Systematic review and meta-analysis of the prevalence of common respiratory viruses in children < 2 years with bronchiolitis reveal a weak role played by the SARS-CoV-2

Sebastien Kenmoe¹, Cyprien Kengne-Nde², Jean Thierry Ebogo-Belobo³, Donatien Serge Mbagá⁴, Abdou Fatawou Modiynji¹⁵, Richard Njouom¹⁺

¹: Department of Virology, Centre Pasteur of Cameroon, 451 Rue 2005, P.O. Box 1274 Yaoundé, Cameroon

²: National AIDS Control Committee, Epidemiological Surveillance, Evaluation and Research Unit, P.O. Box 1459Yaounde, Cameroon

³: Medical Research Centre, Institut of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon

⁴: Department of Microbiology, Faculty of Science, The University of Yaounde I, Yaoundé, Cameroon

⁵: Department of Animals Biology and Physiology, Faculty of Sciences, University of Yaoundé I, P.O. Box 337, Yaoundé, Cameroon

* Correspondence to: Richard Njouom, PhD/HDR

Centre Pasteur of Cameroon, P.O Box: 1274 Yaoundé

Email: njouom@pasteur-yaounde.org / njouom@yahoo.com

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Abstract

Introduction The advent of genome amplification assays has allowed description of new respiratory viruses and to reconsider the role played by certain respiratory viruses in bronchiolitis. This systematic review and meta-analysis was initiated to clarify the prevalence of respiratory viruses in children with bronchiolitis in the coronavirus disease 2019 pandemic context.

Methods We performed an electronic search through Pubmed and Global Index Medicus databases. We included observational studies reporting the detection rate of common respiratory viruses in children with bronchiolitis using molecular assays. Data was extracted and the quality of the included articles was assessed. We conducted sensitivity, subgroups, publication bias, and heterogeneity analyses using a random effect model.

Results The final meta-analysis included 51 studies. Human respiratory syncytial virus (HRSV) was largely the most commonly detected virus 59.2%; 95% CI [54.7; 63.6]). The second predominant virus was Rhinovirus (RV) 19.3%; 95% CI [16.7; 22.0]) followed by Human bocavirus (HBoV) 8.2%; 95% CI [5.7; 11.2]). Other reported viruses included Human Adenovirus (HAdV) 6.1%; 95% CI [4.4; 8.0]), Human Metapneumovirus (HMPV) 5.4%; 95% CI [4.4; 6.4]), Human Parainfluenzavirus (HPIV) 5.4%; 95% CI [3.8; 7.3]), Influenza 3.2%; 95% CI [2.2; 4.3], mild Human Coronavirus (HCoV) 2.9%; 95% CI [2.0; 4.0]), and Enterovirus (EV) 2.9%; 95% CI [1.6; 4.5]). HRSV was the predominant virus involved in multiple detection
and most codetections were HRSV + RV 7.1%, 95% CI [4.6; 9.9]) and HRSV + HBoV 4.5%, 95% CI [2.4; 7.3]).

**Conclusions** The present study has shown that HRSV is the main cause of bronchiolitis in children, we also have Rhinovirus, and Bocavirus which also play a significant role. No study has reported the presence of Severe Acute Respiratory Syndrome Coronavirus-2 in children with bronchiolitis to date.

**Review registration** PROSPERO, CRD42018116067.

**Keywords** Respiratory viruses; Acute Bronchiolitis; Children
Introduction

Bronchiolitis infection is included among the leading cause of hospitalization and death in pediatrics. Bronchiolitis also represents a high economic burden on society and has generated a high hospital cost of around 72 million euros for children under the age of 2 in Portugal between 2000 and 2015. A study by Shi et al. found that lower respiratory infections due to Human Respiratory Syncytial Virus (HRSV), which is the main agent of bronchiolitis, causes approximately 3.2 million hospitalizations and about 60 thousand deaths per year worldwide in children under the age of 5.

Bronchiolitis is generally considered to be a viral illness, and the most common include HRSV, Influenza virus, Rhinovirus (RV), Human Metapneumovirus (HMPV), Enterovirus (EV), Human Coronavirus (HCoV), Human Parainfluenza Virus (HPIV), Human Adenovirus (HAdV), and Human Bocavirus (HBoV).

During the last two decades, the increase in use of Polymerase Chain Reaction (PCR) assays for the detection of respiratory viruses has led to a reassessment of the role played by viruses such as RV in acute respiratory infections. These molecular detection assays have also revealed new respiratory viruses such as HMPV and HBoV, and some RV and HCOV species.

Coronavirus disease 2019 (Covid-19) was first reported in Wuhan, Hubei province in China. This disease is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). This virus is the seventh described species of human coronavirus which includes 4 species associated with mild diseases (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) and 3 species associated with severe pneumonia (SARS-CoV, MERS-CoV and SARS-CoV-2). The World Health Organization declared Covid-19 as a public health emergency of international concern on January 30, 2020 and as a global pandemic on March 11, 2020.
Covid-19 spread rapidly worldwide and is now present in 188 countries in the 5 continents. At August 22, 2020, more than 20 million of cases have been confirmed and about 800 thousand deaths due to Covid-19 were recorded \(^1\). The objective of this study was to report the detection rate of viral agents using PCR and the associated risk factors with bronchiolitis in children \(\leq 2\) years in the era of Covid-19.
Methods

Study design

This systematic review was conducted according to the principles of the Centre for Reviews and Dissemination. The methodological standards of the PRISMA declaration have been applied for this review (S1 Table). The protocol for this review has been registered in the PROSPERO database under number CRD42018116067. This review reports previous published data and ethical clearance was not required.

Electronic search

Pubmed and Global Index Medicus were searched with a combination of keywords related to common respiratory viruses and bronchiolitis. The search strategy applied in Pubmed is available in S2 Table. The search strategy in Pubmed has been adjusted to the Global Index Medicus database. The databases have been consulted since their creation until 06 February 2019 and updated on 16 August 2020.

Manual search

The literature search was supplemented by a review of references of included articles and relevant reviews. We have gradually updated our search strategy iteratively by adding the specific keywords of the searches found manually. No language or geographical restrictions have been applied.
Integration criteria

Observational studies (cohort, case-control, and cross-sectional) published in peer-reviewed journals were used if they reported the detection of respiratory viruses using PCR assays in children < 2 years with bronchiolitis. A wide range of definition of bronchiolitis has been used by the authors of the included articles. Case reports, review, and duplicated studies were excluded.

Study selection

Two investigators (SK and AFM) independently preselected the identified articles on the basis of their titles and abstracts (if necessary). The relevance of eligible studies was assessed using predefined integration criteria.

Data extraction

Data from the included studies were independently extracted by two authors (SK and FBSN) via a preconceived form. The information gathered included: the name of the author, the year of publication, the design of the study, the country, the WHO regions, the sampling method, the period of the study, the definition of bronchiolitis, the exclusion criteria, the sample type, the viral detection assay, age range, mean or median age, percentage of male gender, number of samples tested for each virus, number of positives for each virus, and the data of the evaluation of the study quality. All disagreements were resolved by discussion between two authors and an arbitration by a third author if need be.
Assessing research quality

The quality of the studies was estimated as low (8-10), moderate (5-7), and high (0-4) risk of bias using the Hoy et al. assessment tool (S3 Table).²⁴

Data synthesis

Forest plots, summary tables and a narrative summary were used to present the overall results. The calculation of the prevalence took into account the weight of each study. Each prevalence was estimated using a random-effect meta-analysis given the inseparable heterogeneity of observational studies. A dual arcsine transformation of Freeman-Turkey was used to stabilize the variances in the prevalence calculation. Subgroup analysis was performed to estimate prevalence variations by sample type used for viral detection, WHO region and some viral codetection status. The Cochran Q test and the I² statistic were used to measure heterogeneity between studies.²⁵ Analysis based on studies with a low risk of bias, children ≤ 1 year, hospitalized children, and cross sectional were used for sensitivity analyses. Visual inspection of a funnel plot and the Egger test were used to estimate the risk of publication bias.²⁶ A prediction interval was provided for all meta-analyses to predict future study values. Values of p <0.05 were assimilated as statistically significant. The analyses were conducted using the meta package version 4.9-2 under the R version 3.5.1 software.²⁷,²⁸ The code "metaprop" was used for meta-analyses of prevalence.
Results

Study selection and characteristics

The literature search provided a total of 3777 articles and 154 duplicates were excluded. Selection based on titles and abstracts excluded 3370 irrelevant articles. We therefore examined 253 complete texts and excluded 203 for multiple reasons (Fig 1, S4 Table). We finally stayed with 50 articles (51 studies) that met the inclusion criteria. The different bronchiolitis case definitions are showed in the S5 Table. Children were recruited in included studies between October 1999 and December 2017 (Table 1). The selected studies were published between 2002 and 2020. Almost all children included in these studies were recruited consecutively. Most studies were cross-sectional studies (44; 86.3%), published in the English language (44; 86.3), carried out in Europe (28; 54.9%), carried out on a continuous period (35; 68.6%). Most studies had a prospective recruitment (44; 86.3%), a clear bronchiolitis case definition (45; 88.2%), and low risk of bias (34; 66.7%). Hospitalized boys less than 2 years were predominant in included studies. Analyzed samples were mainly nasopharyngeal secretions (45; 88.2%).

Fig 1. Flow chart of research and selection of studies

Table 1. Sociodemographic and clinical characteristics of included studies

| Characteristics | N = 51 | % |
|-----------------|--------|---|
| %Male; range    | 48.0-72.5 |   |
| Age range       |        |   |
| < 0.5 year      | 2      | 3.9 |
| Period of inclusion of participants; range | 2002-2020 |
|------------------------------------------|------------|
| Year of publication; range               |            |
| Study design                             |            |
| • Case control                           | 2          |
| • Cohort (Baseline data)                 | 5          |
| • Cross sectional                        | 44         |
| Sampling                                 |            |
| • Consecutive sampling                   | 50         |
| • Simple random sampling                 | 1          |
| Timing of data collection                |            |
| • Prospectively                          | 44         |
| • Retrospectively                        | 7          |
| Study bias                               |            |
| • Low risk of bias                       | 34         |
| • Moderate risk of bias                  | 17         |
| Seasonality                              |            |
| Category                                | Count | Percentage |
|-----------------------------------------|-------|------------|
| Continuous study period                 | 35    | 68.6%      |
| Interrupted time series study           | 15    | 29.4%      |
| Unclear/Not reported                    | 1     | 2.0%       |
| WHO region                              |       |            |
| America                                 | 12    | 23.5%      |
| Eastern Mediterranean                   | 3     | 5.8%       |
| Europe                                  | 28    | 54.9%      |
| South-East Asia                         | 1     | 1.9%       |
| Western Pacific                         | 7     | 13.7%      |
| Language                                |       |            |
| English                                 | 44    | 86.3%      |
| Non-English                             | 7     | 13.7%      |
| Hospitalization                         |       |            |
| Hospitalized                            | 40    | 78.4%      |
| Hospitalized/Outpatients                | 4     | 7.8%       |
| Outpatients                             | 5     | 9.8%       |
| Unclear/Not reported                    | 2     | 3.9%       |
| Bronchiolitis definition                |       |            |
| No                                      | 6     | 11.8%      |
Prevalence and codetection rate of viral infections among children < 2 years with bronchiolitis

HRSV was largely the most commonly detected virus (59.2%; 95% CI [54.7; 63.6]). Other viruses included in descending order: RV (19.3%; 95% CI [16.7; 22.0]), HBoV (8.2%; 95% CI [5.7; 11.2]), HAdV (6.1%; 95% CI [4.4; 8.0]), HPIV (5.4%; 95% CI [3.8; 7.3]), HMPV (5.4%; 95% CI [4.4; 6.4]), Influenza (3.2%; 95% CI [2.2; 4.3]), mild HCoV (2.9%; 95% CI [2.0; 4.0]), and EV (2.9%; 95% CI [1.6; 4.5]) (Fig 2, S1 Fig). HRSV was the predominant virus involved in multiple detections. The most codetections were HRSV + RV (7.1%, 95% CI [4.6; 9.9]) and HRSV + HBoV (4.5%, 95% CI [2.4; 7.3]) (S2 Fig). We did not find any major change in our results when we conducted sensitivity analyses that included only children < 1 year, hospitalized children, studies with bronchiolitis case definition, cross sectional studies, and low risk of bias studies (S6 Table). Substantial heterogeneity was detected in overall prevalence and sensitivity analyses for all viruses. Publication bias was detected for HMPV and Influenza meta-analyses (S6 Table and S3-11 Fig).
Fig 2. Global prevalence of Respiratory Viruses in children with bronchiolitis

Subgroup meta-analysis

The subgroup analysis showed a significant difference in the prevalence of HRSV ($p < 0.001$), RV ($p < 0.001$), HBoV ($p < 0.001$), HAdV ($p < 0.001$), HPIV ($p < 0.001$), mild HCoV ($p < 0.001$), and EV ($p < 0.001$) according to the WHO regions (S7 Table). Lower prevalence was observed in Eastern Mediterranean for HRSV, HBoV, and EV; in America for RV and HAdV; and in Europe for HPIV. A significant increase in the prevalence was observed for continuous study period for HMPV ($p = 0.005$), HPIV ($p = 0.005$), and Influenza ($p = 0.014$). The subgroup analysis according to the type of sample revealed a significant increase in nasopharyngeal secretions for HRSV ($p < 0.001$).

Discussion

This systematic review asserts the strong predominance of HRSV in children < 2 years with bronchiolitis. We have shown that HRSV is present in almost two-thirds of bronchiolitis cases. RV and two recently described viruses by the advent of molecular assays (HBoV and HMPV) were among the most common viruses. The HRSV + RV and HRSV + HBoV co-detections were the most frequent.

The predominance pattern of respiratory viruses in bronchiolitis reported in this study is consistent with that reported by several previous narrative reviews.\textsuperscript{79-81} Regardless of multiple factors including detection assays, tested sample types, children’s age, and infection severity
most studies report that HRSV is the major agent in cases of bronchiolitis with rates ranging from 50 to 80%.⁸⁰ RV, that is the second most common virus in this study, has long been considered a cause of benign respiratory infection such as the common cold.⁸² Early investigations of the prevalence of RV in bronchiolitis episodes were conducted using traditional assays including serological testing and culture.⁸³,⁸⁴ The development of serological assays has always been difficult for RV because of the high number of serotypes. It is also well known that RV is insensitive to most cell lines used in viral isolation.⁸⁵,⁸⁶ Therefore the recent widespread use of PCR that would be linked to evidence of this importance of RV in low respiratory tract infections.⁸⁷–⁹² Many other recent works have also highlighted the importance of RV in other respiratory infections such as asthma, wheezing, and long-term respiratory sequelae in pre-school children.⁹³–⁹⁸

HMPV and HBoV were first described in 2001 and 2005 respectively using molecular assays.⁹,¹² As previously reported, our study further reinforces the role assigned to these relatively new agents in bronchiolitis.⁷⁹–⁸¹ Human Bocavirus is commonly reported in asymptomatic children and in co-detection with other viruses, which has long raised the question of its exclusive involvement in the pathogenesis of respiratory infections.⁹⁹–¹⁰⁴ On the other hand, reports have also highlighted HBoV involvement in life-threatening in children with severe respiratory infections, but this still does not establish a role for bocavirus as an important pathogen.¹⁰⁵,¹⁰⁶ The HBoV reported in this study as the third most common virus in children with bronchiolitis therefore deserves further investigation to explain its clinical relevance in bronchiolitis.

This study has shown an increased codetection of either RV or HBoV in children infected with HRSV. This high prevalence of RV or HBoV in HRSV positive patients may indicate an
overlap in the circulation period of these predominant viruses in bronchiolitis. It is known that HBoV and RV are recorded throughout the year, which obviously coincides with the circulation period of HRSV with peaks in winter and early spring.\textsuperscript{107,108}

Respiratory viruses are characterized by their easy transmission through the contact of contaminated objects and through the airway.\textsuperscript{109–111} These viruses are thus cosmopolitan and know no boundaries in their distribution across the different regions of the world. The frequencies of detection of respiratory viruses throughout the various regions of the world are governed by multiple climatic, sociodemographic and cultural factors. We are therefore unable to interpret the pattern of dominance observed in the prevalence of respiratory viruses according to the WHO regions observed during this work. The majority of interrupted time series studies included in this review were conducted in the HRSV peak circulation period.\textsuperscript{112} Contrary to our expectations, the prevalence of HRSV in these interrupted time series studies was not significantly greater than studies conducted over a continuous period. On the other hand, the HMPV, the HPIV and Influenza presented a significant higher prevalence in studies performed in continuous period. This result suggests that the peaks of circulation of HMPV, HPIV and Influenza are different from that of HRSV. In fact the frequency of detection of respiratory viruses can vary from year to another. Some studies have shown an overlap of HRSV and HMPV circulation while others have not.\textsuperscript{113,114} This disagreement observed in the seasonal pattern of these respiratory viruses circulation compromises our ability to interpret our subgroup analysis according to the continuous or interrupted study period.

It is generally recognized that bronchiolitis is a respiratory condition of children up to the age of 2 years.\textsuperscript{115} Several reports have shown that only clinical symptoms are enough for the
management of children with bronchiolitis, and viral testing is generally considered unnecessary.\textsuperscript{116,117} Data on the impact of multiple viral infections on the severity of bronchiolitis is controversial and does not justify recommendations of routinely virological examinations in most guidelines.\textsuperscript{118,119} Increase use of health resources and regularly recorded codetections do not warrant cohorting of infected children and is another reason that hinder the investigation of viral pathogens in bronchiolitis clinical practice.\textsuperscript{117} However, virological tests are still used in children with severe bronchiolitis to reduce the unnecessary use of antibiotics and interventions such as chest X-rays.\textsuperscript{115,120} In children admitted to receive palivizumab, American Academy of Pediatrics recommends HRSV testing for cessation of treatment if the test is positive.\textsuperscript{121} Assessing the respective contribution of respiratory viruses in bronchiolitis, a crucial importance in the orientation of priority actions specific to the choice of viruses in public health, particularly with the imminent introduction of vaccines against some respiratory viruses, especially HRSV.\textsuperscript{122}

It is important to mention that the small number of studies in some subgroups analyses restricts our ability to draw definitive conclusions. A second limitation in our study is that we did not consider multiple other factors that could further explain the variability in the prevalence of viruses in bronchiolitis such as comorbidities, anti-HRSV prophylaxis and the number of virus types sought in studies for multi-species such as mild HCoV and HPIV. It is also known that bronchiolitis case definitions show great variability in terms of age limit and constellation of clinical symptoms according to geographic area and time that we did not consider in this study.\textsuperscript{79,123}

Beyond these limitations, this systematic review and meta-analysis reports the prevalence recorded over two decades of a large panel of common respiratory viruses currently involved...
in bronchiolitis. We report the data obtained in almost all WHO regions from the PCR that currently represent the most commonly used assays in diagnosing respiratory viruses, which is another major asset of this work. We also have conducted multiple sensitivity analyses that further strengthen the robustness of our results on multiple important aspects such as children hospitalization, age range, and design and quality of studies.

The results of this systematic review and meta-analysis further underline the strong majority of HRSV in children with bronchiolitis. This work also highlights the importance of RV and the newly described HMPV and HBoV in children with bronchiolitis. The HRSV + RV and HRSV + HBoV co-detections were the most frequent in children with bronchiolitis.

The high costs of prophylaxis with palivizumab remain a major wall to its widespread use in reducing the burden of bronchiolitis in children. Therefore, to significantly reduce the heavy burden of bronchiolitis due to HRSV in children, the finalization and availability of HRSV vaccines is a high priority. Future studies should explore the involvement of multiple viral infections in the severity of bronchiolitis cases to finally bring down the iron curtain on the need for the use of virologic testing in clinical practice for the better management of children with bronchiolitis. Studies are also needed to clarify the clinical impact of HBoV in bronchiolitis cases.

Legends

Table 1. General characteristics of included studies

Fig 1. Flow chart of research and selection of studies

Fig 2. Global prevalence of Respiratory Viruses in children < 2 years with bronchiolitis
Supporting information

S1 Table. Preferred reporting items for systematic reviews and meta-analyses checklist
S2 Table. Search strategy in Medline (Pubmed)
S3 Table. Items for risk of bias assessment
S4 Table. Main reasons of exclusion of eligible studies
S5 Table. Individual characteristics of included studies
S6 Table. Global prevalence and sensitivity analyses of respiratory viral infections in children < 2 years with bronchiolitis
S7 Table. Subgroup prevalence of respiratory viral infections in children with acute bronchiolitis
S1 Fig. Global prevalence of Respiratory Viruses in children < 2 years with bronchiolitis
S2 Fig. Codetection rate of viral infections among children < 2 years with bronchiolitis
S3 Fig. Funnel plot for publication for HRSV in people with bronchiolitis
S4 Fig. Funnel plot for publication for RV in people with bronchiolitis
S5 Fig. Funnel plot for publication for HBoV in people with bronchiolitis
S6 Fig. Funnel plot for publication for HAdV in people with bronchiolitis
S7 Fig. Funnel plot for publication for HMPV in people with bronchiolitis
S8 Fig. Funnel plot for publication for HPIV in people with bronchiolitis
S9 Fig. Funnel plot for publication for Influenza in people with bronchiolitis
S10 Fig. Funnel plot for publication for EV in people with bronchiolitis
S11 Fig. Funnel plot for publication for HCoV in people with bronchiolitis

Acknowledgments None
References

1. Deshpande S, Northern V. The clinical and health economic burden of respiratory syncytial virus disease among children under 2 years of age in a defined geographical area. Arch Dis Child 2003;88:1065-1069.

2. 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. Eur J Clin Microbiol Infect Dis 2012;31:1975-1981.

3. Mendes-da-Silva A, Gonçalves-Pinho M, Freitas A, Azevedo I. Trends in hospitalization for acute bronchiolitis in Portugal: 2000-2015. Pulmonology June 2018.

4. Schuh S, Kwong JC, Holder L, Graves E, Macdonald EM, Finkelstein Y. Predictors of Critical Care and Mortality in Bronchiolitis after Emergency Department Discharge. The Journal of Pediatrics 2018;199:217-222.e1.

5. Shay DK, Holman RC, Roosevelt GE, Clarke MJ, Anderson LJ. Bronchiolitis-associated mortality and estimates of respiratory syncytial virus-associated deaths among US children, 1979-1997. J Infect Dis 2001;183:16-22.

6. Shi T, McAllister DA, O’Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet July 2017.

7. Gern JE. The ABCs of Rhinoviruses, Wheezing, and Asthma. Journal of Virology 2010;84:7418-7426.

8. Stenberg-Hammar K, Hedlin G, Söderhäll C. Rhinovirus and preschool wheeze. Pediatric Allergy and Immunology 2017;28:513-520.

9. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A 2005;102:12891-12896.

10. Brodzinski H, Ruddy RM. Review of new and newly discovered respiratory tract viruses in children. Pediatr Emerg Care 2009;25:352-360; quiz 361-363.

11. Jartti T, Jartti L, Ruuskanen O, Söderlund-Venermo M. New respiratory viral infections. Curr Opin Pulm Med 2012;18:271-278.

12. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 2001;7:719-724.

13. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. Nat Med 2004;10:368-373.

14. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med January 2020.
15. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. Proc Soc Exp Biol Med 1966;121:190-193.

16. McIntosh K, Becker WB, Chanock RM. Growth in suckling-mouse brain of ‘IBV-like’ viruses from patients with upper respiratory tract disease. Proc Natl Acad Sci U S A 1967;58:2268-2273.

17. Peiris JSM, Lai ST, Poon LLM, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003;361:1319-1325.

18. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. Nat Med 2004;10:368-373.

19. Woo PCY, Lau SKP, Chu C-m., et al. Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia. Journal of Virology 2005;79:884-895.

20. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. New England Journal of Medicine 2012;367:1814-1820.

21. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. The Lancet Infectious Diseases 2020;20:533-534.

22. Centers for Reviews and Dissemination. CRD’s Guidance for Undertaking Reviews in Healthcare: Centers for Reviews and Dissemination. England: York Associates; 2009.

23. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009;6.

24. Hoy D, Brooks P, Woolf A, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. J Clin Epidemiol 2012;65:934-939.

25. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-1558.

26. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-634.

27. Schwarzer G. meta: An R package for meta-analysis. R News 2007;7:40-5.

28. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at https://www.r-project.org/. Accessed 28 September 2018.

29. Amat F, Henquell C, Verdan M, Roszyk L, Mulliez A, Labbé A. Predicting the severity of acute bronchiolitis in infants: Should we use a clinical score or a biomarker?: Is a Biomarker Useful in Acute Bronchiolitis? Journal of Medical Virology 2014;86:1944-1952.
30. Antunes H, Rodrigues H, Silva N, et al. Etiology of bronchiolitis in a hospitalized pediatric population: prospective multicenter study. J Clin Virol 2010;48:134-136.

31. Arabzadeh S, Daeiparizi MH, Molaei HR, Aghaeiafshar A, Salari AA. The Frequency Distribution of Parainfluenza, Adeno and Respiratory Syncytial Virus Infections in Children below 2 Years Old with Bronchiolitis, by Multiplex Polymerase Chain Reaction Method, Afzalipoor Hospital, Kerman, 2006. Journal of Kerman University of Medical Sciences 2008;15:305-311.

32. Atay Ö, Pekcan S, Gökçü B, Özdemir M. Risk Factors and Clinical Determinants in Bronchiolitis of Infancy. Turk Thorac J 2020;21:156-162.

33. Azkur D, Özyayn D, Dibek-Mısırlıoğlu E, et al. Viral etiology in infants hospitalized for acute bronchiolitis. Turk J Pediatr 2014;56:592-596.

34. Bamberger E, Srugo I, Abu Raya B, et al. What is the clinical relevance of respiratory syncytial virus bronchiolitis?: findings from a multi-center, prospective study. European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology 2012;31:3323-30.

35. Bekhof. Co-Infections in Children Hospitalised for Bronchiolitis: Role of Roomsharing. Journal of Clinical Medicine Research 2013.

36. Bekhof J, Wessels M, Ten Velde E, et al. Room Sharing in Hospitalized Children With Bronchiolitis and the Occurrence of Hospital-Acquired Infections: A Prospective Cohort Study. Hosp Pediatr 2019;9:415-422.

37. Brand HK, de Groot R, Galama JMD, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. Pediatric Pulmonology 2012;47:393-400.

38. Calvo C, Pozo F, García-García ML, et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Paediatr 2010;99:883-887.

39. Cangiano G, Nenna R, Frassanito A, et al. Bronchiolitis: Analysis of 10 consecutive epidemic seasons. Pediatric pulmonology 2016;51:1330-1335.

40. Chen Z-R, Ji W, Wang Y-Q, et al. Etiology of acute bronchiolitis and the relationship with meteorological conditions in hospitalized infants in China. Journal of the Formosan Medical Association 2014;113:463-469.

41. Dong-Keon YON, Chae-Yeon MIN, Eun-Kyo HA, et al. Clinical characteristics and genetic variation in respiratory syncytial virus isolated from infants hospitalized due to acute bronchiolitis in Korea during winter season 2016–2017. Allergy, Asthma & Respiratory Disease 2018:110-115.

42. Dumas O, Mansbach JM, Jartti T, et al. A clustering approach to identify severe bronchiolitis profiles in children. Thorax June 2016.

43. Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H. Detection of human coronavirus NL63 in young children with bronchiolitis. Journal of medical virology 2005;75:463-5.
44. Gökçe Ş, Kurugöl Z, Koturoğlu G, Çiček C, Aslan A. Etiology, Seasonality, and Clinical Features of Viral Respiratory Tract Infections in Children Hospitalized With Acute Bronchiolitis: A Single-Center Study. Glob Pediatr Health 2017;4:2333794X17714378.

45. Hendaus MA, Alhammadi AH, Chandra P, Muneer E, Khalifa MS. Identifying agents triggering bronchiolitis in the State of Qatar. International journal of general medicine 2018;11:143-149.

46. Janahi I, Abdulkayoum A, Almeshwesh F, Alkuwari M, Al Hammadi A, Alameri M. Viral aetiology of bronchiolitis in hospitalised children in Qatar. BMC Infect Dis 2017;17:139.

47. Jartti T, Aakula M, Mansbach JM, et al. Hospital length-of-stay is associated with rhinovirus etiology of bronchiolitis. The Pediatric infectious disease journal 2014;33:829-34.

48. Mansbach JM, Hasegawa K, Henke DM, et al. Respiratory syncytial virus and rhinovirus severe bronchiolitis are associated with distinct nasopharyngeal microbiota. J Allergy Clin Immunol 2016;137:1909-1913.e4.

49. Mansbach JM, McAdam AJ, Clark S, et al. Prospective multicenter study of the viral etiology of bronchiolitis in the emergency department. Acad Emerg Med 2008;15:111-118.

50. Midulla F, Nenna R, Scagnolari C, et al. How Respiratory Syncytial Virus Genotypes Influence the Clinical Course in Infants Hospitalized for Bronchiolitis. The Journal of infectious diseases September 2018.

51. Midulla F, Scagnolari C, Bonci E, et al. Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. Arch Dis Child 2010;95:35-41.

52. Miller EK, Gebretsadik T, Carroll KN, et al. Viral Etiologies of Infant Bronchiolitis, Croup and Upper Respiratory Illness During 4 Consecutive Years: The Pediatric Infectious Disease Journal 2013;32:950-955.

53. Nascimento MS, de Souza AV, de Souza Ferreira AV, Rodrigues JC, Abramovici S, da Silva Filho LVF. High Rate of Viral Identification and Coinfections in Infants with Acute Bronchiolitis. Clinics (Sao Paulo) 2010;65:1133-1137.

54. Nenna R, Frassanito A, Petrarca L, Di Mattia G, Midulla F. Age Limit in Bronchiolitis Diagnosis: 6 or 12 Months? Front Pediatr 2020;8:144.

55. Papadopoulos NG, Moustaki M, Tsolia M, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med 2002;165:1285-1289.

56. Papoff P, Moretti C, Cangiano G, et al. Incidence and predisposing factors for severe disease in previously healthy term infants experiencing their first episode of bronchiolitis. Acta Paediatr 2011;100:e17-23.

57. Petrarca L, Nenna R, Frassanito A, et al. Acute bronchiolitis: Influence of viral co-infection in infants hospitalized over 12 consecutive epidemic seasons. Journal of Medical Virology 2018;90:631-638.
58. Piedra F-A, Mei M, Avadhanula V, et al. The interdependencies of viral load, the innate immune response, and clinical outcome in children presenting to the emergency department with respiratory syncytial virus-associated bronchiolitis. PLoS ONE 2017;12:e0172953.

59. Pitrez PMC, Stein RT, Stuermer L, et al. [Rhinovirus and acute bronchiolitis in young infants]. J Pediatr (Rio J) 2005;81:417-420.

60. Praznik A, Vinsek N, Prodan A. Risk factors for bronchiolitis severity: A retrospective review of patients admitted to the university hospital from central region of Slovenia. 2018;12:765-771.

61. Ricart S, Rovira N, Garcia-Garcia JJ, et al. Frequency of apnea and respiratory viruses in infants with bronchiolitis. The Pediatric infectious disease journal 2014;33:988-90.

62. Ricart S, Garcia-Garcia JJ, Anton A, et al. Analysis of Human Metapneumovirus and Human Bocavirus Viral Load: The Pediatric Infectious Disease Journal 2013;32:1032-1034.

63. Ricart S, Marcos MA, Sarda M, et al. Clinical risk factors are more relevant than respiratory viruses in predicting bronchiolitis severity. Pediatric Pulmonology 2013;48:456-463.

64. Richard N, Komurian-Pradel F, Javouhey E, et al. The Impact of Dual Viral Infection in Infants Admitted to a Pediatric Intensive Care Unit Associated with Severe Bronchiolitis: The Pediatric Infectious Disease Journal 2008;27:213-217.

65. Robledo-Aceves M, Moreno-Peregrina MJ, Velarde-Rivera F, et al. Risk factors for severe bronchiolitis caused by respiratory virus infections among Mexican children in an emergency department. Medicine 2018;97:e0057.

66. Salvador Garcia C, Moreno Docon A, Pinero JA, Alfayate Miguelez S, Iborra Bendicho MA. [Aetiology of bronchiolitis in hospitalised children in South-East Spain]. Anales de pediatria (Barcelona, Spain : 2003) 2012;77:386-90.

67. Souza APD de, Leitão LA de A, Luisi F, et al. Lack of association between viral load and severity of acute bronchiolitis in infants. J Bras Pneumol 2016;42:261-265.

68. Teeratakulpisarn J, Ekalaksananan T, Pientong C, Limwattananon C. Human metapneumovirus and respiratory syncytial virus detection in young children with acute bronchiolitis. Asian Pacific journal of allergy and immunology 2007;25:139-45.

69. Tsergouli K, Pappa S, Haidopoulou K, Gogou M, Giannopoulos A, Papa A. Respiratory Syncytial Virus in Greece, 2016-2018. Intervirology 2019;62:210-215.

70. Tsou P, Vadvelan A, Kovvuri M, et al. Association between multiple respiratory viral infections and pediatric intensive care unit admission among infants with bronchiolitis. Arch Pediatr 2020;27:39-44.

71. Uyar M, Kuyucu N, Tezcan S, Aslan G, Tasdelen B. [Determination of the frequency of human bocavirus and other respiratory viruses among 0-2 years age group children diagnosed as acute bronchiolitis]. Mikrobiyoloji bulteni 2014;48:242-58.
72. Vieira SE, Thomazelli LM, de Paulis M, et al. Infections Caused by HRSV A ON1 Are Predominant among Hospitalized Infants with Bronchiolitis in São Paulo City. Biomed Res Int 2017;2017:3459785.

73. Wang Y, Ji W, Hao C, Yan YD, Shao X, Xu J. Comparison of bronchiolitis of human metapneumovirus and human respiratory syncytial virus. Acta virologica 2015;59:98-100.

74. Wollmeister E, Alvarez AE, Bastos JCS, et al. Respiratory syncytial virus in Brazilian infants - Ten years, two cohorts. J Clin Virol 2018;98:33-36.

75. Xepapadaki P, Psarras S, Bossios A, et al. Human Metapneumovirus as a causative agent of acute bronchiolitis in infants. J Clin Virol 2004;30:267-270.

76. Xuan L, Rongfang Z, Zhiping XIE, Hanchun GAO, Zhaojun D, Donghai LIU. Clinical characteristics of bocavirus infection in children with bronchiolitis. Chinese Journal of Experimental and Clinical Virology 2018:187-190.

77. Yon D, Min C-Y, Ha EK, et al. Clinical characteristics and genetic variation in respiratory syncytial virus isolated from infants hospitalized due to acute bronchiolitis in Korea during winter season 2016–2017. 2018.

78. Zhou L, Xiao Q, Zhao Y, Huang A, Ren L, Liu E. The impact of viral dynamics on the clinical severity of infants with respiratory syncytial virus bronchiolitis. Journal of medical virology 2015;87:1276-84.

79. Fretzayas A, Moustaki M. Etiology and clinical features of viral bronchiolitis in infancy. World journal of pediatrics : WJP 2017;13:293-299.

80. Meissner HC. Viral Bronchiolitis in Children. New England Journal of Medicine 2016;374:62-72.

81. Teshome G, Gattu R, Brown R. Acute bronchiolitis. Pediatr Clin North Am 2013;60:1019-1034.

82. Heikkinen T, Järvinen A. The common cold. The Lancet 2003;361:51–59.

83. Ginocchio CC. Detection of respiratory viruses using non-molecular based methods. J Clin Virol 2007;40 Suppl 1:S11-14.

84. Rylander E, Eriksson M, Pershagen G, Nordvall L, Ehrnsr A, Ziegler T. Wheezing bronchitis in children. Incidence, viral infections, and other risk factors in a defined population. Pediatric allergy and immunology 1996;7:6–11.

85. Gern JE, Busse WW. The role of viral infections in the natural history of asthma. Journal of Allergy and Clinical Immunology 2000;106:201-212.

86. Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. Clinical & Experimental Allergy 1992;22:325-336.

87. Berezin EN. Rhinovirus and bronchiolitis. Jornal de pediatria 2006;82:163; author reply 164.
88. Drysdale SB, Mejias A, Ramilo O. Rhinovirus - not just the common cold. J Infect 2017;74 Suppl 1:S41-S46.

89. Korppi M. Rhinovirus bronchiolitis: to be or not to be? Acta Paediatrica 2014;103:997-999.

90. Lau, SKP SK, Yip, Yip CCY CC, Woo, Woo PCY PC, Yuen K-Y. Human rhinovirus C: a newly discovered human rhinovirus species. Emerg Health Threats J 2010;3.

91. McErlean P, Shackelton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. J Clin Virol 2007;39:67-75.

92. Renwick N, Schweiger B, Kapoor V, et al. A Recently Identified Rhinovirus Genotype Is Associated with Severe Respiratory-Tract Infection in Children in Germany. J Infect Dis 2007;196:1754-1760.

93. Zheng X-Y, Xu Y-J, Guan W-J, Lin L-F. Regional, age and respiratory-secretion-specific prevalence of respiratory viruses associated with asthma exacerbation: a literature review. Arch Virol 2018;163:845-853.

94. Liu L, Pan Y, Zhu Y, et al. Association between rhinovirus wheezing illness and the development of childhood asthma: a meta-analysis. BMJ Open 2017;7:e013034.

95. Cox DW, Bizzintino J, Ferrari G, et al. Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. American journal of respiratory and critical care medicine 2013;188:1358-64.

96. Halmo Hurdum S, Zhang G, Khoo SK, et al. Recurrent rhinovirus detections in children following a rhinovirus-induced wheezing exacerbation: A retrospective study. International journal of pediatrics and child health 2015;3:10-18.

97. Turunen R, Koistinen A, Vuorinen T, et al. The first wheezing episode: respiratory virus etiology, atopic characteristics, and illness severity. Pediatr Allergy Immunol 2014;25:796-803.

98. van der Zalm MM, Uiterwaal CSM, Wilbrink B, Koopman M, Verheij TJM, van der Ent CK. The influence of neonatal lung function on rhinovirus-associated wheeze. Am J Respir Crit Care Med 2011;183:262-267.

99. Broccolo F, Falcone V, Esposito S, Toniolo A. Human bocaviruses: Possible etiologic role in respiratory infection. J Clin Virol 2015;72:75-81.

100. Chow BDW, Esper FP. The human bocaviruses: a review and discussion of their role in infection. Clin Lab Med 2009;29:695-713.

101. Christensen A, Nordbo SA, Krokstad S, Rognlien AGW, Døllner H. Human bocavirus commonly involved in multiple viral airway infections. Journal of Clinical Virology 2008;41:34-37.
102. García-García ML, Calvo C, Pozo F, et al. Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. Pediatr Infect Dis J 2008;27:358-360.

103. Kahn J. Human bocavirus: clinical significance and implications. Curr Opin Pediatr 2008;20:62-66.

104. Schildgen O, Müller A, Allander T, et al. Human Bocavirus: Passenger or Pathogen in Acute Respiratory Tract Infections? Clin Microbiol Rev 2008;21:291-304.

105. Edner N, Castillo-Rodas P, Falk L, Hedman K, Söderlund-Venermo M, Allander T. Life-threatening respiratory tract disease with human bocavirus-1 infection in a 4-year-old child. J Clin Microbiol 2012;50:531-532.

106. Ursic T, Steyer A, Kopriva S, Kalan G, Krivec U, Petrovec M. Human Bocavirus as the Cause of a Life-Threatening Infection. Journal of Clinical Microbiology 2011;49:1179-1181.

107. Christensen A, Kesti O, Elenius V, et al. Human bocaviruses and paediatric infections. Lancet Child Adolesc Health April 2019.

108. Jacobs SE, Lamson DM, St. George K, Walsh TJ. Human Rhinoviruses. Clin Microbiol Rev 2013;26:135-162.

109. Dick EC, Jennings LC, Mink KA, Wartgow CD, Inhorn SL. Aerosol transmission of rhinovirus colds. J Infect Dis 1987;156:442-448.

110. Gwaltney JM, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. Ann Intern Med 1978;88:463-467.

111. Hall CB, Douglas RG. Modes of transmission of respiratory syncytial virus. J Pediatr 1981;99:100-103.

112. Obando-Pacheco P, Justicia-Grande AJ, Rivero-Calle I, et al. Respiratory Syncytial Virus Seasonality: A Global Overview. J Infect Dis January 2018.

113. Haas LEM, Thijsen SFT, van Elden L, Heemstra KA. Human Metapneumovirus in Adults. Viruses 2013;5:87-110.

114. Hermos CR, Vargas SO, McAdam AJ. Human Metapneumovirus. Clinics in Laboratory Medicine 2010;30:131-148.

115. Rogers E, Greaves K, Paul SP. A clinical companion to the NICE guide on bronchiolitis. Nurs Child Young People 2017;29:14-16.

116. Bordley W, Viswanathan M, King VJ, et al. Diagnosis and testing in bronchiolitis: A systematic review. Arch Pediatr Adolesc Med 2004;158:119-126.

117. Stollar F, Alcoba G, Gervaix A, Argiroffo CB. Virologic testing in bronchiolitis: does it change management decisions and predict outcomes? European journal of pediatrics 2014;173:1429-35.
118. Brand HK, de Groot R, Galama JMD, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. Pediatric Pulmonology 2012;47:393-400.

119. Sly PD, Jones CM. Viral co-detection in infants hospitalized with respiratory disease: is it important to detect? J Pediatr (Rio J) 2011;87:277-280.

120. Ferronato A, Gilio A, Ferraro A, Paulis M, Vieira S. Etiological diagnosis reduces the use of antibiotics in infants with bronchiolitis. Clinics 2012;67:1007-1011.

121. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics 2014;134:e1474-1502.

122. Noor A, Krilov LR. Respiratory syncytial virus vaccine: where are we now and what comes next? Expert Opin Biol Ther November 2018:1-10.

123. Hancock DG, Charles-Britton B, Dixon D-L, Forsyth KD. The heterogeneity of viral bronchiolitis: A lack of universal consensus definitions. Pediatr Pulmonol 2017;52:1234-1240.

Fig 1. Flow chart of research and selection of studies
Records identified through database searching

Additional records identified through other sources

Records after duplicates removed

Records screened

Full-text articles assessed for eligibility

Studies included in qualitative synthesis

Studies included in quantitative synthesis

Full-text articles excluded, with reasons

- Inappropriate detection assay (49)
- Inappropriate study design (44)

(n = 203)
Fig 2. Global prevalence of Respiratory Viruses in children with bronchiolitis

| Study       | Total | Prevalence (%) | 95% CI   |
|-------------|-------|----------------|----------|
| HRSV (45 studies) | 15351 | 59.17          | [54.66; 63.60] |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 96.8\%$ [96.2\%; 97.2\%], $t^2 = 0.0224, p < 0.001$ |
| RV (36 studies)    | 12967 | 19.29          | [16.67; 22.04] |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 92.8\%$ [90.9\%; 94.2\%], $t^2 = 0.0092, p < 0.0001$ |
| HBoV (24 studies)   | 8706  | 8.23           | [5.65; 11.24]   |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 95.4\%$ [94.1\%; 96.4\%], $t^2 = 0.0148, p < 0.0001$ |
| HAdV (26 studies)   | 6734  | 6.08           | [4.37; 8.03]   |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 88.9\%$ [85.0\%; 91.8\%], $t^2 = 0.0079, p < 0.0001$ |
| HPIV (28 studies)   | 7933  | 5.39           | [3.78; 7.26]   |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 91\%$ [88.1\%; 93.2\%], $t^2 = 0.0090, p < 0.0001$ |
| HMPV (32 studies)   | 9908  | 5.38           | [4.40; 6.44]   |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 70.9\%$ [67.7\%; 83.5\%], $t^2 = 0.0027, p < 0.0001$ |
| Influenza (24 studies) | 6571  | 3.17           | [2.17; 4.34]   |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 82.1\%$ [74.3\%; 87.5\%], $t^2 = 0.0042, p < 0.0001$ |
| HCoV (27 studies)   | 7421  | 2.91           | [1.96; 4.03]   |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 93.5\%$ [76.9\%; 88.1\%], $t^2 = 0.0047, p < 0.0001$ |
| EV (15 studies)     | 4202  | 2.86           | [1.55; 4.52]   |
| Random effect meta-analysis |          |                |          |
| Heterogeneity: $I^2 = 86.1\%$ [78.6\%; 90.9\%], $t^2 = 0.0057, p < 0.0001$ |
| Overall random effect meta-analysis | 79803 | 13.26         | [10.98; 15.71] |
| Residual heterogeneity: $I^2 = 92.7\%$ [92.0\%; 93.3\%], $p = 0$ |