The Epidemiology and Clinical Burden of Human Adenovirus Respiratory Infections Amongst Hospitalized Children Under 5 Years in Jordan

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Background: Human Adenovirus (HAdV) is a significant pathogen associated with severe acute respiratory infections (SARI), especially in children under five. Despite its global impact, its epidemiological and clinical burden in Jordan, particularly post-COVID-19, is limited.

Methods: We conducted a multicenter cross-sectional study across four hospitals in Jordan from November 2022 to April 2023. Nasopharyngeal swabs were collected from children <5 years old hospitalized with respiratory symptoms. HAdV positivity was determined using real-time polymerase chain reaction (RT-PCR). Demographic, clinical, and laboratory data were analyzed to identify predictors of HAdV positivity and complications.

Results: Among 1,000 enrolled participants (median age 9.68 months, 59% male), the HAdV positivity rate was 10.9%, highest in children 49–60 months of age. HAdV-positive cases had higher rates and longer duration of sore throat compared to HAdV-negative cases. Coinfections with RSV or influenza were present in 34.9% of HAdV-positive cases and were associated with increased rates of cough, wheezing, and respiratory crackles. Logistic regression revealed lower odds of HAdV positivity in children under six months (OR 0.31, P<0.001), while invasive ventilation was associated with higher odds of positivity (OR 5.01, P<0.001). HAdV infection without coinfection was associated with reduced odds of complications (OR 0.06, P<0.001).

Conclusions: This is the first comprehensive study in Jordan to document the epidemiologic and clinical burden of HAdV in children post-COVID-19. HAdV remains a major cause of respiratory morbidity, with significant coinfection rates. Further research is needed to explore the non-respiratory manifestations, identify HAdV common local serotypes, and genetic characteristics.

INTRODUCTION

About one-third of pediatric deaths in developing countries have been attributed to severe acute respiratory illnesses (SARI) particularly for children younger than 5 years old [1,2]. The most common pathogens linked to SARIs are viruses including respiratory syncytial viruses (RSV), influenza viruses A and B, parainfluenza viruses, human rhinoviruses, human coronaviruses, and human adenoviruses (HAdV) [3].

HAdV was first isolated from human adenoids in 1953, and two research groups independently described it [4,5]. HAdVs are categorized into seven species (A-G), comprising around 103 HAdVs types, where HAdV-1 to HAdV-51 represent serotypes and HAdV-52 to HAdV-103 indicate genotypes [6]. Among these types, species-B types 3, 4, 7, 14, 55, 21, and 11 are frequently linked to prominent SARI outbreaks [7]. The severity of adenoviral infection depends on the patient's age, environmental factors, and immune status [8].

HAdV virus is easily spread and can be extremely contagious in certain situations [9,10]. HAdVs infect a variety of mucosal sites, such as the surfaces of the ocular, respiratory, gastrointestinal, and genitourinary systems [9,10].

HAdV infections have been acknowledged as major causes of morbidity and mortality in humans, affecting people of all ages and from all over the world. HAdVs are responsible for 5–10% of all lower respiratory tract infections (LRTIs) in children [11,12].The main clinical signs of HAdVs respiratory infections are tonsillitis, fever, cough, and sore throat. Gastrointestinal symptoms like diarrhea and vomiting are also common [13]. In moderate self-limiting cases, HAdVs infection may be latent and asymptomatic, but in severe cases, it has been shown to cause multiple organ failure or even death. In patients with HAdVs-induced pneumonia, 14–60% of patients may experience pulmonary sequelae [14].

There are global variations in the HAdVs positivity rates amongst patients with respiratory infections. For example, the positivity rate for HAdVs in developed countries has been noted to range between approximately 3 to 24% [15–17]. Similar trends in positivity rates were observed in the Middle East. The prevalence of HAdV was found to be 7%, 4.5%, 23.2%, 25.3%, 28.3% in Saudi Arabia, Iraq, Kuwait, Lebanon, and Egypt respectively [18]. A study from Jordan revealed that HAdV positivity was 11.5% among children with a peak occurring in the 7-9 month age group, and primarily presenting as bronchopneumonia [19]. In another study from 2010 to 2013 it was shown that HAdV positivity was 15% among children <2 years [20].

There is no data from Jordan for the last ten years, particularly in the post COVID-19 period [21], and from representative sites on the HAdV positivity rates, clinical manifestations and complications during hospitalization. Previous studies were limited to one site and to those less than 2 years old. Therefore, it was proposed to obtain an updates data from representative sites in Jordan on the epidemiological and clinical burden of HAdV infections in Jordan for children younger 5 years old. This has been achieved through a further analysis of samples, as described below, collected for a study on the burden of pediatric respiratory infections in Jordan for the period between November 2022 and April 2023. The project was initially based on analysis of the collected nasopharyngeal samples for Respiratory Syncytial Virus (RSV), influenza and Sars-Cov2, according to the allocated budget. The first published report from this study revealed a high burden of Respiratory Syncytial virus (RSV) in Jordan, with positivity rates reaching 50.6% [22]. Testing for adenovirus was conducted upon obtaining further budget through a new grant from Mutah University, Jordan, for samples further analysis.

MATERIALS AND METHODS

Study Design

The study is a multi-center cross sectional national project conducted in four centers geographically distributed across Jordan as follows: (1) Princess Rahma Hospital for Children, Irbid,; (2) Zarqa Hospital, Al Zarqa, (3) Jordan University Hospital, Amman; and (4) Al Karak Public Hospital, Al Karak, Inclusion criteria: Diagnosis of acute respiratory infection, which was defined as "an illness presenting with one or more of the following symptoms for less than 7 days: Fever, cough, earache, nasal congestion, rhinorrhea, sore throat, vomiting after coughing, crackles, and labored, rapid or shallow breathing" [23].

Study population

Children <5 years old admitted to the study sites with the following:

(1) At least one sign of an acute infection (temperature ≥38 °C or <35.5 °C, abnormal white blood cell [WBC] count or abnormal differential).

(2) Diagnosis of acute respiratory infection as defined above.

Exclusion criteria:

Not a permanent resident of Jordan.

The full methods have been described previously by Abu-Helalah et. al. [22]. In brief, eligible patients were those who presented with respiratory symptoms to the inpatient clinics at the four study sites, according to above criteria the recruitment of patients took place during weekdays and continued during weekends and holidays to support representative sample and inclusion all eligible subjects during study period.

Sample collection and processing.

Nasopharyngeal (NP) specimens were collected from each patient who met the inclusion criteria and consented to the study. An NP swab was taken and then a Multiplex viral reverse

transcription polymerase chain reaction (RT-PCR) was performed on each nasopharyngeal specimen. Samples were initially analyzed for RSV and influenza infections, and then stored at −80 °C, as described below, for further analysis [24].

Polymerase chain reaction (PCR) was subsequently used to diagnose cases with Human Adenovirus Virus (HAdV) at the included sites. Positive adenovirus included adenovirus with/without other viruses, while adenovirus only refers to cases tested positive for adenovirus and negative for other viruses.

Microbiology - Sample collection and transport

Flocked swabs with plastic shafts were used to collect nasopharyngeal specimens from each patient who met the inclusion criteria. The swabs were then inserted into sterile viral transport media (VTM) and immediately placed on refrigerant gel packs or held at 4 °C prior to transport to the laboratory on the day of collection. Upon arrival at the laboratory, the specimens were immediately processed. Viral nucleic acid was extracted from the specimens and stored at −80 °C for further analysis to identify the target viruses. This study is based on this further analysis of the collected samples, which were stored at −80 °C using TSX Universal Series ULT freezers (Thermo Fisher Scientific®, Waltham, MA, USA) until further analysis.

RNA extraction was performed using the Zybio-Nucleic Acid Isolation System EXM 3000 (Zybio Inc, Chongqing, China) according to the manufacturer's protocol. Extracted RNA was aliquoted and stored at −80 °C using TSX Universal Series ULT freezers (Thermo Fisher Scientific®, Waltham, MA, USA) until further analysis. Storage was for 18 months. For the detection of human Adenovirus, the VIASURE Respiratory Panel III (Certest Biotec, S.L., Zaragoza, Spain) was used, a multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) assay. Amplification and analysis were performed using the QuantStudio™ 5 Real-Time

PCR System, 96-well, 0.2 mL (Applied Biosystems®, Foster, USA). PCR cycling conditions were as follows: reverse transcription at 45 °C for 15 minutes, initial denaturation at 95 °C for 2 minutes, followed by 45 cycles of denaturation at 95 °C for 10 seconds and annealing/extension at 60 °C for 50 seconds.

Power/sample size

The positivity rate for of HAdVs ranged from 3% to 28% based on previous studies [15–18], data from Jordan indicated that the positivity rate for HAdV was estimated at 11.5% [19]. Therefore, we expected 10% to 20% of the subjects to be positive for HAdV when calculating the sample size. 1000 subjects who matched the above clinical criteria were enrolled in the study.

Statistical Methods

Statistical analysis was performed using SPSS version 23, Student's *t*-test and chi-squared test were used as descriptive statistics modes to analyze and compare the categorical variables: demographic characteristics, including the patient characteristics; risk factors; and vaccination details. To identify the predictors of HAdV positivity and predictors of complications Logistic regression analysis was used to identify the predictors of influenza positivity and predictors of at least one complication. Presence of at least one complication was defined as presence of any of the followings: mortality, respiratory failure, bacterial or fungal coinfection, respiratory distress requiring oxygen- either invasive or non-invasive, pneumonia, cardiovascular complications including heart failure or bradycardia or other cardiovascular complications.

Case report form (CRF)

This was described previously. [22] The first part of the form consisted of the study inclusion criteria. Parents/guardians of eligible patients were asked for consent for participation after explaining the study details.

The interview forms included five sections.

1. Background, demographic, and societal data for patients and parents
2. Medical history covering birth history, current medical conditions and use of regular medications. including birth history, existing medical conditions, and current regular medications.
3. Presenting symptoms and signs: details about clinical presentations and their duration were obtained. Other clinical manifestations, such as cardiovascular complications, dehydration, wheezes, cyanosis, low activity level, nasal flaring, hypoxia (SaO_2 < 92%), subcostal/intercostal retractions, tachypnea, pneumothorax/atelectasis, and apnea >10 secs.
4. Laboratory findings: This included white blood cells (WBC) and differential, blood gas, PCR results, chest X-ray findings on arrival, pharyngeal swab, bacterial or fungal coinfections.
5. All utilized medicines during the admission and those prescribed at discharge were recorded.

Ethics

This study was conducted in accordance with the Declaration of Helsinki. Further analysis of the stored samples was approved by the Institutional Review Committee (IRC) for Mutah University Faculty of Medicine Ethics Committee, Reference Number: 912023, dated 20 of November 2023. The original project IRB approval was obtained from the Institutional Review Committee (IRC) for Ministry of Health IRB/REC//2022/295, dated 14 September 2022.

RESULTS

Inpatient data

During the recruitment phase between 15 November 2022 and 14 April 2023, there were 3580 hospitalizations at study sites. Based on the study eligibility criteria, 1755 children were screened; 1008 were eligible for participation. Only eight participants were not included, because consent form was not signed by parents/custodians. A total of 1000 eligible participants were finally enrolled in the study. [22]

The mean age of the study participants was 17.10 (SD: 16.57) months and the median age of 9.68 (Q1–Q3: [3.13–29.83]) months. Around two thirds of study participants (68%) were younger than age of 2 years and 59% of the sample were males.

The key finding of this study was the adenovirus positivity rate of 10.9% (n = 109); 34.9% of them had coinfection with RSV or influenza viruses. The highest positivity rates were reported for children between 49 to 60 months old, followed by those 25 to 36 months old. On the other hand, the lowest positivity rates were observed in children 0 to 6 months of age **(**Fig. 1**).**

Positivity rate by month of admission is shown in Table, Supplemental Digital Content 1. The highest positivity rate was reported in January ($n_{positive}$=23, 15.5%) followed by December ($n_{positive}$=50, 11.0%), while the lowest positivity rates were reported in March ($n_{positive}$=1, 2.78%).

Comparison of the demographic factors and medical histories of the adenovirus-positive and adenovirus-negative individuals was made using chi-squared analysis shown in Table, Supplemental Digital Content 2. The mean age was statistically significantly higher for adenovirus positive cases, when compared with negative cases (23.2 ± 17.4 months vs 16.4 ± 16.3 months, P<0.001). The positivity rate was lower in participants younger than 6 months of age compared with older participants (5.31% vs 14.29%, P<0.001). Furthermore, regarding breastfeeding,

participants who were not breastfed had the highest positivity rate (13.77%), Only one bacterial pneumonia based on culture results, which was positive for Streptococcus pneumoniae.

Table 1 illustrates the range and distribution of symptoms across the study participants. Participants positive for adenovirus had higher rates of sore throat than adenovirus negative participants or adenovirus positive participants who had coinfections with RSV or influenza, or cases negative for adenovirus (33.8%, 19.5%, 15.8%, respectively P=0.013). Duration of sore throat was statistically significantly longer for adenovirus positive cases, when compared with adenovirus negatives cases (Table 2). Interestingly, cases coinfected with RSV or influenza had statistically higher rates of cough and respiratory crackles than those infected with adenovirus only (97.4% vs 87.3%, P=0.046, 50.0% vs 39.4%, P=0.006, respectively).

Table 3 summarizes the complications and other clinical outcomes during hospitalization. Wheeze was significantly higher among the adenovirus-negative participants than the Adenovirus-positive participants (57.9% vs 45.9%, P=0.017). The presence of pneumothorax/atelectasis or the overall need for oxygen with invasive ventilation was only in those co-infected with RSV or influenza but not those only infected with adenovirus (p=0.026, and P<0.001). Finally, the presence of cyanosis was higher among those with coinfection compared with both those with only adenovirus infected or who were negative for adenovirus (18.4% vs 5.63% vs 7.52%, P=0.038).

Predictors of adenovirus positivity

The results of logistic regression analysis of factors associated with adenovirus positivity across all age groups are shown in Table 4. Participants who were less than 6 months old had almost a third the odds of testing positive of adenovirus compared with older participants. Additionally, those with wheezing had lower odds of positivity. On the other hand, those who

needed invasive ventilation had almost five times the likelihood of testing positive for adenovirus.

Predictors of complications

Further logistic regression analysis was performed to identify for predictors of the presence of at least one complication (Table 5).

The presence of asthma significantly increased the odds of having complications (OR =2.48, P=0.016). Testing positive for adenovirus with no co-infection (with RSV and influenza) significantly decreased the odds of getting complications when compared with testing negative (OR=0.06, P<0.001). Conversely, living in Zarqa or Irbid increased the likelihood of complications compared with Amman P<0.001 and P=0.022, respectively). Furthermore, while age and presence of congenital heart disease significantly decreased the likelihood of getting complications (OR=0.94, and OR=0.33, respectively), having bronchial asthma increased significantly the risk of complications (OR=2.48, 1.19-5.19).

DISCUSSION

This study's main finding was that the HAdV sample positivity was 10.9% for children younger than age of 5 with the highest positivity rate among children 49 to 60 months old. HAdV disease is controlled by both innate and adaptive immune responses. The burden is higher in younger children because the protective immunity gets mature enough by the age of 4 to 6 years. The HAdV positivity we found in the in Jordan after the COVID-19 pandemic is consistent with previous studies. Previous studies have shown that although HAdVs have been frequently referred to as a common etiologic agent in common colds, only 3%–6% of common colds in children were attributed to HAdVs. However, these rates varies globally [25,26]. A study from Taiwan revealed a positivity rate of 16.8% for the season 2021/2022,[27] which is higher that rate of 6.9% reported

for the period 2018/2019. [28] The average prevalence rate of HAdV infections in children was 12.7%, with average prevalence rates of 12.82% and 12.58% in the Middle East and North African countries, respectively. The highest prevalence rate (28.3%) was reported in Egypt, whereas the lowest prevalence (5%) was reported in Sudan.[18,29,30]

Our study included a significant number of children with lower respiratory tract infections, 67% had chest X-ray infiltrates. This is consistent with previous studies of hospitalized children showing that the diagnosis in children with adenovirus was pneumonia in 72.7% of cases. [31,32] In our study, the presence of congenital heart disease was negatively associated with complications. This is different to previous studies showing that patients with congenital heart disease have an increased risk of morbidity and mortality from adenovirus infection.[33] Our study had a small number of participants with congenital heart disease (n=42, only 4 with adenovirus infection which may have meant we had insufficient power to identify differences between the groups.

This study also highlighted clinical features associated with adenovirus infection in young children and the impact of coinfection with RSV and/or influenza. Participants positive for adenovirus had higher rates of sore throat and longer duration of this symptom. Studies have shown that acute febrile pharyngitis is the most common adenoviral illness in children and is particularly important as an epidemic illness in closed environments. [23]

In addition to high positivity rates, our study has other important findings that supports our conclusion that adenovirus respiratory infection is a major burden in children in Jordan. This was evident from the complications during hospitalization, with 11% of participants with adenovirus requiring admission to the ICU. According to data from other studies, 7%-22% of adenovirus-infected patients needed admission to ICU. [34–37] Previous studies have shown that

adenovirus infections can cause a more exaggerated immune-inflammatory response than many other pathogens, with a high proportion of children having respiratory distress and faster disease progression. [38,39] HAdVs account for approximately 5%–11% of bronchitis [40] and 5%–18% of bronchiolitis in infants [41]. A large proportion of these cases are complicated by pneumonia. Adenoviral bronchiolitis that occurs early in infancy can be fatal or results in serious residual lung damage and chronic disease [42].

This is the first study from Jordan demonstrating the co-infection rate of adenovirus with influenza or RSV. The coinfection rate was 34.9% among adenovirus positive cases. Our results are consistent with a previous study that showed patients with adenovirus infection commonly have coinfections during the course of the disease.[38] In addition, cases coinfected with RSV or influenza had statistically higher rates of cough and respiratory crackles. Influenza or RSV infections are known to exacerbate clinical symptoms like wheezing, nasal congestion, and coughing because of the virus's ability to cause inflammation and bronchoconstriction. There is conflicting evidence on the clinical impact of co-infections in patients with adenovirus infection. Some studies have demonstrated that patients with only adenovirus infection and those with coinfections have the same clinical severity [43,44]. whereas others have revealed that the clinical features are more significant in the presence of coinfections. [38,39,45].

Of 21 cases suspected with bacterial coinfection, culture positivity was reported only for one patient. In our previous study from Jordan, 23 blood culture cases were positive for Strep. Pneumonia compared with 992 PCR-positive cases for blood samples from patients with lobar pneumonia. This is due to the inappropriate use of antibodies in our region. This emphasizes our

Commented [MA1]: Folkerts G, Busse WW, Nijkamp FP, Sorkness R, Gern JE. Virus-induced airway hyperresponsiveness and asthma. Am J Respir Crit Care Med. 1998 Jun;157(6 Pt 1):1708-20. doi: 10.1164/ajrccm.157.6.9707163. PMID: 9620896.

previous recommendations of the importance of molecular techniques such as PCR testing in the Middle East [46].

The main limitation of this study is that it was conducted during the RSV and influenza seasons and over a relatively short duration (five months). In addition, neither serotyping nor genotyping of adenoviruses. Finally, data collection was limited to respiratory manifestations without including children with other common symptoms of adenovirus infection (e.g., gastrointestinal symptoms or conjunctivitis).

CONCLUSIONS

In conclusion, this national multicenter cross-sectional study, is the first comprehensive study in Jordan to document the epidemiological burden of HAdV in children post-COVID-19 along with clinical manifestations. This study revealed that adenovirus respiratory infections have a significant burden among children under 5 years of age. There is a need for local evidence-based preventive medicine interventions to control this high burden of adenovirus locally. Finally, further research is needed to explore the HAdV non-respiratory manifestations such as gastroenteritis and conjunctivitis, to identify common local serotypes, and to study HAdV genetic characteristics locally.

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Declarations:

Author Contributions: Conceptualization: M.A.-H., M.A.L., S.B.D.; **Methodology:** M.A.-H., M.A.L., S.B.D.; **Validation:** M.A.-H., M.A.L., S.B.D., M.A.-Hn.; **Formal Analysis:** M.A., M.A.-Hn.; **Investigation:** A.A.-T., M.A.L.; **Resources:** M.A.-H., M.A.L.; **Data Curation:** A.A.-T., M.A, M.A.L.; **Writing—Original Draft Preparation:** M.A.-H., S.B.D., M.A.-Hn.; **Writing—Review and Editing:** M.A.-H., S.B.D., M.A.-Hn.; **Visualization:** M.A., M.A.-Hn.; **Supervision:** M.A.-H., M.A.L., S.B.D.; **Project Administration:** M.A.-H., M.A.-Hn.; **Funding Acquisition:** M.A.L. All authors read and agreed to the published version of this manuscript.

Ethics/Ethical Approval: This study was conducted in accordance with the Declaration of Helsinki. Further analysis of the stored samples was approved by the Institutional Review Committee (IRC) for Mutah University Faculty of Medicine Ethics Committee, Reference Number: 912023, dated 20 of November 2023. The original project IRB approval was obtained from the Institutional Review Committee (IRC) for Ministry of Health IRB/REC//2022/295, dated 14 September 2022.

Informed Consent: Written informed consent was obtained from the parents/guardians of the children who were eligible to participate in this study. Prior to signing the consent form, all participants/parents or guardians were provided with relevant information about this study. The

participants without written study consent were not enrolled. As per regulations of the IRB committee, for illiterate parents, verbal consent was obtained in the presence of hospital staff, not part of the study, or in the presence of literate family members.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

REFERENCES

1. Balasubramani K, Prasad KA, Kodali NK, Abdul Rasheed NK, Chellappan S, Sarma DK, et al. Spatial epidemiology of acute respiratory infections in children under 5 years and associated risk factors in India: District-level analysis of health, household, and environmental datasets. Front Public Health. 2022;10:906248.
2. Um S, Vang D, Pin P, Chau D. Trends and determinants of acute respiratory infection symptoms among under-five children in Cambodia: Analysis of 2000 to 2014 Cambodia demographic and health surveys. PLOS Glob Public Health. 2023;3(5):e0001440.
3. Liu T, Li Z, Zhang S, Song S, Julong W, Lin Y, et al. Viral Etiology of acute respiratory tract infections in hospitalized children and adults in Shandong Province, China. Virol J. 2015 Oct 14;12:168.
4. Hilleman MR, Werner JH. Recovery of new agent from patients with acute respiratory illness. Proc Soc Exp Biol Med Soc Exp Biol Med N Y N. 1954 Jan;85(1):183–8.
5. Rowe WP, Huebner RJ, Gilmore LK, Parrott RH, Ward TG. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc Soc Exp Biol Med Soc Exp Biol Med N Y N. 1953 Dec;84(3):570–3.

6. Wen S, Lin Z, Zhang Y, Lv F, Li H, Zhang X, et al. The Epidemiology, Molecular, and Clinical of Human Adenoviruses in Children Hospitalized With Acute Respiratory Infections. Front Microbiol. 2021;12:629971.
7. Wang X, Wang D, Umar S, Qin S, Ling Q, Gray GC, et al. Molecular typing of human adenoviruses among hospitalized patients with respiratory tract infections in a tertiary Hospital in Guangzhou, China between 2017 and 2019. BMC Infect Dis. 2021 Aug 3;21(1):748.
8. Biserni GB, Scarpini S, Dondi A, Biagi C, Pierantoni L, Masetti R, et al. Potential Diagnostic and Prognostic Biomarkers for Adenovirus Respiratory Infection in Children and Young Adults. Viruses. 2021 Sep 21;13(9):1885.
9. Chen Y, Lin T, Wang CB, Liang WL, Lian GW, Zanin M, et al. Human adenovirus (HAdV) infection in children with acute respiratory tract infections in Guangzhou, China, 2010-2021: a molecular epidemiology study. World J Pediatr WJP. 2022 Aug;18(8):545–52.
10. Ismail AM, Zhou X, Dyer DW, Seto D, Rajaiya J, Chodosh J. Genomic foundations of evolution and ocular pathogenesis in human adenovirus species D. FEBS Lett. 2019 Dec;593(24):3583–608.
11. Chen HL, Chiou SS, Hsiao HP, Ke GM, Lin YC, Lin KH, et al. Respiratory adenoviral infections in children: a study of hospitalized cases in southern Taiwan in 2001--2002. J Trop Pediatr. 2004 Oct;50(5):279–84.
12. Yun BY, Kim MR, Park JY, Choi EH, Lee HJ, Yun CK. Viral etiology and epidemiology of acute lower respiratory tract infections in Korean children. Pediatr Infect Dis J. 1995 Dec;14(12):1054–9.

13. Xie L, Zhang B, Xiao N, Zhang F, Zhao X, Liu Q, et al. Epidemiology of human adenovirus infection in children hospitalized with lower respiratory tract infections in Hunan, China. J Med Virol. 2019 Mar;91(3):392–400.
14. Liu X, Ding Y, Hu R, Tang H. Evaluation of Risk Factors for Children with Severe Adenovirus Respiratory Infection: Retrospective Study. Iran J Pediatr. 2024;34(1):e134296.
15. Abbas KZ, Lombos E, Duvvuri VR, Olsha R, Higgins RR, Gubbay JB. Temporal changes in respiratory adenovirus serotypes circulating in the greater Toronto area, Ontario, during December 2008 to April 2010. Virol J. 2013 Jan 7;10:15.
16. Barnadas C, Schmidt DJ, Fischer TK, Fonager J. Molecular epidemiology of human adenovirus infections in Denmark, 2011-2016. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2018 Jul;104:16–22.
17. Bastug A, Altas AB, Koc BT, Bayrakdar F, Korukluoglu G, Bodur H, et al. Molecular characterization of human adenoviruses associated with respiratory infection in Turkey. APMIS Acta Pathol Microbiol Immunol Scand. 2021 Jan;129(1):23–31.
18. Qashqari FSI. Human Mastadenovirus Infections in Children: A Review of the Current Status in the Arab World in the Middle East and North Africa. Child Basel Switz. 2022 Sep 6;9(9):1356.
19. Al-Toum R, Bdour S, Ayyash H. Adenovirus infections in Jordanian hospitalized pediatric patients: Prevalence and clinical features. Jordan Med J. 2009;43(3).
20. Probst V, Rankin DA, Haddadin Z, Hamdan L, Rahman HK, Yanis A, et al. Adenovirus Infection in Hospitalized Children with Acute Respiratory Infection in Jordan. Pediatr Infect Dis J. 2022 Apr 1;41(4):277–83.

21. Lee NJ, Woo S, Rhee JE, Lee J, Lee S, Kim EJ. Increased Trend of Adenovirus Activity After the COVID-19 Pandemic in South Korea: Analysis of National Surveillance Data. Ann Lab Med. 2024 Nov 1;44(6):581–5.
22. Abu-Helalah M, Al-Shatnawi SF, Abu Lubad M, Al-Zayadneh E, Jdaitawi H, Harahsheh M, et al. The Epidemiology, Clinical, and Economic Burdens of Respiratory Syncytial Virus Infections Amongst Hospitalized Children Under 5 Years of Age in Jordan: A National Multi-Center Cross-Sectional Study. Viruses. 2024 Dec;16(12):1867.
23. Shieh WJ. Human adenovirus infections in pediatric population - An update on clinico-pathologic correlation. Biomed J. 2022 Feb;45(1):38–49.
24. Abu-Helalah M, Abu Lubad M, Al-Hanaktah M, Al Tibi A, Alhousani M, Drysdale SB. The Epidemiology and Clinical Burdens of Human Parainfluenza Virus Infections Amongst Hospitalized Children Under 5 Years of Age in Jordan: A National Multi-Center Cross-Sectional Study. Viruses. 2025 Jan 25;17(2):170.
25. Edwards KM, Thompson J, Paolini J, Wright PF. Adenovirus infections in young children. Pediatrics. 1985 Sep;76(3):420–4.
26. Fuchs AM. Differential diagnosis of the common cold: adenovirus, simple cold, bacterial cold and allergy. Eye Ear Nose Throat Mon. 1959 Feb;38(2):129–36.
27. Ali AF, Li W, Wang B, Guo Y. Epidemiological analysis of human adenovirus in children diagnosed with acute respiratory tract infection. 2024;
28. Wang C, Liu J, Mi Y, Chen J, Bi J, Chen Y. Clinical features and epidemiological analysis of respiratory human adenovirus infection in hospitalized children: a cross-sectional study in Zhejiang. Virol J. 2021 Nov 29;18(1):234.

29. Salih MA, Herrmann B, Grandien M, El Hag MM, Yousif BE, Abdelbagi M, et al. Viral pathogens and clinical manifestations associated with acute lower respiratory tract infections in children of the Sudan. Clin Diagn Virol. 1994 Jun;2(3):201–9.

30. van der Zalm MM, Sam-Agudu NA, Verhagen LM. Respiratory adenovirus infections in children: a focus on Africa. Curr Opin Pediatr. 2024 Jun 1;36(3):342–8.

31. Zhong H, Dong X. Analysis of Clinical Characteristics and Risk Factors of Severe Adenovirus Pneumonia in Children. Front Pediatr. 2021;9:566797.

32. Zaki MES, Eid AR, Faried OA. Clinico-pathological study of adenovirus associated with respiratory infections in children. Open Microbiol J. 2020;14(1).

33. Crawford R, Akmyradov C, Dachepally R, Prodhan P. Hospital Outcomes Among Children With Congenital Heart Disease and Adenovirus Pneumonia. Pediatr Infect Dis J. 2024 Aug 1;43(8):720–4.

34. Murtagh P, Giubergia V, Viale D, Bauer G, Pena HG. Lower respiratory infections by adenovirus in children. Clinical features and risk factors for bronchiolitis obliterans and mortality. Pediatr Pulmonol. 2009 May;44(5):450–6.

35. Spaeder MC. Severe adenoviral respiratory infection in children. Intensive Care Med. 2013 Jun;39(6):1157–8.

36. Lai CY, Lee CJ, Lu CY, Lee PI, Shao PL, Wu ET, et al. Adenovirus serotype 3 and 7 infection with acute respiratory failure in children in Taiwan, 2010-2011. PloS One. 2013;8(1):e53614.

37. Hong JY, Lee HJ, Piedra PA, Choi EH, Park KH, Koh YY, et al. Lower respiratory tract infections due to adenovirus in hospitalized Korean children: epidemiology, clinical features, and prognosis. Clin Infect Dis Off Publ Infect Dis Soc Am. 2001 May 15;32(10):1423–9.

38. Liu J, Deng ZH, Luo WJ, Wang WJ, Xu Y, Zhang XH, et al. Clinical characteristics between children with adenovirus infection and children with influenza virus infection. 2023;
39. Mandelia Y, Procop GW, Richter SS, Worley S, Liu W, Esper F. Dynamics and predisposition of respiratory viral co-infections in children and adults. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2021 Apr;27(4):631.e1-631.e6.
40. Lu Z, Zheng Y. Plastic bronchitis associated with adenovirus infection. Lancet Infect Dis. 2018 Apr;18(4):474.
41. García-García ML, Calvo C, Falcón A, Pozo F, Pérez-Breña P, De Cea JM, et al. Role of emerging respiratory viruses in children with severe acute wheezing. Pediatr Pulmonol. 2010 Jun;45(6):585–91.
42. Pichler MN, Reichenbach J, Schmidt H, Herrmann G, Zielen S. Severe adenovirus bronchiolitis in children. Acta Paediatr Oslo Nor 1992. 2000 Nov;89(11):1387–9.
43. Ampuero JS, Ocaña V, Gómez J, Gamero ME, Garcia J, Halsey ES, et al. Adenovirus respiratory tract infections in Peru. PloS One. 2012;7(10):e46898.
44. Palomino MA, Larrañaga C, Villagra E, Camacho J, Avendaño LF. Adenovirus and respiratory syncytial virus-adenovirus mixed acute lower respiratory infections in Chilean infants. Pediatr Infect Dis J. 2004 Apr;23(4):337–41.
45. Farzi R, Pirbonyeh N, Kadivar MR, Moattari A. Prevalence of Influenza Viruses A and B, Adenovirus, Respiratory Syncytial Virus, and Human Metapneumonia Viruses among Children with Acute Respiratory Tract Infection. Adv Virol. 2024;2024:7613948.
46. Abu-Helalah M, Al-Mnayyis A, Alzoubi H, Al-Abdallah R, Jdaitawi H, Nafi O, et al. Epidemiology of Streptococcus pneumoniae Serotypes in Jordan Amongst Children Younger than the Age of 5: A National Cross-Sectional Study. Vaccines. 2023 Sep;11(9):1396.

Legends:

FIGURE 1. Adenovirus Positivity Rate by Age Group.

Table 1 - Presence of symptoms of adenovirus by positivity and coinfection.

Table 2 - Duration of symptoms by adenovirus positivity.

Table 3 - Descriptive statistics of clinical findings by adenovirus positivity and coinfection.

Table 4 - Binary logistic regression analysis of factors associated with a positive adenovirus result across all age groups.

Table 5 - Binary logistic regression analysis of the factors associated with the presence of complications across all age groups.

Table 1 - Presence of symptoms of adenovirus by positivity and coinfection.

	Adenovirus results					
	Negative Adenovirus (N=891)	Positive Adenovirus (N=109)		Positive Adenovirus Only (N=71)	Positive With coinfection** (N= 38)	
Symptoms Count	Count N (%)	Count N (%)	P-value 1*	Count N (%)	Count N (%)	P-value 2*
Fever	881 (98.9%)	107 (98.2%)	0.519	69 (97.2%)	38 (100%)	0.355
Cough	839 (94.2%)	99 (90.8%)	0.173	62 (87.3%)	37 (97.4%)	0.046
Sore throat	174 (19.5%)	30 (27.5%)	0.051	24 (33.8%)	6 (15.8%)	0.013
Rhinorrhea	476 (53.4%)	61 (55.0%)	0.616	40 (56.3%)	21 (55.3%)	0.877
Nasal congestion	402 (45.1%)	52 (47.7%)	0.608	30 (42.3%)	22 (57.9%)	0.259
Poor Feeding	437 (49.1%)	56 (51.4%)	0.646	37 (52.1%)	19 (50.0%)	0.88
Hypoxia/Cyanosis	251 (28.2%)	29 (26.6%)	0.731	19 (26.8%)	10 (26.3%)	0.942
Breathlessness	383 (43.0%)	45 (41.3%)	0.735	29 (40.9%)	16 (42.1%)	0.937
Respiratory crackles	519 (58.3%)	47 (43.1%)	0.003	28 (39.4%)	19 (50.0%)	0.006
Apnea>10 sec	9 (1.01%)	1 (0.92%)	0.927	0 (0.00%)	1 (2.63%)	0.419
Low activity level	480 (53.9%)	57 (52.3%)	0.755	35 (49.3%)	22 (57.9%)	0.659
Tachypnea	315 (35.4%)	27 (24.8%)	0.028	17 (23.9%)	10 (26.3%)	0.087
Post Tussive Vomiting	307 (34.5%)	34 (31.2%)	0.498	20 (28.2%)	14 (36.8%)	0.525

*Chi-square test, statistically significant at $P < 0.05$. P-value 1 is the result of testing the difference between negative and positive adenovirus participants. P-value2 is the result of testing he difference between participants who were adenovirus negative, positive adenovirus only and positive adenovirus with coinfection.

** coinfection refers to RSV or influenza positive with adenovirus positive

Table 2 - Duration of symptoms by adenovirus positivity.

	Adenovirus result						
Symptoms	**Negative**			**Positive**			P-value*
	Mean	Standard Deviation	[95% CI]	Mean	Standard Deviation	[95% CI]	
Fever (days)	3.89	3.65	3.65 - 4.13	4.51	4.31	3.65 - 4.13	0.101
Cough (days)	5.4	5.61	5.03-5.76	5.92	5.59	5.03-5.76	0.36
Sore throat (days)	0.85	2.48	0.69-1.01	2.03	4.61	0.69-1.01	<0.001
Rhinorrhea (days)	3.02	4.3	2.73 - 3.29	3.38	4.88	2.73 - 3.29	0.416
Nasal congestion (days)	2.52	4.5	2.22 - 2.81	2.9	4.89	2.22 - 2.81	0.41
Poor Feeding (days)	1.72	2.61	1.55-1.88	2.14	3.69	1.55-1.88	0.13

Hypoxia/Cyanosis (days)	1	3.15	0.792 - 1.20	1.23	3.66	0.792 - 1.20	0.48
Breathlessness (days)	1.62	2.72	1.44 - 1.79	1.82	3.24	1.44 - 1.79	0.48
Respiratory crackles (days)	2.37	3.18	2.16 - 2.57	2.28	4.08	2.16 - 2.57	0.81
Apnea >10sec (days)	0.24	1.62	0.13 - 34	0.1	0.71	0.13 - 34	0.38
Low activity level (days)	1.94	2.68	1.76 - 2.11	2.04	3.59	1.76 - 2.11	0.72
Tachypnea (days)	1.18	2.21	1.03 - 1.32	0.81	1.76	1.03 - 1.32	0.09
Post Tussive Vomiting (days)	1.06	2.07	0.92 - 1.19	1	1.93	0.92 - 1.19	0.78

* T-test statistically significant at $p < 0.05$.

Table 3 - Descriptive statistics of clinical findings by adenovirus positivity and coinfection**.**

		Adenovirus					
		Adenovirus Negative (N=891)	Adenovirus Positive (N=109)		Positive Only (N=71)	Positive with coinfection** (n=38)	
Clinical		Count N (%)	Count N (%)	P-Value 1*	Count N (%)	Count N (%)	P-Value 2*
Chest X ray infiltrate		573 (64.3%)	74 (67.9%)	0.46	48 (67.6%)	26 (68.4%)	0.759
White blood cell count (x10^9/L) ***	<4.0	14 -1.57%	1 (0.92%)	0.294	1 (1.41%)	0 (0.00%)	0.26
	>10.0	552 (61.95%)	76 (69.7%)		53 (74.7%)	23 (60.5%)	
	4 to 10	325 (36.48%)	32 (29.4%)		17 (24.0%)	15 (39.5%)	
Other Clinical Manifestations:							
· **Cardiovascular**		8 (0.90%)	2 (1.83%)	0.353	2 (2.82%)	0 (0.00%)	0.241
· **Low activity level**		273 (30.6%)	25 (22.9%)	0.097	17 (23.9%)	8 (21.1%)	0.24
· **Apnea >10sec**		9 (1.01%)	1 (0.92%)	0.927	0 (0.00%)	1 (2.63%)	0.419
· **Dehydration**		207 (23.2%)	26 (23.9%)	0.885	18 (25.4%)	8 (21.1%)	0.871
· **Hypoxia (SpO_2 <92%)**		210 (23.6%)	24 (22.0%)	0.718	13 (18.3%)	11 (29.0%)	0.429

· **Subcostal/intercostal retractions**	388 (43.6%)	43 (39.5%)	0.415	26 (36.6%)	17 (44.7%)	0.514
· **Wheeze**	516 (57.9%)	50 (45.9%)	0.017	32 (45.1%)	18 (47.4%)	0.055
· **Tachypnea**	460 (51.6%)	54 (49.5%)	0.681	37 (52.1%)	17 (44.7%)	0.702
· **Cyanosis**	67 (7.52%)	11 (10.1%)	0.345	4 (5.63%)	7 (18.4%)	0.038
· **Pneumothorax/Atelectasis**	2 (0.22%)	1 (0.92%)	0.212	0 (0.00%)	1 (2.63%)	0.026
· **Acute respiratory distress**	258 (29.0%)	24 (22.02%)	0.129	24 (22.0%)	282(28.2%)	0.129
· **Nasal Flaring**	17 (1.91%)	1 (0.92%)	0.463	1 (1.41%)	0 (0.00%)	0.665
· **Required ICU admission**	84 (9.43%)	12 (11.01%)	0.597	8 (11.3%)	4 (10.5%)	0.863

· **Overall oxygen need with Non-invasive ventilation**	55 (6.17%)	4 (3.67%)	0.295	3 (4.23%)	1 (2.63%)	0.546
· **Overall oxygen need with invasive ventilation**	6 (0.67%)	3 (2.75%)	0.03	0 (0.00%)	3 (7.89%)	<0.001

*Chi-square test, statistically significant at $P < 0.05$. P-value 1 is the result of testing the difference between participants adenovirus negative and positive. P-value2 is the result of testing the difference between participants adenovirus negative, adenovirus positive only and adenovirus positive with coinfection.

** coinfection refers to RSV or influenza positive with adenovirus positive.

Table 4 (Word file)

Table 4 - Binary logistic regression analysis of factors associated with a positive adenovirus result across all age groups.

*	Coef.	OR	P-value	[95% Confidence Interval]
Age ≤ 6 months	-1.11	0.33	<0.001	0.2 - 0.55
Wheezing	-0.44	0.64	0.034	0.43 - 0.97
Overall invasive oxygen need	1.55	4.71	0.038	1.09 - 20.3
Constant	-1.58	0.21	<0.001	0.15 - 0.28

** The reference category in all binary variables was assigned to "No" as a reference group*

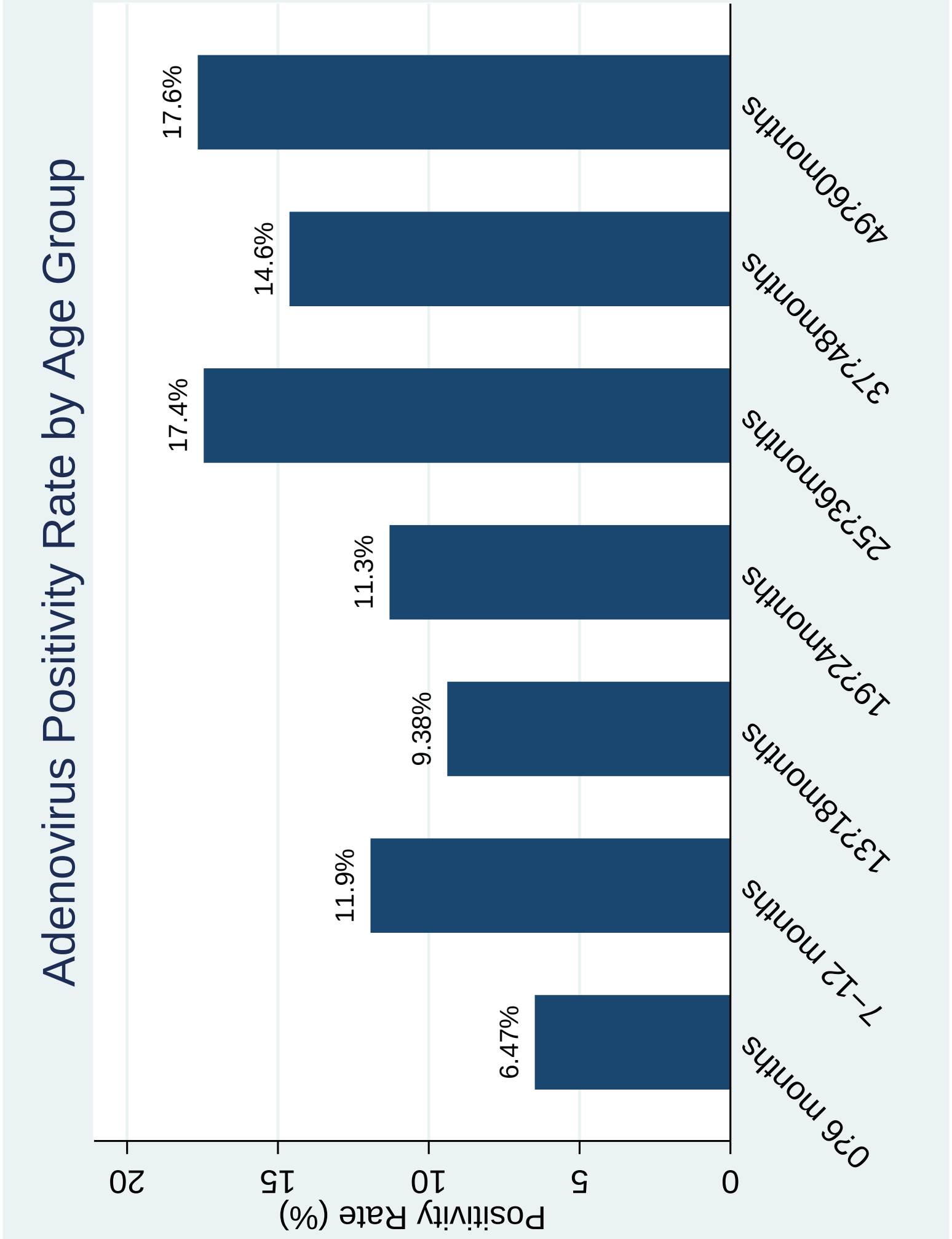
Table 5 - Binary logistic regression analysis of the factors associated with the presence of complications across all age groups.

*	Coef.	OR	P-value	[95% Confidence Interval]
Infection				
Adenovirus without coinfection	-2.74	0.06	<0.001	0.02 - 0.27
Adenovirus with coinfection**	0.55	1.73	0.149	0.82 - 3.67
Adenovirus Negative	Reference			
City				
Zarqa	1.02	2.77	<0.001	1.77 - 4.36
Irbid	0.58	1.79	0.022	1.09 - 2.93
Karak	-0.01	0.99	0.975	0.61 - 1.61
Amman	Reference			
Age (months)	-0.06	0.94	<0.001	0.93 - 0.95
Asthma	0.91	2.48	0.016	1.19 - 5.19
Constant	-1.77	0.17	<0.001	0.1 - 0.3

** The reference category in all binary variables was assigned to "No" as a reference group*

*** coinfection refers to (RSV positive or influenza Positive)*

Figure (EPS)


Adenovirus Positivity Rate by Age Group
Positivity Rate (%)
0
5
10
15
20
0?6 months
7–12 months
13?18months
19?24months
25?36months
37?48months
49?60months
6.47%
11.9%
9.38%
11.3%
17.4%
14.6%
17.6%

SUPPLEMENTAL DIGITAL CONTENT 1. Adenovirus positivity by month

			Adenovirus		Total	P-value*
			NO	YES		
Date month	Nov-2022	Count	289	31	320	0.182
		% within Month	90.3%	9.69%	100%	
	Dec-2022	Count	406	50	456	
		% within Month	89.0%	11.0%	100%	
	Jan-2023	Count	125	23	148	
		% within Month	84.5%	15.5%	100%	
	Feb-2023	Count	36	4	40	
		% within Month	90.0%	10.0%	100%	
	Mar-2023	Count	35	1	36	
		% within Month	97.2%	2.78%	100%	

***Chi-square, statistically significant at P < 0.05.**

SUPPLEMENTAL DIGITAL CONTENT 2. Investigating demographic Factors Associated with Adenovirus results.

Characteristic	Adenovirus				
	Negative (N=891)		Positive (N=109)		P-value*
	Count	Row (N %)	Count	Row (N %)	
Age in months, mean (SE)	16.4 (16.3)		23.2 (17.4)		<0.001
Age in months, median, [Q1-Q3]	9.03 (2.87-28.1)		20.7 (7.00-35.7)		
Age ≤ 6 months					
NO	534	85.71%	89	14.29%	<0.001
YES	357	94.69%	20	5.31%	
Gender, n (%)					0.52
Female patients	372	89.86%	42	10.14%	
Male patients	519	88.57%	67	11.43%	
City, n (%)					
· **Amman**	214	85.60%	36	14.40%	0.006
· **Zarqa**	227	90.80%	23	9.20%	
· **Irbid**	215	86.00%	35	14.00%	
· **Karak**	235	94.00%	15	6.00%	
Living area, n (%)					
· **Rural**	299	91.44%	28	8.56%	0.098
· **Urban**	592	87.96%	81	12.04%	
Admission through, n (%)					

· **Emergency Room referral**		102	89.47%	12	10.53%	0.634
· **Emergency Room without referral**		694	89.32%	83	10.68%	
· **GP**		6	100.00%	0	0.00%	
· **Outpatient clinic**		84	85.71%	14	14.29%	
· **Pediatrician**		5	100.00%	0	0.00%	
Preterm or Full term	full term	753	89.32%	90	10.68%	0.599
	preterm	138	87.90%	19	12.10%	
WGA (Weeks of Gestational Age)	<29	1	50.00%	1	50.00%	0.537
	29-30	3	75.00%	1	25.00%	
	31-32	14	56.25%	2	43.75%	
	33-34	22	88.00%	3	12.00%	
	35-36	98	89.09%	12	10.91%	
	>=37	753	89.32%	90	10.68%	
Delivery	Normal VD,	473	88.41%	62	11.59%	0.453
	Caesarean	418	89.89%	47	10.11%	
Meconium-stained liquor	NO	874	89.00%	108	11.00%	0.463
	YES	17	94.44%	1	5.56%	
NICU	NO	672	88.77%	85	11.23%	0.556
	YES	219	90.12%	24	9.88%	
NICU ventilation	NO	770	89.22%	93	10.78%	0.753
	YES	121	88.32%	16	11.68%	
Surfactant Given	NO	743	88.88%	93	11.12%	0.607
	YES	148	90.24%	16	9.76%	

Breastfed	NO	288	86.23%	46	13.77%	0.022
	Exclusive	305	88.41%	40	11.59%	
	Mixed	298	92.83%	23	7.17%	
Mother smoking during pregnancy	NO	845	88.76%	107	11.24%	0.125
	YES	46	95.83%	2	4.17%	
Patient on special milk/diet	NO	858	88.82%	108	11.18%	0.13
	YES	33	97.06%	1	2.94%	
Overcrowding: (more than 3 more than three people per habitable room)[47]	NO	746	89.13%	91	10.87%	0.949
	YES	145	88.96%	18	11.04%	
Parents smoking cigarettes or Shisha	NO	240	86.64%	37	13.36%	0.123
	YES	651	90.04%	72	9.96%	
Smoking inside home by parents or other households	NO	691	89.51%	81	10.49%	0.446
	YES	200	87.72%	28	12.28%	
Patient regular medications	NO	723	88.93%	90	11.07%	0.719
	YES	168	89.84%	19	10.16%	
Highest completed education of mother	Diploma school	84	94.38%	5	5.62%	0.155
	Primary school	99	90.83%	10	9.17%	
	Secondary school	436	88.80%	55	11.20%	
	University BSc	251	88.38%	33	11.62%	

	University Postgraduate	21	77.78%	6	22.22%	
Highest completed education of Father	Diploma school	72	90.00%	8	10.00%	0.524
	Primary school	124	91.18%	12	8.82%	
	Secondary school	438	88.48%	57	11.52%	
	University BSc	213	90.25%	23	9.75%	
	University Postgraduate	44	83.02%	9	16.98%	
Chronic conditions:						
Asthma	No	842	88.82%	106	11.18%	0.223
	Yes	49	94.23%	3	5.77%	
Bronchopulmonary dysplasia	No	891	89.19%	108	10.81%	0.004
	Yes	0	0.00%	1	100.00%	
Congenital heart disease	No	853	89.04%	105	10.96%	0.77
	Yes	38	90.48%	4	9.52%	
Neuromuscular disease	No	880	89.25%	106	10.75%	0.203
	Yes	11	78.57%	3	21.43%	
Other comorbidities**	No	793	89.30%	95	10.70%	0.564
	Yes	98	87.50%	14	12.50%	
Cystic fibrosis	No	891	89.19%	108	10.81%	0.004

	Yes	0	0.00%	1	100.00%	
Other congenital disease* **	No	881	89.17%	107	10.83%	0.519
	Yes	10	83.33%	2	16.67%	
Eczema (Atopy)	NO	873	89.36%	104	10.64%	0.092
	YES	18	78.26%	5	21.74%	
Patient Chronic Conditions	NO	698	89.26%	84	10.74%	0.761
	YES	193	88.53%	25	11.47%	
Patient attending kindergarten or day care	NO	813	89.44%	96	10.56%	0.277
	YES	78	85.71%	13	14.29%	
Parent with history of atopic eczema	NO	822	89.45%	97	10.55%	0.238
	YES	69	85.19%	12	14.81%	
Siblings attending kindergarten or daycare	NO	717	89.29%	86	10.71%	0.697
	YES	174	88.32%	23	11.68%	
Parent with history of asthma	NO	821	89.63%	95	10.37%	0.076
	YES	70	83.33%	14	16.67%	
Siblings with history of asthma	NO	815	88.78%	103	11.22%	0.277
	YES	76	92.68%	6	7.32%	
Siblings <5 years living in the same household	NO	367	89.95%	41	10.05%	0.473
	YES	524	88.51%	68	11.49%	

*Chi-square test, statistically significant at $p< 0.05$. For 'Age' the t-test was used.

** Other comorbidities: including congenital heart disease, Ventricular Septal Defect (VSD), Antenatally Diagnosed Congenital Pulmonary Airway Malformation (CPAM) Type 1 with extra lobar sequestration, and Complete Atrioventricular (AV) Canal, along with Atrial Septal Defect (ASD) and VSD leading to heart failure. Additionally, Down syndrome, Global Developmental Delay (GDD), and epilepsy. Other conditions include Reactive Airway Disease (RAD), Retinopathy of Prematurity (ROP), renal anomalies, seizures and Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD).

***In terms of other congenital diseases, these included: chest deformity, Gastroesophageal Reflux Disease (GERD), Pierre Robin Syndrome, horseshoe kidney, laryngomalacia, single kidney, single lung, hydrocephalus, and Vesicoureteral Reflux (VUR).