CI 0.75-0.96), lack of breast feeding (aOR 0.72, 95% CI 0.59-0.88), lower vitamin D level (aOR 0.86, 95% CI 0.76-0.98), UMC (aOR 1.71, 95% CI 1.37-2.13), virus detection (1.24, 95% CI 1.03-1.48), and diagnoses of pneumonia (aOR 2.07, 95% CI 1.65-2.60) or suspected sepsis (aOR 2.44, 95% CI 1.94-3.06)(table 2). Since early deaths would distort this observation, a sensitivity analysis excluding children who died was performed and still showed a significant association. Younger age (aOR 0.23, 95% CI 0.17-0.31), lower gestational age (0.90, 95% CI 0.82-0.98), lack of breast feeding (0.67, 95% CI 0.40-0.94), lower vitamin D levels (0.82, 95% CI 0.70-0.97), UMC (1.95, 95% CI 1.49-2.56), virus detection (aOR 1.34, 95% CI 1.04-1.71), sex (aOR 0.83, 95% CI 0.70-0.99), and diagnosis of pneumonia (aOR 2.94, 95% CI 2.22-3.89) or sepsis (aOR 0.21, 95% CI 0.15-0.27) were associated with oxygen therapy (table 2). Diagnosis of pneumonia (3.12, 95% CI 2.16-4.50), younger age (0.47, 95% 0.30-0.74), UMC (aOR 2.51, 95% CI 1.71-3.68), and lack of breastfeeding (aOR 0.63, 95% CI 0.44-0.91), were associated with an ICU stay (table 2). MV was associated with a diagnosis of pneumonia (aOR 3.33, 95% CI 1.85-6.0), or bronchopneumonia (aOR 2.03, 95% CI 1.05-3.90), younger age (0.28, aOR (0.15-0.53), and UMC (1.91, 95% CI 1.11-3.28), but was less likely with the diagnosis of suspected sepsis (0.48, 95% 0.26-0.89) (table 2). The number of deaths was too low to support a multivariable analysis. However, in a univariate model, younger age, lack of breast feeding, underlying medical condition, virus negativity, and diagnoses of

pneumonia or suspected sepsis were all associated with death, while bronchiolitis or bronchopneumonia were less likely to be associated with death (table 2).

DISCUSSION

Our study revealed that respiratory viruses are the predominant pathogens among young hospitalized children who present with fever and/or respiratory symptoms in Amman, Jordan. The most common diagnoses were bronchopneumonia, suspected sepsis, bronchiolitis, pneumonia, and pertussis-like cough. Only one-fifth of the children enrolled had no virus detected. Consistently, during each of the three winter seasons, ~95% of children hospitalized during winter months tested positive for a virus, with RSV and HRV being the most common viruses identified. Both RSV and HRV rates were higher than expected compared to US-based studies.[17,22,23] Specifically, RSV rates were similar to rates from Turkey, Norway, and Austria [24-28]; higher than the US and Netherlands;[22,29] and lower than UK, Spain, and Denmark.[30-33] Rates of influenza, HMPV, and PIV1-3 in our cohort were similar to US reports[34-37]. Our estimates of the burden of influenza, HRV, HMPV, PIV 1-3, and RSV fill a gap in knowledge from the Middle East.[7,8]

Hospitalized children who were virus-negative were more likely to be younger, febrile, and symptomatic with non-respiratory symptoms. Moreover, the overall illness severity of virus-negative children was greater than those with a confirmed viral cause, with more frequent suspected sepsis, seizures, ICU admission, and/or mortality. In contrast, virus-positive children were more likely to present with severe lower respiratory tract infection (LRTI) as indicated by higher frequency of cough and shortness of breath, wheezing, flaring, and cyanosis on exam, and increased oxygen therapy requirement. These clinical differences were dramatic, and may serve as helpful clinical predictors of likely viral diagnosis prior to the availability of more definitive laboratory findings. In addition, 68% of the children who died had at least one respiratory virus detected, noting the association of severe disease with viral detection in these children. Since antibiotic use was nearly universal in children with positive detection of respiratory viruses, targeted interventions for antibiotic stewardship may also be warranted.

Since RSV was the most common virus detected, we compared the clinical presentations of RSV only to single detection of other respiratory viruses. RSV only children were more likely to present with cough and shortness of breath; have flaring, wheezing, and cyanosis on physical exam; and less likely to present with fever, diarrhea, and seizures compared to other respiratory viruses. Interestingly, the clinical presentations of RSV and HMPV were indistinguishable, other than age: HMPV only children were older. A surveillance study of RSV-positive children in India found the presence of cough, fast-breathing, crepitation, and hypoxia to be independent predictors of RSV infection.[38] Durani et al. found the combination of cough, wheezing, and retractions to be good predictors of RSV infection.[38,39] Our study also found cough to be a strong predictor for the presence of virus, including RSV. In addition, the RSV-positive children in our study were more likely to require oxygen therapy, suggesting these children presented with more severe LRT disease. We also documented RSV being more severe than other viruses in a previous pilot study.[14] Therefore, if preventive measures such as vaccines become available for RSV, Jordanian children would benefit greatly.

We examined independent risk factors for several measures of illness severity: LOS, oxygen therapy, ICU admission, MV, and death. Universally, younger age, UMC, and the diagnosis of pneumonia were associated with all five illness severity markers. Lack of breastfeeding was associated with all severity markers except for MV. Therefore, the promotion of breastfeeding is an important public health intervention for reducing the severity of respiratory illnesses. Our study found that lower vitamin D levels were associated with longer LOS and higher probability of oxygen use. Two studies identified an association between lower infant vitamin D levels and increased risk of ARI, and one study found lower levels of vitamin D in children admitted into the ICU with bronchiolitis or pneumonia compared to those admitted to the wards.[40-42] Observational evidence supports further interventional studies to determine if vitamin D supplementation could reduce respiratory illness severity in this population.

A principal strength of our study was its basis of over three full years of surveillance data from a public hospital serving the poor and lower middle class children of a large city in the Middle East North Africa (MENA) region. Prior to this study, very few large prospective studies of respiratory viral diseases of children have been completed in this setting. Our ongoing surveillance enabled us to test samples during the time of the first MERS-CoV outbreak in 2012, confirming that none of the children in this study were admitted with MERS-CoV.[43] Our state-of-the-art molecular diagnostics give one of the best assessments to date of respiratory viral etiologies of hospitalized children in any MENA nation. Limitations included having only five days of surveillance per week, though we did weighted analysis in our population burden estimates to adjust for missing days. Also, we did not test for all respiratory viruses (e.g., parainfluenza 4, non-MERS coronaviruses), so viral burden is underestimated. Lastly, this study is limited to children from low- and middle-income population, and thus is not generalizable to the entire population of Jordan.

Respiratory viral burden is likely to be substantial for the entire MENA region.[7] Prevention strategies such as breastfeeding promotion, vitamin D supplementation, and future RSV vaccines could reduce regional ARI burden. Further studies to include a more generalizable population are needed.

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References:

1. Madhi SA, Klugman KP. Acute Respiratory Infections. 2006.
2. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ 2008;86:408-16.
3. Rudan I, O'Brien KL, Nair H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health 2013;3:10401.
4. Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med 2015;372:835-45.
5. Benet T, Sanchez Picot V, Messaoudi M, et al. Microorganisms Associated With Pneumonia in Children <5 Years of Age in Developing and Emerging Countries: The GABRIEL Pneumonia Multicenter, Prospective, Case-Control Study. Clin Infect Dis 2017.
6. Benet T, Picot VS, Awasthi S, et al. Severity of Pneumonia in Under 5-Year-Old Children from Developing Countries: A Multicenter, Prospective, Observational Study. Am J Trop Med Hyg 2017;97:68-76.
7. Nair H, Brooks WA, Katz M, et al. Global Burden of Respiratory Infections Due to Seasonal Influenza in Young Children: A Systematic Review and Meta-Analysis. The Lancet 2011;378:1917-30.
8. Nair H, Nokes DJ, Gessner BD, et al. Global Burden of Acute Lower Respiratory Infections Due to Respiratory Syncytial Virus in Young Children: A Systematic Review and Meta-Analysis. Lancet 2010;375:1545-55.
9. Rudan I, Lawn J, Cousens S, et al. Gaps in Policy-Relevant Information on Burden of Disease in Children: A Systematic Review. The Lancet 2005;365:2031-40.
10. Naghipour M, Cuevas LE, Bakhshinejad T, Dove W, Hart CA. Human Bocavirus in Iranian Children with Acute Respiratory Infections. Journal of Medical Virology 2007;79:539-43.
11. Al-Sonboli N HC, Al-Aeryani A, Banajeh SM, Al-Aghbari N, Dove W, Cuevas LE. Respiratory Syncytial Virus and Human Metapneumovirus in Children with Acute Respiratory Infections in Yemen. The Pediatric Infectious Disease Journal 2005;24:734-6.
12. Halasa N, Williams J, Faouri S, et al. Natural history and epidemiology of respiratory syncytial virus infection in the Middle East: Hospital surveillance for children under age two in Jordan. Vaccine 2015;33:6479-87.
13. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap) - A Metadata-Driven Methodology and Workflow Process for Providing Translational Research Informatics Support. Journal of Biomedical Informatics 2009;42:377-81.
14. Khuri-Bulos N, Williams JV, Shehabi AA, et al. Burden of Respiratory Syncytial Virus in Hospitalized Infants and Young Children in Amman, Jordan. Scandinavian Journal of Infectious Diseases 2010;42:368-74.
15. Miller EK, Khuri-Bulos N, Williams JV, et al. Human Rhinovirus C Associated with Wheezing in Hospitalized Children in the Middle East. Journal of Clinical Virology 2009;46:85-9.
16. Khuri-Bulos N, Lang RD, Blevins M, et al. Vitamin D Deficiency among Newborns in Amman, Jordan. Global Journal of Health Science 2014;6:162-71.
17. Hall CB, Weinberg GA, Iwane MK, et al. The Burden of Respiratory Syncytial Virus Infection in Young Children. New England Journal of Medicine 2009;360:588-98.
18. Steve Brooks AG, Galin Jones, Xiao-Li Meng. Handbook of Markov Chain Monte Carlo: Chapman and Hall/CRC 2011.
19. Patil A, Huard D, Fonnesbeck CJ. PyMC: Bayesian Stochastic Modelling in Python. Journal of statistical software 2010;35:1-81.
20. Gelman A, Rubin DB. Inference from Iterative Simulation Using Multiple Sequences. Statistical Science 1992;7:457-72.
21. Bayesian Data Analysis. Third ed: Chapman and Hall/CRC; 2013.

22. Hall CB, Weinberg GA, Blumkin AK, et al. Respiratory Syncytial Virus–Associated Hospitalizations Among Children Less Than 24 Months of Age. Pediatrics 2013.
23. Miller EK, Lu X, Erdman DD, et al. Rhinovirus-Associated Hospitalizations in Young Children. The Journal of Infectious Diseases 2007;195:773-81.
24. Hacımustafaoğlu M CS, Bozdemir SE, Ozgür T, Ozcan I, Güray A, Cakır D. RSV Frequency in Children Below 2 Years Hospitalized for Lower Respiratory Tract Infections. The Turkish Journal of Pediatrics 2013;55:130-9.
25. Weigl JAI, Puppe W, Schmitt HJ. Incidence of Respiratory Syncytial Virus-Positive Hospitalizations in Germany. European Journal of Clinical Microbiology and Infectious Diseases 2001;20:452-9.
26. Fjaerli H-O, Farstad T, Bratlid D. Hospitalisations for respiratory syncytial virus bronchiolitis in Akershus, Norway, 1993–2000: a population-based retrospective study. BMC Pediatr. 2004 Dec 17;4:25.
27. Resch B, Gusenleitner W, Mandl C, Müller W. Epidemiology of respiratory syncytial virus infection in Southern Austria. Pediatr Infect Dis J. 2000 Jun;19(6):587–8.
28. Resch B GW, Müller W. The Impact of Respiratory Syncytial Virus Infection: A Prospective Study in Hospitalized Infants Younger than 2 Years. Infection 2002;30:193-7.
29. Zomer-Kooijker K, Uiterwaal CSPM, van der Gugten AC, Wilbrink B, Bont LJ, van der Ent CK. Decreased Lung Function Precedes Severe Respiratory Syncytial Virus Infection and Post-Respiratory Syncytial Virus Wheeze in Term Infants. European Respiratory Journal 2014;44:666.
30. Gil-Prieto R, Gonzalez-Escalada A, Marín-García P, Gallardo-Pino C, Gil-de-Miguel A. Respiratory Syncytial Virus Bronchiolitis in Children up to 5 Years of Age in Spain: Epidemiology and Comorbidities: An Observational Study. Medicine 2015;94:e831.
31. Hervás D, Reina J, Yañez A, del Valle JM, Figuerola J, Hervás JA. Epidemiology of Hospitalization for Acute Bronchiolitis in Children: Differences Between RSV and Non-RSV Bronchiolitis. European Journal of Clinical Microbiology & Infectious Diseases 2012;31:1975-81.
32. Haerskjold A, Kristensen K, Kamper-Jørgensen M, Nybo Andersen A-M, Ravn H, Graff Stensballe L. Risk Factors for Hospitalization for Respiratory Syncytial Virus Infection: A Population-based Cohort Study of Danish Children. Pediatr Infect Dis J. 2016 Jan;35(1):61–5.
33. Kristensen K DT, Frederiksen PS, Ibsen J, Iyore E, Jensen AM, Kjaer BB, Olofsson K, Pedersen P, Poulsen S. Epidemiology of Respiratory Syncytial Virus Infection Requiring Hospitalization in East Denmark. The Pediatric Infectious Disease Journal 1998;17:996-1000.
34. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-Based Surveillance for Hospitalizations Associated With Respiratory Syncytial Virus, Influenza Virus, and Parainfluenza Viruses Among Young Children. Pediatrics 2004;113:1758.
35. Williams JV, Edwards KM, Weinberg GA, et al. Population-Based Incidence of Human Metapneumovirus in Hospitalized Children. The Journal of infectious diseases 2010;201:1890-8.
36. Edwards KM, Zhu Y, Griffin MR, et al. Burden of Human Metapneumovirus Infection in Young Children. The New England journal of medicine 2013;368:633-43.
37. Poehling KA, Edwards KM, Weinberg GA, et al. The Underrecognized Burden of Influenza in Young Children. New England Journal of Medicine 2006;355:31-40.
38. Saha S, Pandey BG, Choudekar A, et al. Evaluation of Case Definitions for Estimation of Respiratory Syncytial Virus Associated Hospitalizations Among Children in a Rural Community of Northern India. Journal of Global Health 2015;5:010419.
39. Durani Y, Friedman MJ, Attia MW. Clinical Predictors of Respiratory Syncytial Virus Infection in Children. Pediatrics International 2008;50:352-5.
40. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D Deficiency in Young Children with Severe Acute Lower Respiratory Infection. Pediatric Pulmonology 2009;44:981-8.

41. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of Subclinical Vitamin D Deficiency with Severe Acute Lower Respiratory Infection in Indian Children Under 5y. European Journal of Clinical Nutrition 0000;58:563-7.
42. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A. Association of Subclinical Vitamin D Deficiency in Newborns with Acute Lower Respiratory Infection and their Mothers. uropean Journal of Clinical Nutrition 2007;63:473-7.
43. Khuri-Bulos N, Payne DC, Lu X, et al. Middle East Respiratory Syndrome Coronavirus Not Detected in Children Hospitalized with Acute Respiratory Illness in Amman, Jordan, March 2010 to September 2012. Clinical Microbiology and Infection 2014;20:678-82.

Figure Legends:

Figure 1. The viral pathogens detected in our Jordanian surveillance study. Figure 1A. The total number of pathogens detected by individual virus, with co-detection of viral pathogens within each virus. Figure 1B. The proportion of viral pathogens detected by age group by single infection, co-infection, and no viral pathogen. Figure 1C is the proportion of individual virus detection, co-infection, and no viral pathogen by admission diagnoses.

Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

Figure 2. The distribution of the total number of viruses detected over three-years by individual month in Amman, Jordan.

Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

Figure 3. Hospitalization rates by age group and years

Supplemental Figure 1. The comparison of presenting symptoms and physical findings by single detection of the individual viruses. * denotes $p<0.001$ when comparing RSV-only to the each individual virus.

Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

	All (N=3168)	Virus-Negative (n=587)	Virus-Positive (n=2581)	>1 Virus (n=944)	RSV Only§ (n=728)	HRV Only (n=541)	AdV Only (n=106)	HMPV Only (n=128)	PIV 1-3 Only (n=84)	Flu A-C Only (n=50)
Age (Months)*	3·5 (1·6, 8·5)	2·4 (1·1, 7·8)	3·8† (1·8, 8·6)	3·9 (1·8, 8·4)	3·4 (1·7, 7·1)	3·3 (1·6, 8·6)	6·1 (1·9, 12·1)	6·1† (2·9, 9·8)	5·3 (1·9, 11·1)	6·6 (1·7, 14·7)
Sex (Male)	1912 (60)	357 (61)	1555 (60)	568 (60)	433 (59)	332 (61)	69 (65)	77 (60)	52 (62)	24 (48)
Breastfeeding	2661 (84)	494 (84)	2167 (84)	794 (84)	627 (86)	444 (82)	91 (86)	103 (80)	68 (81)	40 (80)
No Underlying Medical Condition	2793 (88)	502 (86)	2291 (89)	851 (90)	665 (91)	458 (85)†	93 (88)	113 (88)	70 (83)	41 (82)
Smoke Exposure	2425 (77)	451 (77)	1974 (76)	720 (76)	558 (77)	411 (76)	82 (77)	98 (77)	65 (77)	40 (80)
Antibiotics Prior to Hospitalization	1286 (41)	187 (32)	1099 (43)†	420 (44)	339 (47)	174 (32)†	48 (45)	66 (52)	31 (37)	21 (42)
Antibiotics during Hospitalization	2891 (92)[a]	532 (91)[b]	2359 (92)[c]	872 (93)	659 (91)	488 (91)	96 (91)	119 (94)	79 (94)	46 (92)
Preterm Birth	450 (14)	88 (15)	362 (14)	141 (15)	89 (12)	80 (15)	15 (14)	20 (16)	12 (14)	5 (10)
Oxygen Therapy	1013 (32)[d]	136 (23)[e]	877 (34)†[f]	331 (35)	305 (42)	152 (29)†	22 (21)†	39 (31)	19 (23)†	9 (18)
Mechanical Ventilation	111 (4)[g]	22 (4)[e]	89 (3)[h]	30 (3)	29 (4)	20 (4)	3 (3)	2 (2)	4 (5)	1 (2)
Any ICU Stay	284 (9)[i]	66 (11)[e]	218 (9)[j]	86 (9)	63 (9)	53 (10)	4 (4)	4 (3)	5 (6)	3 (6)
LOS (Days)*	5 (3, 7)[k]	5 (3, 8)[e]	5 (3, 7)[l]	5 (3, 7)	5 (3, 7)	5 (3, 8)	4 (2-7)	4 (3-6)	5 (3-8)	5 (3-6)
Death	31 (1)[m]	10 (2)[n]	21 (1)[o]	6 (1)	5 (1)	8 (1)	1 (1)	0 (0)	0 (0)	1 (2)
Vitamin D Level (ng/mL)*	16·5[p] (5·2, 26·0)	16·0[q] (6·0, 25·6)	16·7[r] (5·0, 26·0)	15·8 (4·4, 25·9)	15·3 (3·7, 25·6)	18·5† (7·9, 26·2)	19·9 (6·7, 31·3)	16·8 (8·6, 26·3)	17·9 (7·9, 26·3)	17·3 (4·1, 25·7)

Abbreviations: RSV, Respiratory syncytial virus; HRV, Human rhinovirus; AdV, Adenovirus; HMPV, Human Metapneumovirus; PIV, Parainfluenza; Flu, Influenza; ICU, Intensive Care Unit; LOS, length of stay

Data are n (%) or *median (IQR). †p-value<0·001; calculated using Pearson chi-square test for categorical variables and Wilcoxon rank-sum test for continuous variables. § RSV Only was the reference group for comparison between viruses.

[a]n=3147; [b]n=586; [c]n=2561; [d]n=3137; [e]n=585; [f]n=2552; [g]n=3136; [h]n=2551; [i]n=3140; [j]n=2555; [k]n=3139; [l]n=2554; [m]n=3136; [n]n=583; [o]n=2553; [p]n=2688; [q]n=506; [r]n=2182

Table 1· Demographic characteristics and clinical outcomes of study population

	UNIVARIATE				MULTIVARIABLE		
	Risk of Death				Length of Stay		
	N	Death N=31 (%)	Alive N=3105 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3136	1·71 (1·23, 3·98)	3·5 (1·6, 8·5)	0·007	0·47	<0·01	0·38-0·59
Gestational age in weeks	3136	40 (37,40)	40 (38,40)	0·52	0·93	0.036	0·86-0.99
Birth Weight	3134	2·80 (2·44, 3·38)	3·0 (2·5,3·5)	0·25	0·85	0.012	0.75-0.96
Smoke exposure	3135	71%	77%	0·46	1.00	0.961	0.85-1.16
Sex Male: Female	3136	48%	60%	0·17	1.05	0.520	0.91-1.20
Breastfeeding	3136	65%	84%	0·003	0·72	0·001	0·59-0·88
Vitamin D level	2661	20·4 (4·7, 25·0)	16·4 (5·2, 26·0)	0·75	0.86	0.021	0.76-0.98
UMC	3136	42%	11%	<0·01	1.71	<0·01	1.37-2.13
Virus positive	3136	68%	82%	0·049	1.24	0.022	1.03-1.48
Pneumonia	3136	32%	12%	<0·01	2.07	<0·01	1.65-2.60
Sepsis	3136	52%	29%	0·01	2.44	<0·01	1.94-3.06
Bronchiolitis	3136	3%	17%	0·04	0.80	0.056	0.63-1.01
Bronchopneumonia	3136	13%	32%	0·02	0.98	0.857	0.80-1.21
	Oxygen Therapy						
	N	Oxygen N=1013 (%)	No Oxygen N=2124 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3137	2·9 (1·4, 6·5)	4·0 (1·7, 9·2)	<0·01	0·23	<0·01	0·17-0·31
Gestational age in weeks	3137	40 (37, 40)	40 (38, 40)	<0·01	0·90	0·02	0.82-0.98
Birth Weight	3135	3·0 (2·5, 3·4)	3·0 (2·6, 3·5)	0·03	0·96	0.63	0.81-1.13
Smoke exposure	3136	75%	77%	0·31	0.86	0.16	0.71-1.06
Sex - male	3137	58%	61%	0·51	0.83	0.048	0.70-0.998
Breastfeeding	3137	82%	85%	0·01	0.67	0.005	0.40-0.94
Vitamin D level, ng/mL	2664	14·9 (4, 25)	17·3 (6, 26·4)	<0·01	0.82	0·02	0·70-0·97
UMC	3137	16%	10%	<0·01	1.95	<0·01	1.49-2.56
Virus positive	3137	87%	79%	<0·01	1.34	0.02	1.04-1.71
Pneumonia	3137	21%	9%	<0·01	2.94	<0·01	2.22-3.89
Sepsis	3137	20%	33%	<0·01	0·21	<0·01	0.15-0.27
Bronchiolitis	3137	21%	15%	<0·01	1·05	0.72	0.79-1.41
Bronchopneumonia	3137	28%	34%	<0·01	1.06	0.69	0.80-1.39
	ICU Admission						
	N	ICU Stay N=284 (%)	No ICU Stay N=2856 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3140	2·1 (1·1, 6·1)	3·6 (1·7, 8·6)	<0·01	0·47	<0·01	0·30-0·74
Gestational age in weeks	3140	40 (37,40)	40 (38, 40)	0·017	0·93	0.32	0·81-1·07
Birth Weight	3138	2·9 (2·44,3·30)	3·0 (2·6,3·5)	0·001	0·87	0.28	0·67-1·12
Smoke exposure	3139	74%	77%	0·35	0·89	0.46	0.65-1.22
Sex	3140	55%	61%	0·53	0·80	0·11	0·60-1·05
Breastfeeding	3140	76%	85%	<0·01	0·63	0·01	0.44-0.91
Vitamin D level, ng/mL	2666	18·2 (5·3, 25·5)	16·4 (5·2, 26)	0·81	1·08	0·55	0·83-1·41
UMC	3140	23%	11%	<0·01	2.51	<0·01	1·71-3·68
Virus positive	3140	77%	82%	0·036	0.84	0·31	0·59-1·18
Pneumonia	3140	28%	11%	<0·01	3·12	<0·01	2·16-4·50
Sepsis	3140	43%	28%	<0·01	1.14	0.53	0.76-1.71
Bronchiolitis	3140	11%	18%	<0·01	0·78	0.36	0·46-1·32
Bronchopneumonia	3140	19%	33%	<0·01	0.81	0.37	0.50-1.30
	MV						
	N	MV N=111 (%)	No MV N=3025 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3136	3·2 (1·4, 6·7)	3·5 (1·6, 8·5)	0.16	0·28	<0·01	0·15-0·53
Gestational age in weeks	3136	40 (37,40)	40 (38,40)	0·28	0·88	0·19	0·72-1·07
Birth Weight	3134	3·0 (2·5, 3·3)	3·0 (2·5,3·5)	0·18	0.998	0·99	0·69-1·44
Smoke exposure	3135	77%	77%	0·99	0.99	0·97	0·63-1·57
Gender	3136	59%	60%	0·70	0·89	0·56	0·60-1·32
Breastfeeding	3136	86%	84%	0·48	1·53	0·17	0·84-2.81
Vitamin D level, ng/mL	2663	20·1 (5·7, 26·6)	16·4 (5·2, 25·9)	0·35	1·31	0·14	0·92-1·85
UMC	3136	20%	11%	<0.01	1.91	0·02	1·11-3·28
Virus positive	3136	80%	81%	0·75	0·88	0·62	0·52-1·48
Pneumonia	3136	23%	12%	<0·01	3·33	<0·01	1·85-6.0
Sepsis	3136	22%	29%	0·09	0·48	0·02	0·26-0·89
Bronchiolitis	3136	19%	17%	0·64	1·61	0·17	0·81-3·20
Bronchopneumonia	3136	33%	32%	0·78	2.03	0·03	1·05-3·90

Abbreviations: UMC, Underlying Medical Condition; ICU, Intensive Care Unit; MV, Mechanical Vent

Table 2. Univariate and multivariable analysis of factors associated with length of stay, risk of death, oxygen therapy, ICU admission, and mechanical ventilation

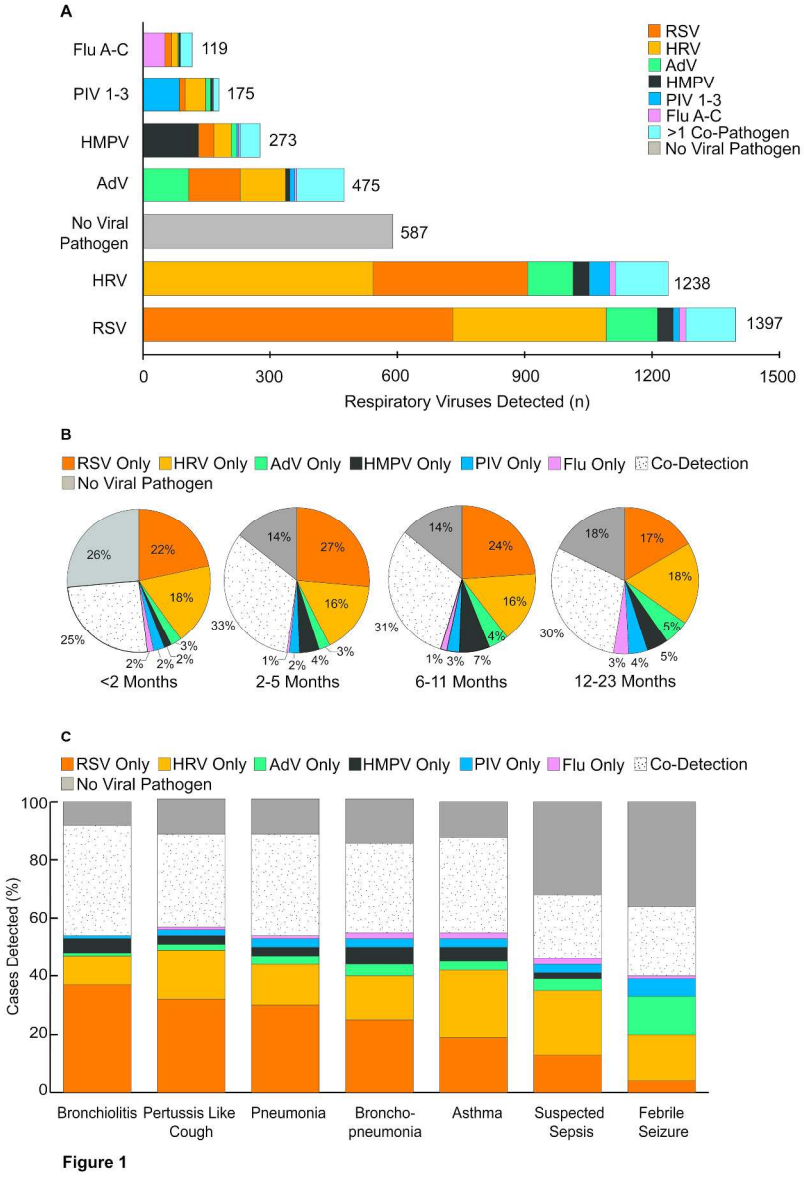

Figure 1. The viral pathogens detected in our Jordanian surveillance study. Figure 1A. The total number of pathogens detected by individual virus, with co-detection of viral pathogens within each virus. Figure 1B. The proportion of viral pathogens detected by age group by single infection, co-infection, and no viral pathogen. Figure 1C is the proportion of individual virus detection, co-infection, and no viral pathogen by admission diagnoses.

Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

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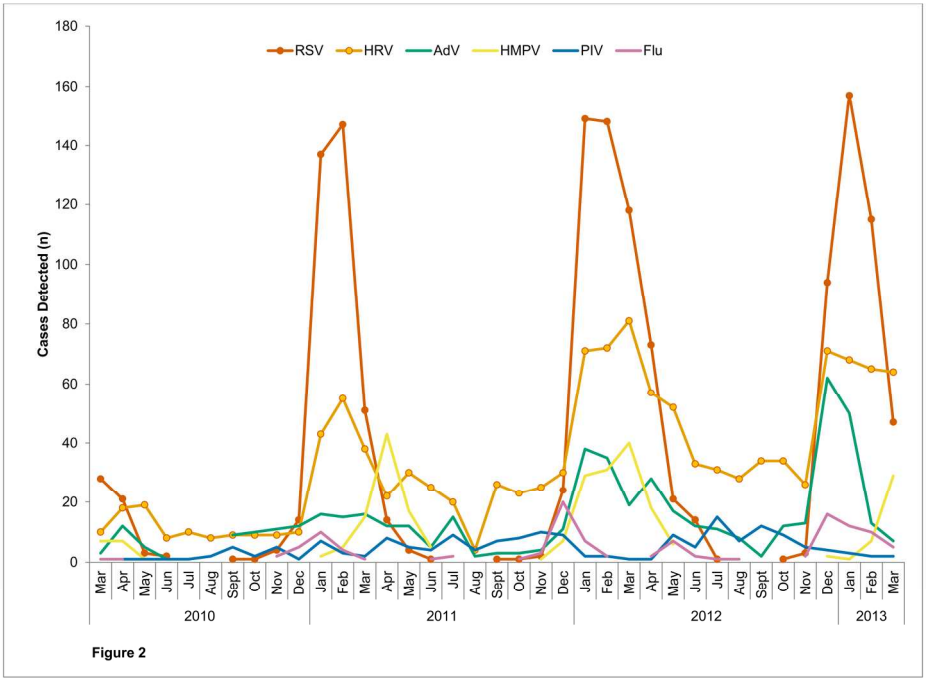

Figure 2. The distribution of the total number of viruses detected over three-years by individual month in Amman, Jordan.
Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

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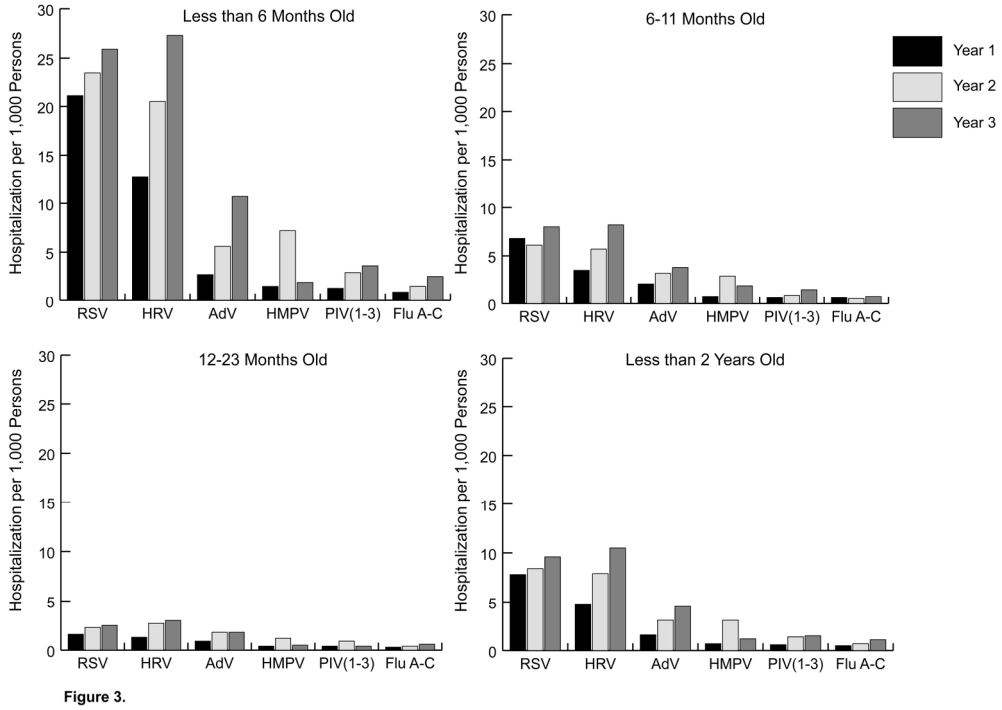

Figure 3.

Figure 3. Hospitalization rates by age group and years

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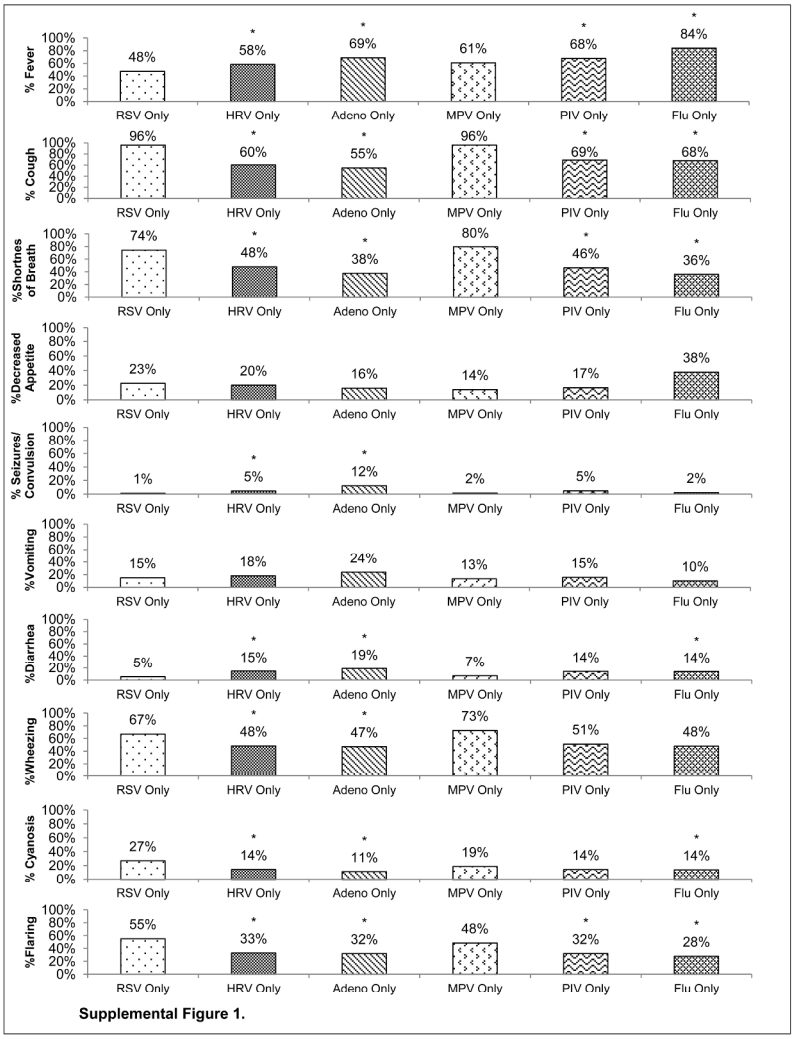

Supplemental Figure 1.

279x361mm (300 x 300 DPI)

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Severe outcomes associated with respiratory viruses in newborns and infants: a prospective viral surveillance study in Jordan

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Contributors:
Najwa Khuri-Bulous (NKB), Natasha Bassam Halasa (NBH), Samir Faouri (SF), Asem Shehabi (AS), Sten Vermund (SHV), and John Vance Williams (JVW) were involved in study design, reviewing the data, and interpretation of the data. NKB, NBH, SF, Lindsey Lawrence (LL), and AS oversaw data and clinical sample collection. Li Wang (LW), Bhinnata Piya (BP), and Christopher Fonnesbeck (CF) were involved in analyzing the data, and BP reviewed the data and created figures. NBH wrote the article, which was revised by SHV, NKB, and JVW and approved by all authors.

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Dr. Halasa received grants from Sanofi, Pfizer, Astra Zeneca, and Biocryst.
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Patient consent: Obtained

Ethics Approval: The study was given ethical approval by the University of Jordan, the Jordanian Ministry of Health, and Vanderbilt University.

Data sharing statement: No additional data are available.

ABSTRACT

Objective: To assess virus-specific hospitalization rates, risk factors for illness severity, and seasonal trends in children hospitalized with acute respiratory infections (ARI).

Design: Prospective cohort study.

Setting: A government hospital serving low- and middle-income population in Amman, Jordan.

Participants: Children under two years old hospitalized with fever and/or respiratory symptoms (n=3168) from 3/16/2010-3/31/2013. Children with chemotherapy-associated neutropenia and newborns who had never been discharged after birth were excluded from the study.

Outcome Measures: Hospitalization rates and markers of illness severity: admission to intensive care unit (ICU), mechanical ventilation (MV), oxygen therapy, length of stay (LOS), and death.

Results: Of the 3168 subjects, 2581 (82%) had at least one respiratory virus detected, with respiratory syncytial virus (RSV) being the most predominant pathogen isolated. During admission, 1013 (32%) received oxygen therapy, 284 (9%) were admitted to ICU, 111 (4%) were placed on MV, and 31 (1%) children died. Oxygen therapy was higher in RSV only subjects compared to human rhinovirus only (42% vs. 29%, p<0.001), adenovirus only (42% vs. 21%, p<0.001), and human parainfluenza virus only (42% vs. 23% p<0.001) subjects. The presence of an underlying medical condition was associated with oxygen therapy (aOR 1.95, 95% CI 1.49-2.56), ICU admission (aOR 2.51, 95%CI 1.71-3.68), MV (aOR 1.91, 95% CI 1.11-3.28), and longer LOS (aOR1.71, 95% CI 1.37-2.13). Similarly, younger age was associated with oxygen therapy (0.23, 95% CI 0.17-0.31), ICU admission (aOR 0.47, 95% CI 0.30-0.74), MV (0.28, 95% CI 0.15-0.53), and longer LOS (aOR 0.47, 95% CI 0.38-0.59). Pneumonia was strongly associated with longer LOS (aOR 2.07, 95% CI 1.65-2.60), oxygen therapy (aOR 2.94, 95% CI 2.22-3.89), ICU admission (aOR 3.12, 95% CI 2.16-4.50), and MV (aOR 3.33, 95% CI 1.85-6.00). Virus-specific hospitalization rates ranged from 0.5-10.5 per 1000 children.

Conclusion: Respiratory viruses are associated with severe illness in Jordanian children hospitalized with ARI. Prevention strategies such as extended breastfeeding, increased access to palivizumab, and RSV vaccine development could help decrease hospitalization rates and illness severity, particularly in young children with underlying medical conditions.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This three-year prospective surveillance study uses real-time reverse-transcriptase polymerase chain reaction to test a cohort of over 3000 children for 11 major respiratory viruses.
- To our knowledge, this study is the first to report virus-specific hospitalization rates and illness severity in Jordanian children hospitalized with acute respiratory infections (ARI).
- We fit a multivariable logistic model to analyze the risk factors for severe illness, and used a Bayesian hierarchical model to derive the population prevalence of each respiratory virus.
- This study is limited to children from low- and middle-income population seeking care at a single government hospital, potentially underestimating the true burden of ARI in Jordan.

INTRODUCTION

Acute respiratory infections (ARI) are the leading cause of death beyond the neonatal period in children under five years of age worldwide.[1] Historically, bacterial pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type B have been noted as predominant etiologies;[1-3] however, respiratory viruses are frequently implicated as primary causes as well. A recent study conducted in the U.S. found that respiratory viruses were much more common in children hospitalized with pneumonia than bacteria, with at least one virus detected in 65% of enrolled children.[4] A multicenter study conducted in four developing countries also noted that even though *Streptococcus pneumonia* was associated with increased risk of pneumonia deaths, respiratory viruses were frequently associated with severe, hypoxic pneumonia. [5,6]

Earlier studies have estimated that there were 66,000-199,000 RSV-related deaths in 2005 and 28,000-111,5000 influenza-related deaths in 2008 in children under five, however, these systematic reviews did not include countries in the Middle East due to the paucity of population-based studies in the region.[7,8] The seasonality of respiratory viral diseases other than RSV and influenza and their overall burden in low and middle-income countries (LMICs) also remain poorly defined.[9] Additionally, prior published viral surveillance studies from the region are limited by their retrospective nature, the use of convenience samples over short time periods, and the lack of use of modern molecular techniques such as real-time reverse-transcriptase polymerase chain reaction (RT-PCR).[10,11] Thus, the true burden and severity of virus-driven ARIs in Middle Eastern children are less defined.

We conducted a prospective surveillance study that tracked ARI-associated hospitalizations over a three-year period among Jordanian children. The objectives of this study were to estimate virus-specific hospitalization rates, identify risk factors for illness severity, and characterize seasonal variations of respiratory viral infections in Jordan.

METHODS

Study Design and Participants

In this prospective surveillance study, we enrolled children under two years old who were hospitalized with fever and/or respiratory symptoms between March 16, 2010 and March 31, 2013 at Al-Bashir Hospital in Amman, Jordan (detailed inclusion/exclusion criteria previously reported).[12] Subjects were recruited five days a week (Sunday through Thursday) within 48 hours of hospital admission.[12] Children with chemotherapy-associated neutropenia and newborns who had never been discharged after birth were excluded from the study.

The institutional review boards at the University of Jordan, the Jordanian Ministry of Health, and Vanderbilt University approved the study. Written informed consent was obtained from parents or guardians.

Setting

Al-Bashir Hospital is one of the three major government hospitals in Amman, which is Jordan's largest city and capital.[12] We estimate that during the study period, the hospital provided care for ≈55% of all children in Amman (author SF, unpublished data). Additionally, the pediatric wards admitted 17,557 children, 11,230 (64%) of whom were under two years of age.

Data and Specimen Collection

After consent, trained local research staff obtained blood either by heel stick or venipuncture, as well as nasal and throat swabs. Standardized questionnaires were used to record demographic, clinical, and socioeconomic data. Parents were queried in Arabic, and bilingual research staff transcribed the information into an English-language case report form at the time of the interview. After discharge, charts were abstracted for the following: antibiotic use, blood, urine, and cerebrospinal fluid (CSF) cultures, chest radiography, oxygen use, intensive care unit (ICU) stay, mechanical ventilation (MV), length of stay (LOS) in the hospital, and discharge status.

All data were entered into a standardized, secure REDCap™ (Research Electronic Data Capture, Vanderbilt University, Nashville, TN, USA) database.[13] We performed data quality checks on a minimum of 10% of the charts and verified data from all case report forms after entry.

Variables

Admission to ICU, oxygen therapy, MV placement, LOS, and death were considered markers of severe illness. ICU admission included children who were transferred in during their hospital stay or were admitted directly. Smoke exposure included both cigarette and/or hookah pipe (nargila) smokers in household. Underlying medical conditions (UMCs) were classified as the following: diabetes, heart disease, Down syndrome, kidney disease, sickle cell disease, cystic fibrosis, cancer, genetic/metabolic, cerebral palsy, neurological, mental retardation/developmental delay, seizure disorder, chronic diarrhea (e.g. >2 weeks), gastroesophageal reflux disease, immunodeficiency, asthma/reactive airway disease, liver disease, or other. Subjects were categorized as suspected sepsis if they had the admission diagnosis of rule out sepsis or febrile neonate. Covariates included age, gestational age at enrollment, birth weight, sex (male), smoke exposure, breastfeeding status, UMC, vitamin D levels, viral detection, pneumonia, sepsis, bronchiolitis, and bronchopneumonia. Cycle threshold (Ct) values were used as a proxy for viral load, i.e., lower Ct values indicated higher viral load.

Laboratory Testing

Nasal and throat swabs were collected and combined in transport medium (M4RT®, Remel, USA), aliquoted into MagMAX™ Lysis/Binding Solution Concentrate (Life Technologies, USA), snap-frozen, and then stored at -80℃. Aliquots were shipped on dry ice to Nashville, Tennessee, USA, and were tested by RT-PCR for 11 viruses: RSV; human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A, B, and C; parainfluenza (PIV) virus 1, 2, and 3; adenovirus (AdV), and Middle East respiratory syndrome coronavirus (MERS-CoV).[14-16] Blood was placed directly onto filter paper and air dried for ≥30 minutes before storage at room temperature and kept dry through shipment to ZRT Laboratory (Beaverton, OR, USA) for 25(OH)D (vitamin D) level measurement (techniques published previously).[17]

Statistical Analysis

Descriptive statistics were presented as frequency (percentage) or median and interquartile range (IQR) where appropriate. Categorical variables were compared using Pearson Chi-square tests. Continuous variables were compared using Mann-Whitney U test. For comparisons of continuous variables for ≥3 groups, Kruskal-Wallis tests were used. We fit a multivariable logistic model to analyze the risk factors for oxygen therapy, ICU admission, MV, or longer LOS. Risk factors included breastfeeding, vitamin D level, age at enrollment, gestational age, sex, UMC, smoke exposure (both cigarette and hookah), viral detection, and four admission diagnoses (pneumonia, suspected sepsis, bronchiolitis, and bronchopneumonia) based on literature review.[18] All analyses were performed using statistical software R version 3.1.2 (http://www.R-project.org/). Bonferroni adjustments were made to account for multiple comparisons in univariate analyses.

Models for each virus were fitted using Markov chain Monte Carlo.[19,20] Models were run for 100,000 iterations, with the first 90,000 iterations conservatively discarded as burn-in. Models were checked for convergence using the Gelman-Rubin diagnostic.[21,22]

Rate Calculation

Al-Bashir Hospital admissions data were used to estimate the population prevalence of each respiratory virus. These data were filtered to exclude admissions of individuals not residing in greater Amman. We used a Bayesian hierarchical model to derive estimates for each of the three years of the study. [21] Estimates of the under 2-year-old Jordanian population were obtained from the World Bank online database, and the proportion of the population residing in Amman (35%) was taken from the 2012 national census. These values were used in a binomial model to estimate the population of children <2 years of age in greater Amman in 2010-2012. A binomial data likelihood was specified:

$$y_{a,t}^{v} \sim Binomial\left(n_{a,t}, p_{a,t}^{v}\right)$$

where $y_{a,t}^{v}$ is the number of children of age *a* in year *t* at Al-Bashir observed with virus *v*, while $n_{a,t}$ is the population of children age *a* in year *t* eligible for sampling, and $p_{a_t}^{v}$ the prevalence of virus *v* among children age

a in year *t*. The sampled population $n_{a,t}$ was similarly estimated from a binomial model that sampled from $N_{a,t}$ the total Amman population of age *a* in year *t*:

$$n_{a,t} \sim Binomial(N_{a,t}, \pi)$$

Here, π is the proportion of the Amman population that is eligible to be sampled, which accounts for the market share m and the sampling intensity (5 days out of 7):

$$\pi = m(5/7)$$

The market share for Al-Bashir Hospital was modeled as a random variable, and given a uniform prior distribution between 50% and 60%, based on prior information. Prevalence was given diffuse beta(1,5) priors for all models. Note that the model structure implies all individuals carrying the virus in question seek hospital care.

Data sharing statement: Technical appendix, statistical code, and dataset available upon request with proper ethical approval.

Patient and Public Involvement: were not involved in this study design or input.

RESULTS

Study Population

From March 16, 2010 through March 31, 2013, we screened and confirmed eligibility for 3793 patients. Of the 3793 patients, 618 had parent/guardian refusal, three were determined to be ineligible after enrollment due to age, and four were diagnosed with meningitis. Our final sample consisted of 3168 subjects.

Demographics and Clinical Characteristics

The median age was 3.5 months, (range 0.07-23.96 months), 60% were male, 12% had UMCs, and 14% were premature (<37 weeks gestation) (table 1). Of the 375 subjects with UMCs, heart disease was the most common, 146/357 (39%). Nearly 90% of the children's parents self-identified as Jordanian, and 7% reported Palestinian as their nationality. Primary and secondary education was the highest attainment in 41% and 44% of the mothers respectively. The median birth weight was 3.0 kg, and 28% were born by cesarean section. The median number of siblings was two, 1.6% attended daycare, and 77% were exposed to smoke (73% and 18% to cigarette and hookah, respectively).

Before hospitalization, 41% of the children had received antibiotics, and 92% were administered an antibiotic during their hospital stay (table 1). The seven main admission diagnoses included: bronchopneumonia (32%), suspected sepsis (28%), bronchiolitis (17%), pneumonia (12%), pertussis-like cough (7%), asthma/reactive airway disease (5%), and febrile seizure (3%). The median length of stay was five days, with 9% admitted to the ICU, 4% on MV, and 32% receiving oxygen therapy (table 1). Of the 2688 (85%) subjects who were tested for vitamin D levels, 49% had vitamin D deficiency (<20 ng/mL). The median level was 16.5 ng/mL (IQR 5.2, 26)(table 1). During the study period, 31 (1%) children died (table 1). Among the 31 subjects, 21 (68%) had at least one respiratory virus detected (8 HRV-positive; 5 RSV-positive; 1 AdV-positive; 1 Flu-positive; and 6 co-infection cases).

Viral Detection

At least one virus was detected in 2581 (81.5%) of the 3168 children. RSV (1397, 44%) was the most common virus detected, followed by HRV (1238, 39%), AdV (475, 15%), HMPV (273, 9%), PIV1-3 (175, 6%), and Flu A-C (119, 4%) (figure 1A). MERS-CoV was not detected in any sample. Viral co-detection was common, with 944/2581 (37%) having at least one other virus co-detected, ranging from 48% with RSV to 78% with AdV (figure 1A). Single virus detection (i.e., excluding the children with viral co-detection) was 728 for RSV only, 541 for HRV only, 106 for AdV only, 128 for HMPV only, 84 for PIV1-3 only, and 50 for Flu A, B, and C only. Comparison of Ct values for single virus detection with co-detection revealed a slightly higher viral load for single detection for RSV (25.2 vs. 26.4, p<0.001); HRV (30.1 vs. 31.5, p<0.001); HMPV (29.0 vs. 32.0,

p<0.001); and PIV (28.8 vs 32.4), p<0.01) cases. No significant differences in Ct values were seen for Flu and AdV cases. Figure 1B shows the viral distribution by age, with viral co-detection common in all age groups. The percentage of viral detection by admission diagnoses are displayed in Figure 1C. The frequency and distribution of all viruses over the three-year period are displayed in Figure 2, with >95% viral detection in the winter months, predominately RSV.

Bacterial Detection

Reports of blood, urine, and CSF cultures were available for 764, 769, and 614 subjects respectively. Of these subjects, 38/764 (5.0%) had positive blood cultures, 118/769 (15.3%) had positive urine cultures, and 4/614 (0.7%) had positive CSF cultures. Of the 150 subjects who tested positive for at least one bacterial culture, 104 (69.3%) also had viral co-detection.

Hospitalization Rates

Of our 3168 subjects, 3048 (96.2%) resided in Amman. The highest rates of hospitalization were due to RSV in years one (7.8 per 1000 children) and two (8.4 per 1,000 children) and HRV in year 3 (9.6 per 1,000). Hospitalization rates were higher in children under six months old for all viruses compared to the older age groups, and those who were 6-11 months had higher rates compared to 12-23 months, based on non-overlapping credible intervals (figure 3).

Clinical Outcomes

Virus-positive children were significantly more likely to require oxygen therapy (34% vs 23%, p<0.001) and receive an antibiotic prior to admission (43% vs 32%, p<0.001) (table 1) compared to virus-negative subjects. They were also more likely to present with cough (82% vs. 42%, p<0.001) and shortness of breath (63% vs. 34%, p<0.001); have wheezing (60% vs. 37%, p<0.001), flaring (45% vs. 24%, p<0.001), and cyanosis (21% vs. 14%, p<0.001) on physical examination; and have the diagnoses of bronchopneumonia (34% vs. 23%, p<0.001), bronchiolitis (19% vs. 7%, p<0.001), and pneumonia (14% vs. 7%, p<0.001) compared to virus-negative children. In contrast, virus-negative children were significantly more likely to be younger (table 1); present with fever (64% vs. 54%, p<0.001), vomiting (23% vs. 15%, p<0.001), diarrhea (15% vs. 9%, p<0.001), poor appetite (29% vs. 20%, p<0.001), and seizures (7% vs. 3%, p<0.001); have a diagnosis of suspected sepsis (50% vs. 24%, p<0.001) and febrile seizure (5% vs. 2%, p<0.001)..

When comparing RSV only with other single detections, i.e., HRV only, AdV only, HMPV only, PIV only and Flu only, children with RSV only were significantly younger than children with HMPV only (3.4 months vs. 6.1 months, p<0.001) (table 1). RSV only subjects were significantly more likely to require oxygen therapy compared to HRV only (42% vs. 29%, p<0.001), AdV only (42% vs. 21%, p<0.001), and PIV only children (42% vs. 23%, p<0.001) (table 1). Additionally, RSV only children were significantly more likely to have lower vitamin D levels (15.3 ng/mL vs. 18.5 ng/mL, p<0.001), receive antibiotics prior to hospitalization (47% vs. 32%, p<0.001), and less likely to have UMCs than HRV only children (9% vs. 15%, p<0.001) (table 1)**.** RSV only subjects were also significantly less likely to present with fever and more likely to present with a cough, shortness of breath, and have flaring on exam compared to HRV, AdV, PIV, and Flu-only children *(supplemental figure 1)*. RSV only subjects significantly were less likely to have seizures/convulsions but more likely to have wheezing on exam compared to HRV only and AdV only children *(supplemental figure 1)*. RSV only subjects significantly were less likely to present with diarrhea but more likely to have cyanosis on exam compared to HRV only, Flu only, and AdV only children *(supplemental figure 1)*.

Risk Factors for Illness Severity

Longer LOS was associated with younger age (aOR 0.47, 95% CI 0.38-0.59), lower gestational age (aOR 0.93, 95% CI 0.86-0.99), lower birth weight (aOR 0.85, 95% CI 0.75-0.96), lack of breastfeeding (aOR 0.72, 95% CI 0.59-0.88), lower vitamin D level (aOR 0.86, 95% CI 0.76-0.98), UMC (aOR 1.71, 95% CI 1.37-2.13), virus detection (1.24, 95% CI 1.03-1.48), and diagnoses of pneumonia (aOR 2.07, 95% CI 1.65-2.60) or suspected sepsis (aOR 2.44, 95% CI 1.94-3.06)(table 2). Since early deaths would distort this observation, a sensitivity analysis excluding children who died was performed and still showed a significant association. Younger age (aOR 0.23, 95% CI 0.17-0.31), lower gestational age (0.90, 95% CI 0.82-0.98), lack of breast feeding (0.67, 95% CI 0.40-0.94), lower vitamin D levels (0.82, 95% CI 0.70-0.97), UMC (1.95, 95% CI 1.49-2.56), virus detection (aOR 1.34, 95% CI 1.04-1.71), sex (aOR 0.83, 95% CI 0.70-0.99), and diagnosis of pneumonia (aOR 2.94, 95% CI 2.22-3.89) or sepsis (aOR 0.21, 95% CI 0.15-0.27) were associated with

oxygen therapy (table 2). Diagnosis of pneumonia (3.12, 95% CI 2.16-4.50), younger age (0.47, 95% 0.30-0.74), UMC (aOR 2.51, 95% CI 1.71-3.68), and lack of breastfeeding (aOR 0.63, 95% CI 0.44-0.91), were associated with an ICU stay (table 2). MV was associated with a diagnosis of pneumonia (aOR 3.33, 95% CI 1.85-6.0), or bronchopneumonia (aOR 2.03, 95% CI 1.05-3.90), younger age (0.28, aOR (0.15-0.53), and UMC (1.91, 95% CI 1.11-3.28), but was less likely with the diagnosis of suspected sepsis (0.48, 95% 0.26-0.89) (table 2). The number of deaths was too low to support a multivariable analysis. However, in a univariate model, younger age, lack of breast feeding, underlying medical condition, virus negativity, and diagnoses of pneumonia or suspected sepsis were all associated with death, while bronchiolitis or bronchopneumonia was less likely to be associated with death (table 2).

DISCUSSION

Our study revealed that respiratory viruses are the predominant pathogens among young hospitalized children who present with fever and/or respiratory symptoms in Amman, Jordan. The most common diagnoses were bronchopneumonia, suspected sepsis, bronchiolitis, pneumonia, and pertussis-like cough. Only one-fifth of the children enrolled had no virus detected. Consistently, during each of the three winter seasons, ~95% of children hospitalized during winter months tested positive for a virus, with RSV and HRV being the most common viruses identified. Both RSV and HRV rates were higher than expected compared to US-based studies.[18,23,24] Specifically, RSV rates were similar to rates from Turkey, Norway, and Austria [25-29]; higher than the US and Netherlands;[23,30] and lower than UK, Spain, and Denmark.[31-34] Rates of influenza, HMPV, and PIV1-3 in our cohort were similar to US reports[35-38]. Our estimates of the burden of influenza, HRV, HMPV, PIV 1-3, and RSV fill a gap in knowledge from the Middle East.[7,8]

Hospitalized children who were virus-negative were more likely to be younger, febrile, and symptomatic with non-respiratory symptoms. Moreover, the overall illness severity of virus-negative children was greater than those with a confirmed viral cause, with more frequent suspected sepsis, seizures, ICU admission, and/or mortality. In contrast, virus-positive children were more likely to present with severe lower respiratory tract infection (LRTI) as indicated by the higher frequency of cough and shortness of breath, wheezing, flaring, and cyanosis on exam, and increased oxygen therapy requirement. These clinical differences were dramatic and may serve as helpful clinical predictors of likely viral diagnosis prior to the availability of more definitive laboratory findings. In addition, 68% of the children who died had at least one respiratory virus detected, noting the association of severe disease with viral detection in these children. Since antibiotic use was nearly universal in children with positive detection of respiratory viruses, targeted interventions for antibiotic stewardship may also be warranted.

Since RSV was the most common virus detected, we compared the clinical presentations of RSV only to single detection of other respiratory viruses. RSV only children were more likely to present with cough and shortness of breath; have flaring, wheezing, and cyanosis on physical exam; and less likely to present with fever, diarrhea, and seizures compared to other respiratory viruses. Interestingly, the clinical presentations of RSV and HMPV were indistinguishable, other than age: HMPV only children were older. A surveillance study of RSV-positive children in India found the presence of cough, fast-breathing, crepitation, and hypoxia to be independent predictors of RSV infection.[39] Durani et al. found the combination of cough, wheezing, and retractions to be good predictors of RSV infection.[39,40] Our study also found cough to be a strong predictor of the presence of virus, including RSV. In addition, the RSV-positive children in our study were more likely to require oxygen therapy, suggesting these children presented with more severe LRT disease. We also documented RSV being more severe than other viruses in a previous pilot study.[14] Therefore, if preventive measures such as vaccines become available for RSV, Jordanian children would benefit greatly.

We examined independent risk factors for several measures of illness severity: LOS, oxygen therapy, ICU admission, MV, and death. Universally, younger age, UMC, and the diagnosis of pneumonia were associated with all five illness severity markers. Lack of breastfeeding was associated with all severity markers except for MV. Therefore, the promotion of breastfeeding is an essential public health intervention for reducing the severity of respiratory illnesses. Our study found that lower vitamin D levels were associated with longer LOS and a higher probability of oxygen use. Two studies identified an association between lower infant vitamin D levels and increased risk of ARI, and one study found lower levels of vitamin D in children admitted into the

ICU with bronchiolitis or pneumonia compared to those admitted to the wards.[41-43] Observational evidence supports further interventional studies to determine if vitamin D supplementation could reduce respiratory illness severity in this population.

A principal strength of our study was its basis of over three full years of surveillance data from a public hospital serving the poor and lower-middle class children of a large city in the Middle East North Africa (MENA) region. Prior to this study, very few large prospective studies of respiratory viral diseases of children have been completed in this setting. Our ongoing surveillance enabled us to test samples during the time of the first MERS-CoV outbreak in 2012, confirming that none of the children in this study were admitted with MERS-CoV.[44] Our state-of-the-art molecular diagnostics give one of the best assessments to date of respiratory viral etiologies of hospitalized children in any MENA nation. Limitations included having only five days of surveillance per week, though we did weighted analysis in our population burden estimates to adjust for missing days. Also, we did not test for all respiratory viruses (e.g., parainfluenza 4, non-MERS coronaviruses), so the viral burden is underestimated. Lastly, this study is limited to children from low- and middle-income population seeking care at a single health facility in Amman, and thus is not generalizable to the entire population of Jordan.

The respiratory viral burden is likely to be substantial for the entire MENA region.[7] Prevention strategies such as breastfeeding promotion, vitamin D supplementation, and future RSV vaccines could reduce regional ARI burden. Further studies that include groups representing the broader population in Jordan are needed.

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Figure Legend:

Figure 1. The viral pathogens detected in our Jordanian surveillance study. Figure 1A. The total number of pathogens detected by individual virus, with co-detection of viral pathogens within each virus. Figure 1B. The proportion of viral pathogens detected by age group by single infection, co-infection, and no viral pathogen. Figure 1C is the proportion of individual virus detection, co-infection, and no viral pathogen by admission diagnoses.

Figure 2. The distribution of the total number of viruses detected over three-years by individual month in Amman, Jordan.

Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

Figure 3. Hospitalization rates by age group and years

References:

1. Madhi SA, Klugman KP. Acute Respiratory Infections. 2006.
2. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ 2008;86:408-16.
3. Rudan I, O'Brien KL, Nair H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health 2013;3:10401.
4. Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med 2015;372:835-45.
5. Benet T, Sanchez Picot V, Messaoudi M, et al. Microorganisms Associated With Pneumonia in Children <5 Years of Age in Developing and Emerging Countries: The GABRIEL Pneumonia Multicenter, Prospective, Case-Control Study. Clin Infect Dis 2017.
6. Benet T, Picot VS, Awasthi S, et al. Severity of Pneumonia in Under 5-Year-Old Children from Developing Countries: A Multicenter, Prospective, Observational Study. Am J Trop Med Hyg 2017;97:68-76.
7. Nair H, Brooks WA, Katz M, et al. Global Burden of Respiratory Infections Due to Seasonal Influenza in Young Children: A Systematic Review and Meta-Analysis. The Lancet 2011;378:1917-30.
8. Nair H, Nokes DJ, Gessner BD, et al. Global Burden of Acute Lower Respiratory Infections Due to Respiratory Syncytial Virus in Young Children: A Systematic Review and Meta-Analysis. Lancet 2010;375:1545-55.
9. Rudan I, Lawn J, Cousens S, et al. Gaps in Policy-Relevant Information on Burden of Disease in Children: A Systematic Review. The Lancet 2005;365:2031-40.
10. Naghipour M, Cuevas LE, Bakhshinejad T, Dove W, Hart CA. Human Bocavirus in Iranian Children with Acute Respiratory Infections. Journal of Medical Virology 2007;79:539-43.
11. Al-Sonboli N HC, Al-Aeryani A, Banajeh SM, Al-Aghbari N, Dove W, Cuevas LE. Respiratory Syncytial Virus and Human Metapneumovirus in Children with Acute Respiratory Infections in Yemen. The Pediatric Infectious Disease Journal 2005;24:734-6.
12. Halasa N, Williams J, Faouri S, et al. Natural history and epidemiology of respiratory syncytial virus infection in the Middle East: Hospital surveillance for children under age two in Jordan. Vaccine 2015;33:6479-87.
13. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap) - A Metadata-Driven Methodology and Workflow Process for Providing Translational Research Informatics Support. Journal of Biomedical Informatics 2009;42:377-81.
14. Khuri-Bulos N, Williams JV, Shehabi AA, et al. Burden of Respiratory Syncytial Virus in Hospitalized Infants and Young Children in Amman, Jordan. Scandinavian Journal of Infectious Diseases 2010;42:368-74.
15. Miller EK, Khuri-Bulos N, Williams JV, et al. Human Rhinovirus C Associated with Wheezing in Hospitalized Children in the Middle East. Journal of Clinical Virology 2009;46:85-9.
16. Schuster JE, Khuri-Bulos N, Faouri S, et al. Human Metapneumovirus Infection in Jordanian Children: Epidemiology and Risk Factors for Severe Disease. Pediatr Infect Dis J 2015.
17. Khuri-Bulos N, Lang RD, Blevins M, et al. Vitamin D Deficiency among Newborns in Amman, Jordan. Global Journal of Health Science 2014;6:162-71.
18. Hall CB, Weinberg GA, Iwane MK, et al. The Burden of Respiratory Syncytial Virus Infection in Young Children. New England Journal of Medicine 2009;360:588-98.
19. Steve Brooks AG, Galin Jones, Xiao-Li Meng. Handbook of Markov Chain Monte Carlo: Chapman and Hall/CRC 2011.
20. Patil A, Huard D, Fonnesbeck CJ. PyMC: Bayesian Stochastic Modelling in Python. Journal of statistical software 2010;35:1-81.
21. Gelman A, Rubin DB. Inference from Iterative Simulation Using Multiple Sequences. Statistical Science 1992;7:457-72.
22. Bayesian Data Analysis. Third ed: Chapman and Hall/CRC; 2013.
23. Hall CB, Weinberg GA, Blumkin AK, et al. Respiratory Syncytial Virus–Associated Hospitalizations Among Children Less Than 24 Months of Age. Pediatrics 2013.
24. Miller EK, Lu X, Erdman DD, et al. Rhinovirus-Associated Hospitalizations in Young Children. The Journal of Infectious Diseases 2007;195:773-81.
25. Hacımustafaoğlu M CS, Bozdemir SE, Ozgür T, Ozcan I, Güray A, Cakır D. RSV Frequency in Children Below 2 Years Hospitalized for Lower Respiratory Tract Infections. The Turkish Journal of Pediatrics 2013;55:130-9.

26. Weigl JAI, Puppe W, Schmitt HJ. Incidence of Respiratory Syncytial Virus-Positive Hospitalizations in Germany. European Journal of Clinical Microbiology and Infectious Diseases 2001;20:452-9.
27. Fjaerli H-O, Farstad T, Bratlid D. Hospitalisations for Respiratory Syncytial Virus Bronchiolitis in Akershus, Norway, 1993–2000: A Population-Based Retrospective Study. BMC Pediatrics 2004;4:25-.
28. Resch BMDG, W. M.D.; Mandl, C. M.D.; Muller, W. M.D. Epidemiology of Respiratory Syncytial Virus Infection in Southern Austria. The Pediatric Infectious Disease Journal 2000;19:587.
29. Resch B GW, Müller W. The Impact of Respiratory Syncytial Virus Infection: A Prospective Study in Hospitalized Infants Younger than 2 Years. Infection 2002;30:193-7.
30. Zomer-Kooijker K, Uiterwaal CSPM, van der Gugten AC, Wilbrink B, Bont LJ, van der Ent CK. Decreased Lung Function Precedes Severe Respiratory Syncytial Virus Infection and Post-Respiratory Syncytial Virus Wheeze in Term Infants. European Respiratory Journal 2014;44:666.
31. Gil-Prieto R, Gonzalez-Escalada A, Marín-García P, Gallardo-Pino C, Gil-de-Miguel A. Respiratory Syncytial Virus Bronchiolitis in Children up to 5 Years of Age in Spain: Epidemiology and Comorbidities: An Observational Study. Medicine 2015;94:e831.
32. Hervás D, Reina J, Yañez A, del Valle JM, Figuerola J, Hervás JA. Epidemiology of Hospitalization for Acute Bronchiolitis in Children: Differences Between RSV and Non-RSV Bronchiolitis. European Journal of Clinical Microbiology & Infectious Diseases 2012;31:1975-81.
33. Haerskjold AMK, Kim MD, DMSc; Kamper-Jørgensen, Mads MScPH, PhD; Nybo Andersen, Anne-Marie MD, PhD; Ravn, Henrik MSc, PhD; Graff Stensballe, Lone MD, PhD. Risk Factors for Hospitalization for Respiratory Syncytial Virus Infection: A Population-based Cohort Study of Danish Children. The Pediatric Infectious Disease Journal 2016;35:61-5.
34. Kristensen K DT, Frederiksen PS, Ibsen J, Iyore E, Jensen AM, Kjaer BB, Olofsson K, Pedersen P, Poulsen S. Epidemiology of Respiratory Syncytial Virus Infection Requiring Hospitalization in East Denmark. The Pediatric Infectious Disease Journal 1998;17:996-1000.
35. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-Based Surveillance for Hospitalizations Associated With Respiratory Syncytial Virus, Influenza Virus, and Parainfluenza Viruses Among Young Children. Pediatrics 2004;113:1758.
36. Williams JV, Edwards KM, Weinberg GA, et al. Population-Based Incidence of Human Metapneumovirus in Hospitalized Children. The Journal of infectious diseases 2010;201:1890-8.
37. Edwards KM, Zhu Y, Griffin MR, et al. Burden of Human Metapneumovirus Infection in Young Children. The New England journal of medicine 2013;368:633-43.
38. Poehling KA, Edwards KM, Weinberg GA, et al. The Underrecognized Burden of Influenza in Young Children. New England Journal of Medicine 2006;355:31-40.
39. Saha S, Pandey BG, Choudekar A, et al. Evaluation of Case Definitions for Estimation of Respiratory Syncytial Virus Associated Hospitalizations Among Children in a Rural Community of Northern India. Journal of Global Health 2015;5:010419.
40. Durani Y, Friedman MJ, Attia MW. Clinical Predictors of Respiratory Syncytial Virus Infection in Children. Pediatrics International 2008;50:352-5.
41. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D Deficiency in Young Children with Severe Acute Lower Respiratory Infection. Pediatric Pulmonology 2009;44:981-8.
42. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of Subclinical Vitamin D Deficiency with Severe Acute Lower Respiratory Infection in Indian Children Under 5y. European Journal of Clinical Nutrition 0000;58:563-7.
43. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A. Association of Subclinical Vitamin D Deficiency in Newborns with Acute Lower Respiratory Infection and their Mothers. uropean Journal of Clinical Nutrition 2007;63:473-7.
44. Khuri-Bulos N, Payne DC, Lu X, et al. Middle East Respiratory Syndrome Coronavirus Not Detected in Children Hospitalized with Acute Respiratory Illness in Amman, Jordan, March 2010 to September 2012. Clinical Microbiology and Infection 2014;20:678-82.

	All (N=3168)	Virus-Negative (n=587)	Virus-Positive (n=2581)	>1 Virus (n=944)	RSV Only§ (n=728)	HRV Only (n=541)	AdV Only (n=106)	HMPV Only (n=128)	PIV 1-3 Only (n=84)	Flu A-C Only (n=50)
Age (Months)*	3·5 (1·6, 8·5)	2·4 (1·1, 7·8)	3·8† (1·8, 8·6)	3·9 (1·8, 8·4)	3·4 (1·7, 7·1)	3·3 (1·6, 8·6)	6·1 (1·9, 12·1)	6·1† (2·9, 9·8)	5·3 (1·9, 11·1)	6·6 (1·7, 14·7)
Sex (Male)	1912 (60)	357 (61)	1555 (60)	568 (60)	433 (59)	332 (61)	69 (65)	77 (60)	52 (62)	24 (48)
Breastfeeding	2661 (84)	494 (84)	2167 (84)	794 (84)	627 (86)	444 (82)	91 (86)	103 (80)	68 (81)	40 (80)
No Underlying Medical Condition	2793 (88)	502 (86)	2291 (89)	851 (90)	665 (91)	458 (85)†	93 (88)	113 (88)	70 (83)	41 (82)
Smoke Exposure	2425 (77)	451 (77)	1974 (76)	720 (76)	558 (77)	411 (76)	82 (77)	98 (77)	65 (77)	40 (80)
Antibiotics Prior to Hospitalization	1286 (41)	187 (32)	1099 (43)†	420 (44)	339 (47)	174 (32)†	48 (45)	66 (52)	31 (37)	21 (42)
Antibiotics during Hospitalization	2891 (92)[a]	532 (91)[b]	2359 (92)[c]	872 (93)	659 (91)	488 (91)	96 (91)	119 (94)	79 (94)	46 (92)
Preterm Birth	450 (14)	88 (15)	362 (14)	141 (15)	89 (12)	80 (15)	15 (14)	20 (16)	12 (14)	5 (10)
Oxygen Therapy	1013 (32)[d]	136 (23)[e]	877 (34)†[f]	331 (35)	305 (42)	152 (29)†	22 (21)†	39 (31)	19 (23)†	9 (18)
Mechanical Ventilation	111 (4)[g]	22 (4)[e]	89 (3)[h]	30 (3)	29 (4)	20 (4)	3 (3)	2 (2)	4 (5)	1 (2)
Any ICU Stay	284 (9)[i]	66 (11)[e]	218 (9)[j]	86 (9)	63 (9)	53 (10)	4 (4)	4 (3)	5 (6)	3 (6)
LOS (Days)*	5 (3, 7)[k]	5 (3, 8)[e]	5 (3, 7)[l]	5 (3, 7)	5 (3, 7)	5 (3, 8)	4 (2-7)	4 (3-6)	5 (3-8)	5 (3-6)
Death	31 (1)[m]	10 (2)[n]	21 (1)[o]	6 (1)	5 (1)	8 (1)	1 (1)	0 (0)	0 (0)	1 (2)
Vitamin D Level (ng/mL)*	16·5[p] (5·2, 26·0)	16·0[q] (6·0, 25·6)	16·7[r] (5·0, 26·0)	15·8 (4·4, 25·9)	15·3 (3·7, 25·6)	18·5† (7·9, 26·2)	19·9 (6·7, 31·3)	16·8 (8·6, 26·3)	17·9 (7·9, 26·3)	17·3 (4·1, 25·7)

Abbreviations: RSV, Respiratory syncytial virus; HRV, Human rhinovirus; AdV, Adenovirus; HMPV, Human Metapneumovirus; PIV, Parainfluenza; Flu, Influenza; ICU, Intensive Care Unit; LOS, length of stay

Data are n (%) or *median (IQR). †p-value<0·001; calculated using Pearson chi-square test for categorical variables and Wilcoxon rank-sum test for continuous variables. § RSV Only was the reference group for comparison between viruses.

[a]n=3147; [b]n=586; [c]n=2561; [d]n=3137; [e]n=585; [f]n=2552; [g]n=3136; [h]n=2551; [i]n=3140; [j]n=2555; [k]n=3139; [l]n=2554; [m]n=3136; [n]n=583; [o]n=2553; [p]n=2688; [q]n=506; [r]n=2182

Table 1· Demographic characteristics and clinical outcomes of study population

	UNIVARIATE				MULTIVARIABLE		
	Risk of Death				Length of Stay		
	N	Death N=31 (%)	Alive N=3105 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3136	1·71 (1·23, 3·98)	3·5 (1·6, 8·5)	0·007	0·47	<0·01	0·38-0·59
Gestational age in weeks	3136	40 (37,40)	40 (38,40)	0·52	0·93	0.036	0·86-0.99
Birth Weight	3134	2·80 (2·44, 3·38)	3·0 (2·5,3·5)	0·25	0·85	0.012	0.75-0.96
Smoke exposure	3135	71%	77%	0·46	1.00	0.961	0.85-1.16
Sex Male: Female	3136	48%	60%	0·17	1.05	0.520	0.91-1.20
Breastfeeding	3136	65%	84%	0·003	0·72	0·001	0·59-0·88
Vitamin D level	2661	20·4 (4·7, 25·0)	16·4 (5·2, 26·0)	0·75	0.86	0.021	0.76-0.98
UMC	3136	42%	11%	<0·01	1.71	<0·01	1.37-2.13
Virus positive	3136	68%	82%	0·049	1.24	0.022	1.03-1.48
Pneumonia	3136	32%	12%	<0·01	2.07	<0·01	1.65-2.60
Sepsis	3136	52%	29%	0·01	2.44	<0·01	1.94-3.06
Bronchiolitis	3136	3%	17%	0·04	0.80	0.056	0.63-1.01
Bronchopneumonia	3136	13%	32%	0·02	0.98	0.857	0.80-1.21
	Oxygen Therapy						
	N	Oxygen N=1013 (%)	No Oxygen N=2124 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3137	2·9 (1·4, 6·5)	4·0 (1·7, 9·2)	<0·01	0·23	<0·01	0·17-0·31
Gestational age in weeks	3137	40 (37, 40)	40 (38, 40)	<0·01	0·90	0·02	0.82-0.98
Birth Weight	3135	3·0 (2·5, 3·4)	3·0 (2·6, 3·5)	0·03	0·96	0.63	0.81-1.13
Smoke exposure	3136	75%	77%	0·31	0.86	0.16	0.71-1.06
Sex - male	3137	58%	61%	0·51	0.83	0.048	0.70-0.998
Breastfeeding	3137	82%	85%	0·01	0.67	0.005	0.40-0.94
Vitamin D level, ng/mL	2664	14·9 (4, 25)	17·3 (6, 26·4)	<0·01	0.82	0·02	0·70-0·97
UMC	3137	16%	10%	<0·01	1.95	<0·01	1.49-2.56
Virus positive	3137	87%	79%	<0·01	1.34	0.02	1.04-1.71
Pneumonia	3137	21%	9%	<0·01	2.94	<0·01	2.22-3.89
Sepsis	3137	20%	33%	<0·01	0·21	<0·01	0.15-0.27
Bronchiolitis	3137	21%	15%	<0·01	1·05	0.72	0.79-1.41
Bronchopneumonia	3137	28%	34%	<0·01	1.06	0.69	0.80-1.39
	ICU Admission						
	N	ICU Stay N=284 (%)	No ICU Stay N=2856 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3140	2·1 (1·1, 6·1)	3·6 (1·7, 8·6)	<0·01	0·47	<0·01	0·30-0·74
Gestational age in weeks	3140	40 (37,40)	40 (38, 40)	0·017	0·93	0.32	0·81-1·07
Birth Weight	3138	2·9 (2·44,3·30)	3·0 (2·6,3·5)	0·001	0·87	0.28	0·67-1·12
Smoke exposure	3139	74%	77%	0·35	0·89	0.46	0.65-1.22
Sex	3140	55%	61%	0·53	0·80	0·11	0·60-1·05
Breastfeeding	3140	76%	85%	<0·01	0·63	0·01	0.44-0.91
Vitamin D level, ng/mL	2666	18·2 (5·3, 25·5)	16·4 (5·2, 26)	0·81	1·08	0·55	0·83-1·41
UMC	3140	23%	11%	<0·01	2.51	<0·01	1·71-3·68
Virus positive	3140	77%	82%	0·036	0.84	0·31	0·59-1·18
Pneumonia	3140	28%	11%	<0·01	3·12	<0·01	2·16-4·50
Sepsis	3140	43%	28%	<0·01	1.14	0.53	0.76-1.71
Bronchiolitis	3140	11%	18%	<0·01	0·78	0.36	0·46-1·32
Bronchopneumonia	3140	19%	33%	<0·01	0.81	0.37	0.50-1.30
	MV						
	N	MV N=111 (%)	No MV N=3025 (%)	p-value	Adjusted OR	p-value	95% CI
Median age in months	3136	3·2 (1·4, 6·7)	3·5 (1·6, 8·5)	0.16	0·28	<0·01	0·15-0·53
Gestational age in weeks	3136	40 (37,40)	40 (38,40)	0·28	0·88	0·19	0·72-1·07
Birth Weight	3134	3·0 (2·5, 3·3)	3·0 (2·5,3·5)	0·18	0.998	0·99	0·69-1·44
Smoke exposure	3135	77%	77%	0·99	0.99	0·97	0·63-1·57
Gender	3136	59%	60%	0·70	0·89	0·56	0·60-1·32
Breastfeeding	3136	86%	84%	0·48	1·53	0·17	0·84-2.81
Vitamin D level, ng/mL	2663	20·1 (5·7, 26·6)	16·4 (5·2, 25·9)	0·35	1·31	0·14	0·92-1·85
UMC	3136	20%	11%	<0.01	1.91	0·02	1·11-3·28
Virus positive	3136	80%	81%	0·75	0·88	0·62	0·52-1·48
Pneumonia	3136	23%	12%	<0·01	3·33	<0·01	1·85-6.0
Sepsis	3136	22%	29%	0·09	0·48	0·02	0·26-0·89
Bronchiolitis	3136	19%	17%	0·64	1·61	0·17	0·81-3·20
Bronchopneumonia	3136	33%	32%	0·78	2.03	0·03	1·05-3·90

Abbreviations: UMC, Underlying Medical Condition; ICU, Intensive Care Unit; MV, Mechanical Vent

Table 2. Univariate and multivariable analysis of factors associated with length of stay, risk of death, oxygen therapy, ICU admission, and mechanical ventilation

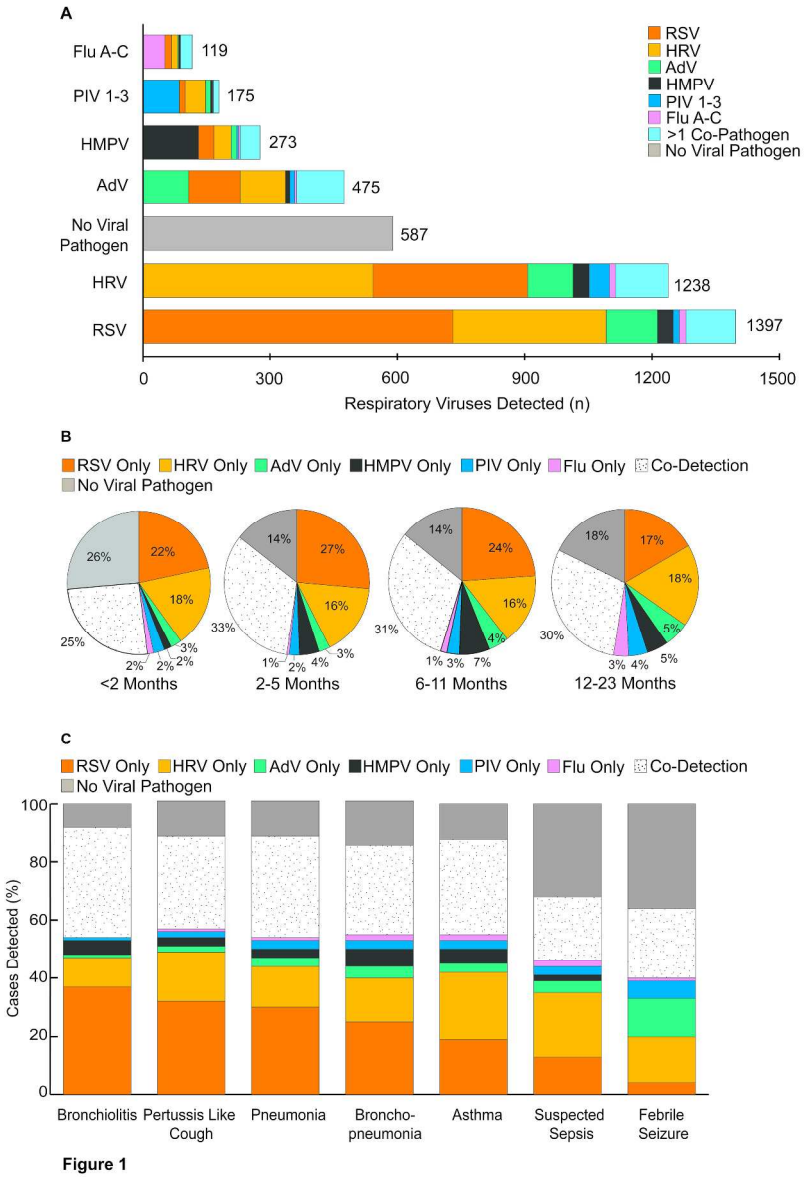

Figure 1

Figure 1. The viral pathogens detected in our Jordanian surveillance study. Figure 1A. The total number of pathogens detected by individual virus, with co-detection of viral pathogens within each virus. Figure 1B. The proportion of viral pathogens detected by age group by single infection, co-infection, and no viral pathogen. Figure 1C is the proportion of individual virus detection, co-infection, and no viral pathogen by admission diagnoses.

Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

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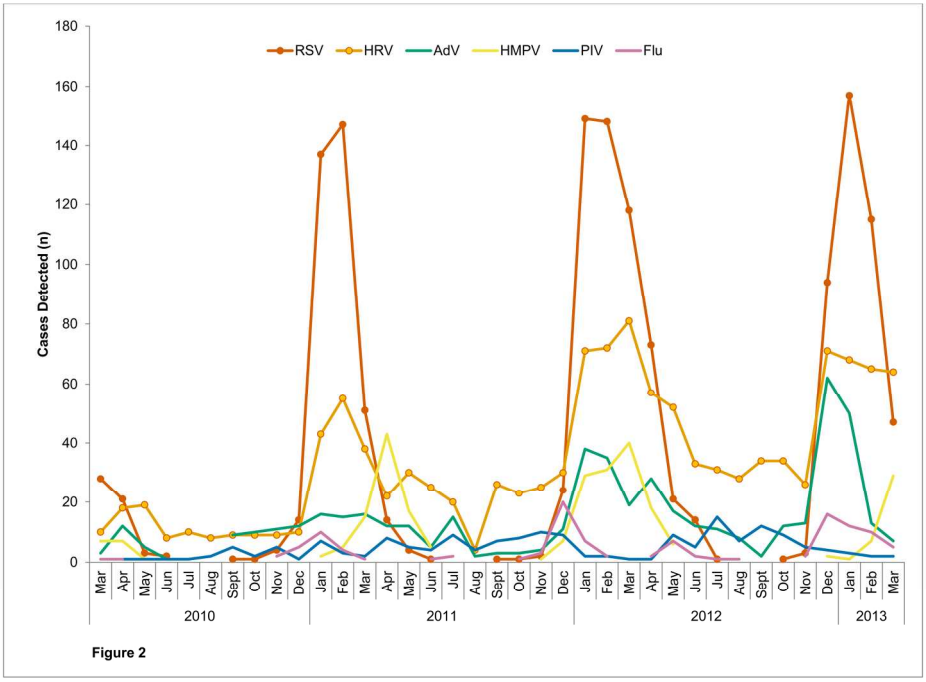

Figure 2. The distribution of the total number of viruses detected over three-years by individual month in Amman, Jordan.
Abv: Respiratory syncytial virus (RSV); human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (flu) A , B, and C; parainfluenza (PIV) virus 1, 2, and 3; and adenovirus (AdV).

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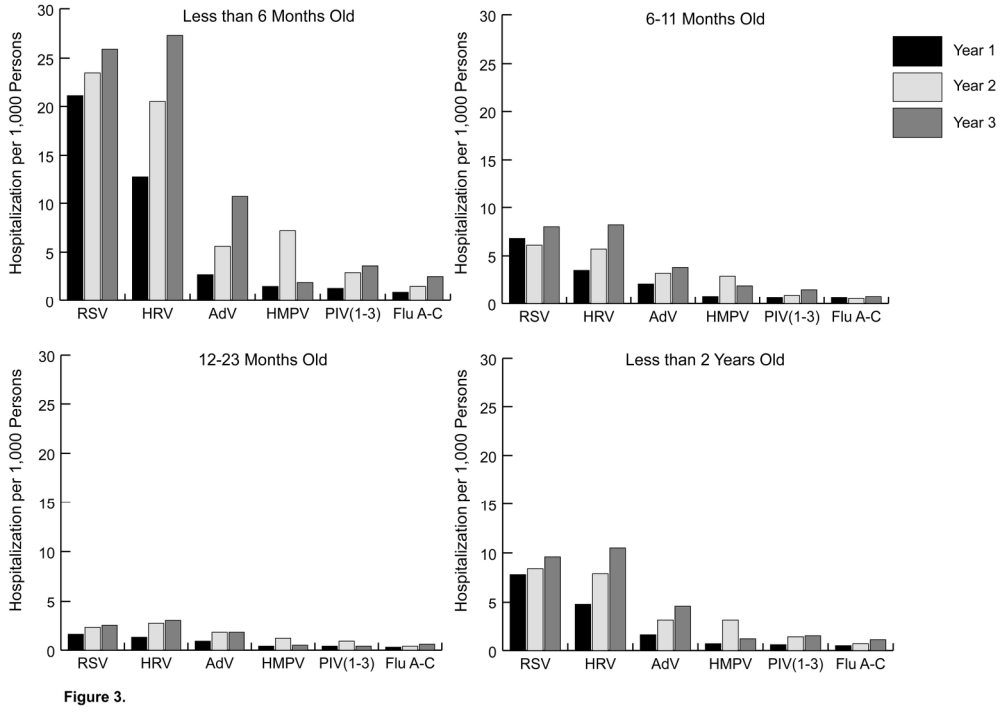

Figure 3.

Figure 3. Hospitalization rates by age group and years

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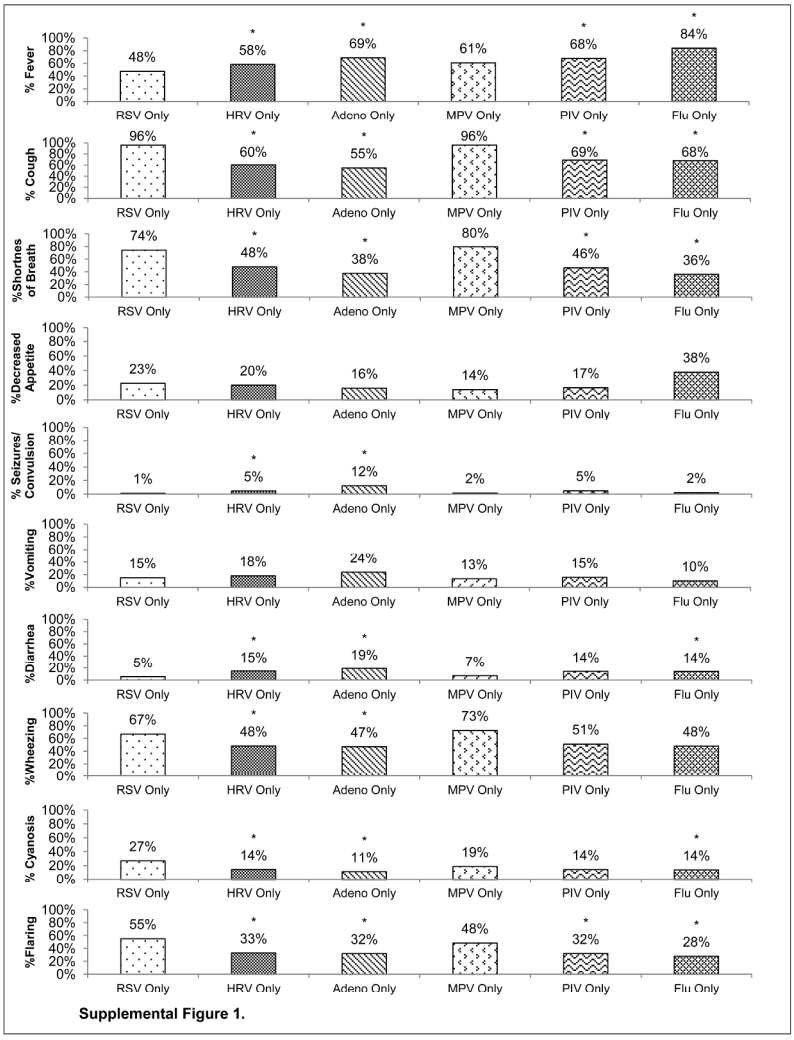

Supplemental Figure 1.

279x361mm (300 x 300 DPI)