

Duration of wheezy episodes in early childhood is independent of the microbial trigger

Christian J. Carlsson, MD,^a Nadja H. Vissing, MD, PhD,^a Astrid Sevelsted, MSc,^a Sebastian L. Johnston, FRCP, PhD,^b Klaus Bønnelykke, MD, PhD,^a and Hans Bisgaard, MD, DMSc^a *Copenhagen, Denmark, and London, United Kingdom*

Background: Wheezy episodes in young children are often triggered by viral and bacterial respiratory tract infections, but there is little evidence supporting the hypothesis that symptom duration depends on the specific microbial trigger.

Objective: We sought to investigate whether the duration of wheezy episodes in young children depends on the microbial trigger.

Methods: Two hundred eighty-three children from the Copenhagen Prospective Study on Asthma in Childhood₂₀₀₀ at-risk birth cohort were prospectively examined for common airway pathogenic bacteria and viruses during acute wheezy episodes in the first 3 years of life. Findings were related to symptomatic duration of episodes, as monitored in daily diary cards from birth.

Results: Eight hundred thirty-seven samples were investigated for viruses, bacteria, or both. Both viruses and bacteria were identified in 55% of episodes, bacteria were identified exclusively in 31% of episodes, and viruses were identified exclusively in 10% of episodes. The median duration of acute symptoms was 9 days (interquartile range, 5-16 days), and duration was independent of bacterial or viral species.

Conclusions: The duration of wheezy episodes was independent of pathogenic airway bacterial or viral species. This suggests that symptom burden from infections is dependent on other factors, such as environmental exposures or host factors. The common term viral wheeze seems inappropriate in view of the finding of pathogenic bacteria in 86% of wheezy episodes. (J Allergy Clin Immunol 2015;■■■■-■■■■.)

Key words: Respiratory tract infections, wheezy episodes, pediatrics

Abbreviations used

COPSAC₂₀₀₀: Copenhagen Prospective Studies on Asthma in Childhood₂₀₀₀

RSV: Respiratory syncytial virus

Infections with respiratory tract viruses are known triggers of wheezy episodes in children, with reported infection rates of 62% to 95% during episodes¹⁻⁶ and 40% outside of an episode.⁵ Rhinoviruses, respiratory syncytial virus (RSV), and coronaviruses seem to be the most prevalent viruses during wheezy episodes.¹⁻⁶ Several other respiratory tract viruses have been implicated, with lesser relative contributions.^{1,3-6} In our recent study we highlighted that common pathogenic bacteria and respiratory tract viruses were equally closely associated with wheezy episodes,⁵ suggesting that bacteria should also be considered an important trigger.

The aim of this study was to address the question of whether the duration of wheezy episodes is attributable specifically to the infecting species. If not, this would suggest host or environmental factors to be responsible for the disease course, as suggested by our recent finding of an interaction between the 17q21 gene risk variant, exposure to rhinovirus, and risk of persistent wheeze.⁷ Therefore we have compared differences in the duration of wheezy episodes associated with various respiratory tract viral and bacterial species in the Copenhagen Prospective Studies on Asthma in Childhood 2000 (COPSAC₂₀₀₀) at-risk birth cohort, a cohort of subjects followed from birth with daily diary cards and acute visits to our clinic during wheezy episodes.^{5,8,9}

METHODS

COPSAC₂₀₀₀ is a single-center prospective birth cohort study following 411 children of mothers with doctor-diagnosed asthma.⁸ Criteria for inclusion have been published previously^{8,10} and are summarized in the **Methods** section in this article's Online Repository at www.jacionline.org.

Children in COPSAC₂₀₀₀ attended the research clinic during acute wheezy episodes, at which time airway aspirates for microbiological diagnosis were collected. Wheezy symptoms were recorded in daily diaries from 1 month to 3 years of age.^{8,9} Parents were taught to record their child's symptoms, with emphasis on the lower airways, at comprehensive educational sessions conducted at planned half-yearly visits. Wheezy symptoms were defined for the parents as any symptom significantly affecting the child's breathing, such as noisy breathing (wheeze or whistling sounds), shortness of breath, or persistent troublesome cough affecting the child's sleep or activity. Daily symptoms were recorded as composite dichotomized scores (yes/no) each day; parents were taught to make a global assessment. The complexity of symptoms was detailed in a book that was given to the parents (www.copsac.com/content/literature-parents). Diary cards were collected and reviewed by doctors at the planned half-yearly clinic visits.

From ^aCOPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, and ^bthe Airway Disease Infection Section, National Heart and Lung Institute, MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, Imperial College London.

The Lundbeck Foundation (grant no. A1694); The Ministry of Health (grant no. 903516); Danish Council for Strategic Research (grant no.0603-00280B); The Danish Council for Independent Research (grant nos. 10-082884 and 271-08-0815), and The Capital Region Research Foundation have provided core support for COPSAC. Thermo Fisher Scientific Inc sponsored the IgE analyses. No pharmaceutical company was involved in the study. The funding agencies did not have any role in design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication November 16, 2014; revised May 11, 2015; accepted for publication May 12, 2015.

Corresponding author: Hans Bisgaard, MD, DMSc, COPSAC, Copenhagen Prospective Studies of Asthma in Childhood, Herlev and Gentofte Hospital, Ledreborg Allé 34, DK-2820 Gentofte, Denmark. E-mail: bisgaard@copsac.com.

0091-6749/\$36.00

© 2015 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2015.05.003>

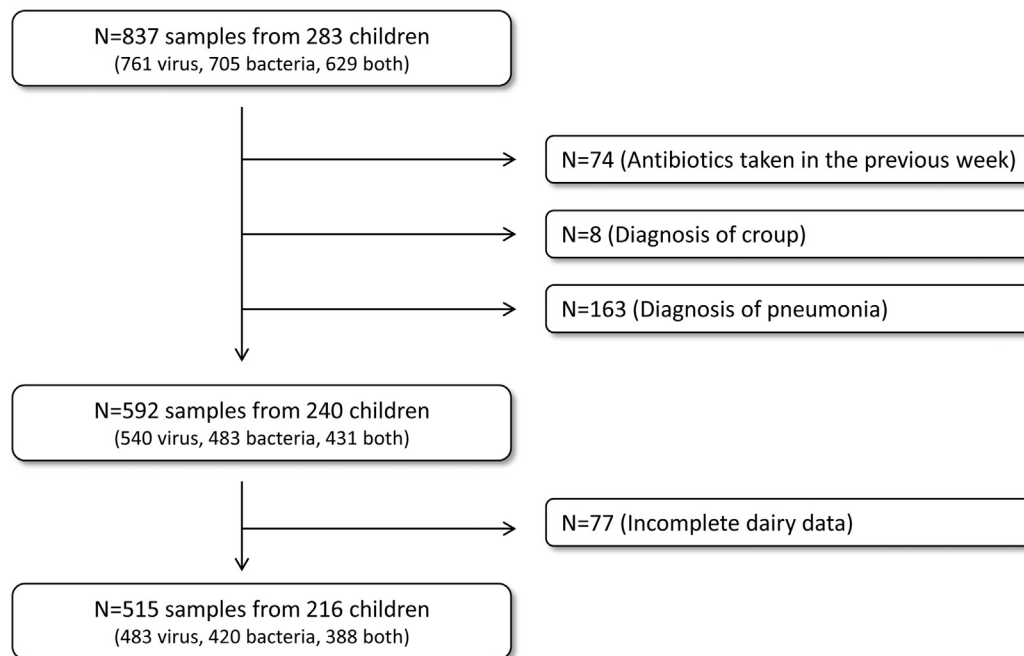


FIG 1. Study base selection of samples from young children with wheezy episodes analyzed for viruses and bacteria.

TABLE I. Viral and bacterial findings in pharyngeal aspirates obtained during wheezy episodes in young children

	No.	Percentage
Viruses		
Picornaviruses	159/540	29
RSV	89/540	16
Coronaviruses	69/540	13
Other virus (next 5)	129/540	24
Parainfluenza viruses	42/540	8
Influenza viruses	36/540	7
Human metapneumoviruses	17/540	3
Adenoviruses	5/540	1
Bocavirus	43/540	8
Any virus (of above)	350/540	65
Multiple virus (of above)	183/540	34
Bacteria		
<i>S pneumoniae</i>	229/483	47
<i>H influenzae</i>	200/483	41
<i>M catarrhalis</i>	238/483	49
Any bacteria (of above)	412/483	85
Multiple bacteria (of above)	205/483	42
Tested for coinfection		
Only virus	42/431	10
Only bacteria	132/431	31
Coinfection	237/431	55
No pathogens	20/431	5

Wheezy episodes were defined as 3 consecutive days during which the child had wheezing symptoms. The parents were requested to bring the child to the clinical research unit for examination by the research physician within 24 hours after each episode (ie, on the fourth consecutive day of symptoms). For some episodes, wheezy symptoms were not recorded on the day of aspiration. These episodes were still included in the analysis if there had been symptoms up to the day before aspiration. At each acute visit, the children were examined by physicians trained in pediatrics and clinical

research for the diagnosis and treatment of wheezy episodes in accordance with predefined standard operating procedures. Aspirates from acute respiratory episodes with clinical signs indicative of pneumonia or croup were excluded from this study, as described in the [Methods](#) section in this article's Online Repository.

Hypopharyngeal aspirates were obtained for routine bacterial cultures without any *a priori* species selection (we detected *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*) and nasopharyngeal aspirates for PCR virus identification (picornaviruses, RSV, coronaviruses, parainfluenza viruses, influenza viruses, human metapneumoviruses, adenoviruses, and bocavirus) at all acute visits, as described in the [Methods](#) section in this article's Online Repository.

Various baseline host and environmental factors were investigated for a potential confounding effect on the association between microbial triggers and duration of wheezy episodes. Allergic sensitization was determined from specific IgE measurements and skin prick tests at the ages of 6 months, 1.5 years, and 4 years. Asthma was diagnosed throughout the first 3 years of life in accordance with international guidelines, as further detailed in the [Methods](#) section in this article's Online Repository. Allelic discrimination at the *ORMDL3* locus rs7216389 at chromosome 17q21 was performed with an Applied Biosystems (Foster City, Calif) Custom TaqMan SNP Genotyping assay (c/n 4332072) on a 7700 Sequence Detection System. The variant was in Hardy-Weinberg equilibrium ($P > .05$). We collected occipital hair samples at 1 year of age for determination of trace amounts of nicotine using gas chromatography-mass spectrometry, as previously described.¹¹

Microbial findings during wheezy episodes were summarized in frequency tables across all episodes with available microbial data. Coinfection was defined as identification of at least 1 respiratory tract virus and at least 1 of the investigated bacteria (*S pneumoniae*, *H influenzae*, and *M catarrhalis*).

The main outcome was duration of acute wheezy episodes during which microbial sampling was performed. The effect of various infectious agents on episode duration was investigated by using generalized estimating equations to adjust for interobservational correlations caused by multiple sampling from the same child. We assumed negative binomial distribution of the duration of episodes. Each infectious agent was investigated separately as a dichotomous variable (present/not present) independent of other findings in the sample. Results are shown both unadjusted and adjusted for confounders.

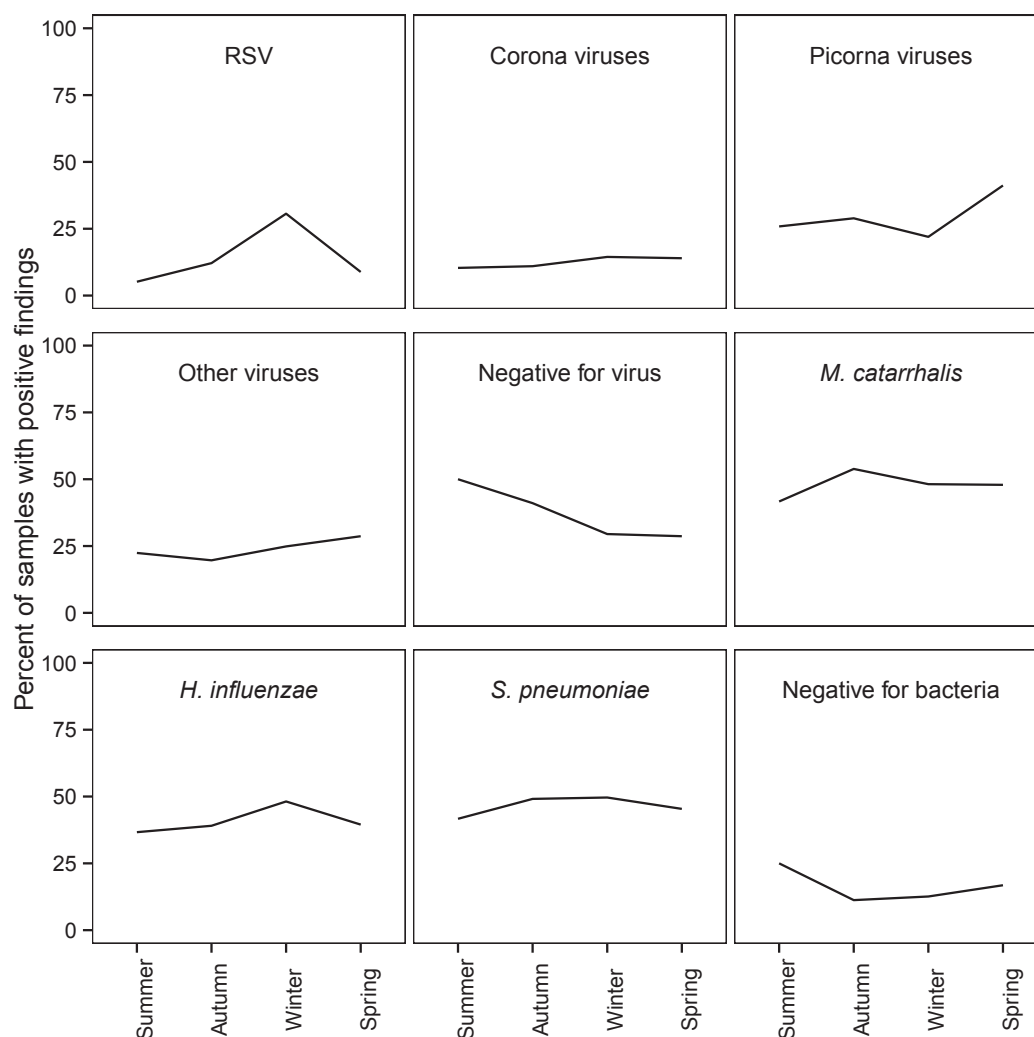


FIG 2. Seasonal variation in the prevalence of viruses and bacteria found during wheezy episodes in young children. The y-axis shows the proportion of aspirates with relevant findings within each month, and the x-axis represents calendar months.

Distribution of potentially confounding environmental and host risk factors (sex; age at episode; father's asthma; mother's educational level; nicotine in the hair at age 1 year; sensitization at the ages of 6 months, 18 months, and 4 years; asthma from 0-3 years of age; and chromosome 17q21 variant) were summarized for the study base of wheezy episodes and investigated for their independent effect on duration of wheezy episodes, also by using generalized estimating equations.

The effect of infection with virus only, infection with bacteria only, or coinfection compared with no infection was investigated in a subanalysis. A significance level of .05 was used in all analyses. All analyses were conducted with SAS statistical software (version 9.3; SAS, Institute, Cary, NC).

To visualize the effect of various agents on symptom duration, not limiting the analyses to consecutive days with symptoms, we plotted the percentage of diaries in which symptoms were recorded on the day of sampling and for each day 30 days before and after sampling.

RESULTS

The clinical follow-up rate of the COPSAC cohort was 95% at age 1 year, 90% at age 2 years, and 85% at age 3 years.

The study base (Fig 1) included 837 samples taken during wheezy episodes from 283 children in the first 3 years of life. Cultures were

excluded if antibiotics had been taken within the previous week of sampling ($n = 74$). Samples taken in relation to a clinical diagnosis of pneumonia ($n = 163$) were excluded, and samples taken in relation to a clinical diagnosis of croup ($n = 8$) were also excluded, leaving 592 eligible samples from 240 children. Of these, 540 samples were investigated for viruses, 483 were investigated for bacteria, and 431 were investigated for both.

During the first 3 years of life, 96 children contributed 1 aspirate, 112 contributed 2 to 4 aspirates, and 32 contributed 5 or more aspirates with either viruses or bacteria. One hundred seventy-one (42%) of 411 children had no eligible aspirates obtained during wheezy episodes. Details on numbers of eligible bacterial and viral aspirates per child are displayed in Fig E1 in this article's Online Repository at www.jacionline.org.

Although we encouraged the parents to bring their children to the research clinic after 3 consecutive days of wheezy symptoms, this was not always the case. If parents and children attended the clinic after only 1 to 2 days of troublesome lung symptoms ($n = 23$), aspirates were still obtained according to the standard operating procedures described above.

TABLE II. Effect of environmental and host factors on duration of wheezy episodes in young children

	No. of episodes	No. of children	IRR (95% CI 95), P value
Sex	515	216	
Female	288	115	0.97 (0.72-1.30), .85
Male	227	101	Reference
Age	515	216	
0-1 y	227	136	1.22 (0.82-1.81), .33
1-2 y	205	139	1.13 (0.75-1.70), .56
2-3 y	83	62	Reference
Father with a diagnosis of asthma	485	205	
Yes	100	33	0.93 (0.70-1.25), .65
No	385	172	Reference
Maternal educational level	505	210	
At most college	333	137	1.01 (0.73-1.41), .94
Medium duration	127	50	0.99 (0.60-1.62), .963
University	45	23	Reference
Environmental tobacco exposure (nicotine in hair at age 1 y >1 ng/mg vs <1 ng/mg)	491	207	
Yes	206	90	1.16 (0.86-1.56), .34
No	285	117	Reference
Sensitization at any point (6 mo, 18 mo, and 4 y)	512	214	
Yes	183	52	0.82 (0.64-1.04), .10
No	329	162	Reference
Asthma during age 0-3 y (yes vs no)	478	198	
Yes	183	52	1.74 (1.32-2.30), <.001
No	295	146	Reference
Chromosome 17q21 gene variant	509	211	
TT	170	72	1.05 (0.67-1.66), .83
TC	227	88	0.87 (0.56-1.35), .54
CC	112	51	Reference

IRR, Incidence rate ratio.

Table I shows the distribution of viruses and bacteria in the samples taken during wheezy episodes. Picornaviruses (of which 84% were rhinovirus), RSV, and coronaviruses were the most prevalent viral agents present in 29%, 17%, and 13% of samples, respectively, with lesser relative contributions of other viruses. *S pneumoniae*, *M catarrhalis*, and *H influenzae* were found in 47%, 49%, and 41% of the samples, respectively, whereas *S aureus* and *S pyogenes* were found in an insignificant proportion of samples and excluded from further analyses. In 55% of wheezy episodes, coinfection with both viruses and bacteria was identified. Exclusively bacterial or viral infection was found in 31% and 10% of episodes, respectively. In 5% of samples, no pathogen was identified. There was no increased risk of coinfection with any type of pathogen: infection with virus did not cause greater risk of bacterial infection and *vice versa* ($P = .59$, χ^2 test).

There were no apparent differences in age at first infection between investigated viruses and bacteria, and there were no obvious age-dependent differences in the prevalence of any of the pathogens (see details in Figs E2 and E3 in this article's Online Repository at www.jacionline.org).

Fig 2 shows the seasonal variation of viruses and bacteria found during acute respiratory episodes. RSV exhibited a marked peak

during the winter months, whereas all other viruses and bacteria were found in stable proportions throughout the year. For both viruses and bacteria, a larger percentage of aspirates was without pathogen findings during the summer months.

During the first 3 years of follow-up, symptom diary data were completed for 387 children on a total of 374,264 days of the potential 450,867 days of observation (coverage rate, 83%). The median age for starting the diary recordings was 19 days (interquartile range, 13-29 days). Wheezy symptoms were recorded on 6.6% (24,708) of the days. Twenty-four children had no diary data for the first 3 years of life. Forty-five children never recorded any symptom days throughout the first 3 years of follow-up.

Five hundred fifteen (91%) of the 592 eligible aspirates were taken from episodes with complete diary data 1 month before and 1 month after the sample collection. Thus we analyzed a total of 515 samples in 216 children (Fig 1).

The median duration of the 515 wheezy episodes was 9 days (interquartile range, 5-16 days). The effects of various environmental and host factors on duration of episodes are shown in Table II. A diagnosis of asthma from 0 to 3 years of age was associated with significantly longer duration of wheezy episodes (incidence rate ratio, 1.74 [1.32-2.30]; $P \leq .001$), whereas there was no evidence of association with the other risk factors.

There was no significant difference in duration of wheezy episodes associated with any specific viral or bacterial trigger (Table III). Similarly, symptom duration was independent of the presence of solely viral or bacterial infection or coinfection compared with episodes with no pathogens detected (see Table E1 in this article's Online Repository at www.jacionline.org). Adjusting the analyses for the potential confounding effect of various environmental and host factors did not change the results (Table III and see Table E1).

Fig 3, A, shows the proportion of diaries on which symptoms were recorded for each day during the 30-day period before and after an aspirate with positive findings for respiratory tract viruses. In the immediate period surrounding discovery of positive viral aspirates, there were no discernible differences in the portion of children with wheezy symptoms between different viral species; all viruses triggered wheezy episodes of similar symptom profiles. The levels of baseline respiratory symptoms before and after the wheezy episodes was also similar among the investigated viruses; no species caused more sustained symptoms than others. It was evident that the majority of families attended the research clinic for clinical examination and airway aspiration (day 0) after a period of a few symptom days, which is in accordance with our protocol. Again, this was true for all the most common respiratory tract viruses.

Fig 3, B, shows the proportion of diaries in which symptoms were recorded for each day during the 30-day period before and after aspiration, with positive findings for pathogenic respiratory bacteria. There were no differences in disease course and remission between bacterial species. The level of baseline respiratory symptoms before and after the wheezy episode was similar among the investigated bacteria.

Fig 3, C, shows the proportion of diaries in which symptoms were recorded for each day during the 30-day period before and after aspiration with positive findings for only viral infection, only bacterial infection, coinfection, or no identified pathogens. Symptom duration was similar for the 3 groups with positive microbiological findings. Symptomatic episodes with no

TABLE III. Duration of wheezy episodes in young children in relation to various viral and bacterial triggers

	Negative			Positive			Results from GEE models	
	Median days (IQR)	No. of episodes	No. of children	Median days (IQR)	No. of episodes	No. of children	Unadjusted IRR (95% CI), <i>P</i> value (n = 483 episodes)	Confounder-adjusted IRR* (95% CI), <i>P</i> value (n = 403 episodes)
Virus								
Picornaviruses	10 (6-16)	345	184	9 (6-17)	138	101	0.91 (0.74-1.13), .40	0.88 (0.71-1.08), .22
RSV	9 (6-17)	402	192	11 (7-15)	81	73	0.94 (0.76-1.16), .54	0.99 (0.78-1.26), .95
Coronaviruses	10 (6-17)	422	202	9 (5-13)	61	47	1.04 (0.68-1.58), .85	1.00 (0.70-1.42), .99
Other virus	10 (6-16)	366	182	10 (6-17)	117	88	1.05 (0.86-1.29), .61	1.06 (0.87-1.30), .55
Any virus	9 (6-17)	171	117	10 (6-17)	312	171	0.97 (0.78-1.22), .81	0.95 (0.74-1.21), .67
Bacteria								
<i>S pneumoniae</i>	9 (6-16)	224	134	11 (6-19)	196	130	1.22 (0.99-1.49), .058	1.18 (0.94-1.48), .16
<i>H influenzae</i>	10 (6-18)	243	154	11 (6-17)	177	113	0.98 (0.79-1.22), .88	1.00 (0.78-1.27), .99
<i>M catarrhalis</i>	11 (6-18)	215	128	10 (6-17)	205	135	1.02 (0.82-1.27), .85	1.03 (0.84-1.27), .76
Any bacteria	9 (5-16)	62	47	11 (6-17)	358	180	1.22 (0.99-1.51), .059	1.17 (0.94-1.45), .15

GEE, Generalized estimating equations; IQR, interquartile range; IRR, incidence rate ratio.

*Sex; age at episode; father's asthma; mother's educational level; nicotine in the hair at age 1 year; sensitization at the ages of 6 months, 18 months, and 4 years asthma from 0-3 years of age; and chromosome 17q21 variant.

identified pathogens were rare and thus appeared to have a more erratic course, with fewer baseline symptoms before the wheezy episode.

DISCUSSION

Pathogenic bacteria were more common than viruses during wheezy episodes, and hence the common term viral wheeze should be abandoned. Duration of wheezy episodes was independent of specific pathogenic respiratory bacterial or viral species. This suggests that symptom burden from infections in terms of duration of wheezy episodes is dependent on other factors than the specific triggering agent.

Strengths and weaknesses of the study

The intense clinical surveillance of the COPSAC₂₀₀₀ birth cohort is a major strength of this study. All children attended the COPSAC research clinic instead of other health care facilities. Experienced research physicians made clinical diagnoses and performed sampling at the clinic in accordance with standard procedures. This approach reduced the risk of misclassification of illness and variation in sampling quality. The physicians at the clinic distinguished clinical pneumonia from wheezy episodes on the basis of the presence of tachypnea, fever, and crepitation on auscultation without wheeze in accordance with standard operating procedures. This clinical differentiation between wheeze and pneumonia can be debated. However, the children were assessed by the same doctors in accordance with standard operating procedures; the sampling for viruses and bacteria was independent of such a distinction, and the clinical diagnosis was independent of microbiological results.

Another strength of this study is the prospective monitoring of lung symptoms in daily diaries since neonatal age. This ensured reliable monitoring of episode duration. The validity of the mother's symptom observation and recording was probably improved based on the fact that they all had a history of asthma.

The incidence of viruses in wheezy episodes in this study is comparable with that of other recent studies of viral infections during wheezy episodes of infants and preschool children.^{2-4,6}

It is a limitation of this study that we do not have data on symptom severity. It is our interpretation that symptom duration is a surrogate marker of symptom severity, but this needs to be studied.

According to the study protocol, we instructed the parents to bring their child to the research clinic after 3 consecutive days of symptoms. This potentially hampers our ability to analyze episode duration. Some parents brought their child to the research clinic after only 1 to 2 days of symptoms, and therefore we have some samples (n = 23) from episodes of shorter duration included in the analysis. We compared microbiological findings in wheezy episodes with duration in the lower quartile (1-5 days) to episodes with duration in the upper quartile (17- >28 days), and did not find any differences between the groups (data not shown).

The use of traditional culturing methods is a limitation. Unfortunately, bacterial DNA sequencing data were not available in the current study.

Another limitation of our findings is that the investigations were carried out in a high-risk population. The selection for maternal asthma and exclusion of premature newborns limit the generalizability of the findings, which required replication in unselected cohorts.

Meaning of this study

Viral and bacterial infections are triggers of wheezy episodes in young children.^{1,5} This study shows similar symptom duration of acute wheezy episodes from the most common respiratory bacteria and viruses. These findings suggest that the particular pathogenic species is not important for the course of such episodes. This conclusion is supported by our recent findings of similar relative distributions of specific bacterial and viral species in symptomatic and asymptomatic children.⁵ The notion that different species cause different clinical outcomes might be confounded by prevalence, and there is little evidence to signify that the role of the infectious agent among pathogenic respiratory bacteria and viruses is dependent on species.

Previous reports have been in conflict as to whether the presence of viral infection augments the severity and duration of lower respiratory tract symptoms, and differential symptom burden for specific viruses has been reported in some

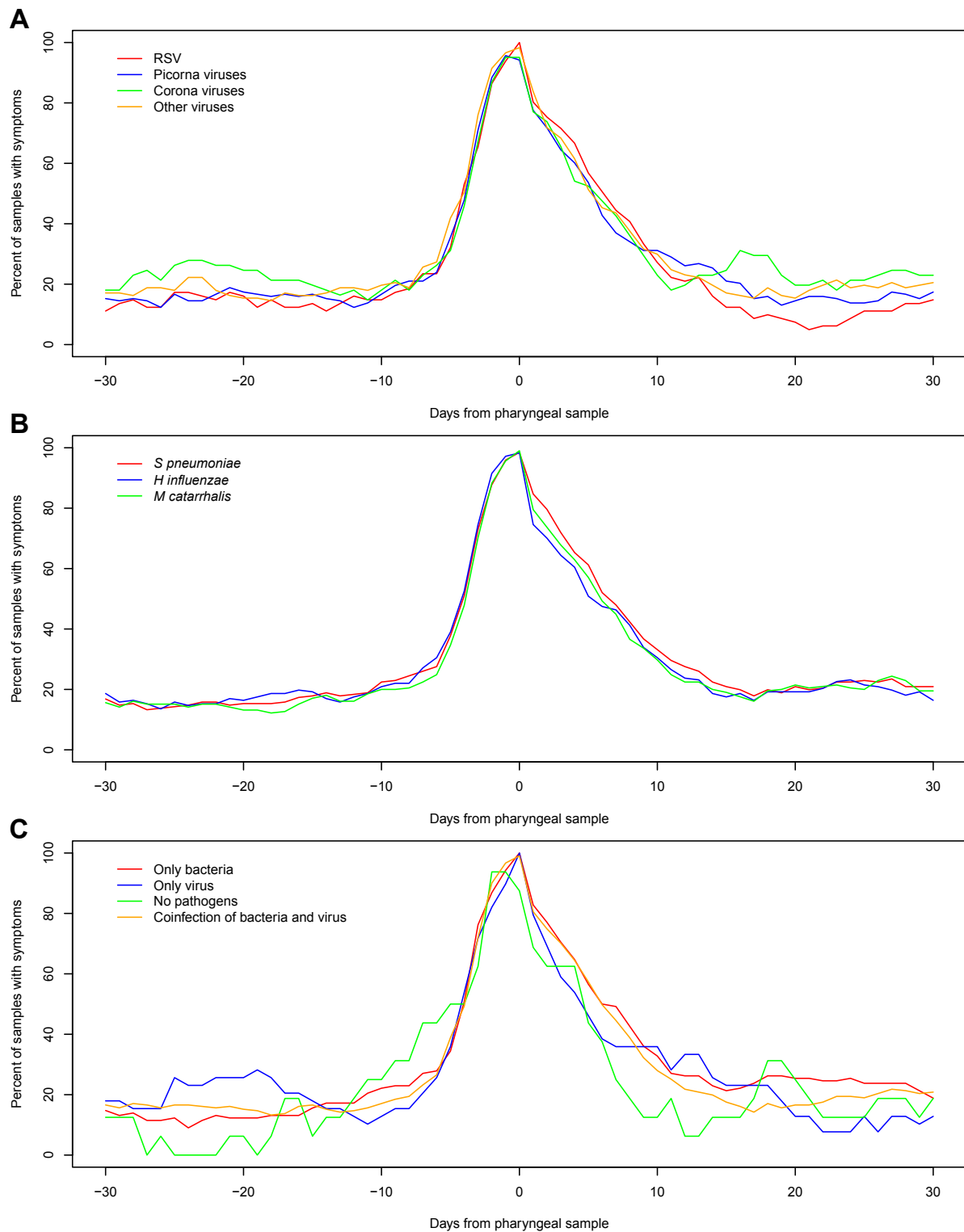


FIG 3. A, Days with wheezy symptoms before and after pharyngeal sampling (day 0) with positive findings of various respiratory tract viruses. B, Days with wheezy symptoms before and after pharyngeal sampling (day 0) with positive findings of various respiratory bacteria. C, Days with wheezy symptoms before and after pharyngeal sampling (day 0) with no microbial findings, only viral findings, only bacterial findings, or both viruses and bacteria detected.

studies.^{1,12,13} Schoolchildren were reported to have a lesser decrease in peak expiratory flow and a lower subjective symptom score when infected with coronaviruses than with other

respiratory tract viruses.¹ Others reported picornaviruses and adenoviruses to be more prevalent in near-fatal adult asthma than in less severe exacerbations.¹² One important difference

from our study is that these phenotypes are much different from wheezy episodes in young children.

Wheezy episodes are a much debated diagnosis to the point that the debate often confuses the need to understand, prevent, and treat this entity. Wheeze is the most common reason for acute hospitalization, health care use, and medicine use in young children in westernized countries.¹⁴ The terminology is misleading because evidence shows that wheeze is an unusual symptom for parents to report even before severe exacerbations¹⁵ and that the quantitative global assessment of significant troublesome lung symptoms in the first 3 years of life is a better predictor of asthma than assessment of wheeze alone.¹⁶ The common term viral wheeze is further obsolete given the fact that bacteria seem more prevalent in such episodes than viruses.

In the COPSAC birth cohort studies we have instead educated parents to record global respiratory distress, be it wheeze, breathlessness, cough, or any other terms used by laypersons. Symptoms severely affecting the well-being of the child are the key to recordings in the diary cards. Although we find the term wheezy episodes to be a misnomer, we need to use the term to be able to communicate our research.

Our results suggest that although microbiological pathogens act as instigators of respiratory distress in children, other factors determine the duration of the symptomatic episode. The mechanisms through which viruses and bacteria provoke exacerbations are yet unclear, but current evidence suggests that allergic sensitization and altered immune response might be more important than the virulence of the specific agent.¹⁷ It has been suggested that deficiencies in antiviral activity and the integrity of the airway epithelial barrier might make asthmatic patients more likely to have severe viral respiratory tract infections of the lower airway.¹⁷ In this study we found asthma at age 3 years to be associated with episodes of longer duration. This finding was not unexpected because the presence of persistent symptoms is one of the diagnostic criteria for asthma. None of the other host factors investigated had any confounding effect on duration of episodes.

RSV showed a peak in winter, with no other species-specific variation with respect to season or age. However, there was a general decrease in the proportion of infections per wheezy episode during summer. The latter is an unexpected and interesting observation, but the interpretation is uncertain. It is a common notion that viruses are the predominant microbial agents responsible for acute respiratory symptoms in young children. However, we recently found bacterial infections to be significantly associated with acute wheezy episodes in children up to 3 years of age, which is similar to but independent of the well-known association with viral infections.⁵ In this study we showed a high prevalence of concurrent infection with both viruses and bacteria (55%) and of exclusively bacterial infection (31%) in acute symptomatic episodes. Surprisingly, only a few aspirates were found with only viral species (10%). This is of apparent clinical interest because acute episodes with bacteria might be susceptible to antibiotic therapy or prophylaxis.

Conclusion

We found no significant differences in duration of wheezy episodes from the most common pathogenic respiratory tract viruses and bacteria. Although triggered by viruses and bacteria, the course of wheezy episodes seems independent of infecting

species and might therefore be controlled by other factors, such as intrinsic host factors or environmental exposures.

We thank the children and families of the COPSAC₂₀₀₀ cohort study for their support and commitment. We acknowledge and appreciate the unique efforts of the COPSAC research team and T. Kebabdz and J. Aniscenko for the virologic analyses.

Key messages

- The duration of wheezy episodes in young children is independent of the specific viral or bacterial triggers.
- The common term viral wheeze seems inappropriate in view of the finding of pathogenic bacteria in the majority of wheezy episodes.

REFERENCES

1. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995;310:1225-9.
2. Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *Am J Respir Crit Care Med* 1999; 159:785-90.
3. Kusel MM, de Klerk NH, Holt PG, Kebabdz T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006;25:680-6.
4. Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A, et al. Viruses and bacteria in acute asthma exacerbations—a GA²LEN-DARE systematic review. *Allergy* 2011;66:458-68.
5. Bisgaard H, Hermansen MN, Bønnelykke K, Stokholm J, Baty F, Skjott NL, et al. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *BMJ* 2010;341:c4978.
6. Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den Hoogen B, Osterhaus AD, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 2004;10: 1095-101.
7. Calışkan M, Bochkov YA, Kreiner-Møller E, Bønnelykke K, Stein MM, Du G, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med* 2013;368:1398-407.
8. Bisgaard H. The Copenhagen Prospective Study on Asthma in Childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study. *Ann Allergy Asthma Immunol* 2004;93:381-9.
9. Bisgaard H, Hermansen MN, Lohland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med* 2006; 354:1998-2005.
10. Bisgaard H, Hermansen MN, Buchvald F, Lohland L, Halkjaer LB, Bønnelykke K, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357:1487-95.
11. Sørensen M, Bisgaard H, Stage M, Loft S. Biomarkers of exposure to environmental tobacco smoke in infants. *Biomarkers* 2007;12:38-46.
12. Tan WC, Xiang X, Qiu D, Ng TP, Lam SF, Hegele RG. Epidemiology of respiratory viruses in patients hospitalized with near-fatal asthma, acute exacerbations of asthma, or chronic obstructive pulmonary disease. *Am J Med* 2003;115:272-7.
13. Olenec JP, Kim WK, Lee WM, Vang F, Pappas TE, Salazar LE, et al. Weekly monitoring of children with asthma for infections and illness during common cold seasons. *J Allergy Clin Immunol* 2010;125:1001-6.e1.
14. Kocevkar VS, Bisgaard H, Jönsson L, Valovirta E, Kristensen F, Yin DD, et al. Variations in pediatric asthma hospitalization rates and costs between and within Nordic countries. *Chest* 2004;125:1680-4.
15. Bisgaard H, Swern AS, Knorr B. "To wheeze or not to wheeze": that is not the question—the sequel. *J Allergy Clin Immunol* 2012;130:531-2.
16. Skjott N, Bønnelykke K, Bisgaard H. "To wheeze or not to wheeze": that is not the question. *J Allergy Clin Immunol* 2012;130:403-7.e5.
17. Busse WW, Lemanske RF, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet* 2010;376:826-34.

METHODS

Between August 1998 and December 2001, we enrolled 411 neonates at 1 month of age. Infants with a severe congenital anomaly, a gestational age of less than 36 weeks, a need for mechanical ventilation, or a lower respiratory tract infection were excluded, as previously described in detail.^{E1-E3} The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the local ethics committee (COPSAC₂₀₀₀: KF 01-289/96) and the Danish Data Protection Agency (COPSAC₂₀₀₀: 2008-41-1754). Both parents provided written informed consent before enrollment.

Samples collected during acute respiratory episodes with clinical signs indicative of pneumonia were excluded from this study. Clinical pneumonia was defined by tachypnea, fever, and abnormal auscultation independent of identified pathogens and radiographic or laboratory findings. Episodes with symptoms judged by the study physician to be characteristic for croup were also excluded. Sampling for viruses and bacteria was independent of such distinctions, and clinical diagnosis was independent of microbiological outcomes. A sensitivity analysis including aspirates taken with a clinical diagnosis of pneumonia did not significantly alter our results (data not shown).

Bacterial cultures

The research doctor performed aspiration of the hypopharynx under aseptic conditions with a soft suction catheter passed through the nose. Aspiration was done intermittently, ensuring no suction was applied during retraction through the oropharynx and nasopharynx. The catheter was flushed with 1 mL of saline into a vessel to flush out secretions from the tube. Samples were transported at room temperature to the microbiology laboratories within 2 hours of collection. *S pneumoniae*, *Haemophilus influenzae*, and *M catarrhalis* were identified, according to standard procedures.^{E4}

Detection of viruses

The research doctor aspirated the upper rhinopharynx under aseptic conditions with a soft suction catheter. We used PCR of nasopharyngeal samples to detect picornaviruses (mostly rhinoviruses); RSV; coronaviruses 229E and OC43; parainfluenza viruses 1 to 3; influenza viruses AH1, AH3, and B; human metapneumoviruses; adenoviruses; and bocavirus, as previously described.^{E4} Bocavirus was detected on 2 μ L of random primed cDNA by using the PCR primers HBOV 01.2 TAT-GGC-CAA-GGC-AAT-CGT-CCA-AG and HBOV 02.2 GCC-GCG-TGA-ACA-TGA-GAA-ACA-GA with cycling conditions of 94°C, 56°C, and 72°C each for 20 seconds for 35 cycles. The positive control was the complete coding genome of bocavirus plasmid DNA.

Sensitization

Atopic sensitization was determined from specific IgE at the ages of 6 months, 1.5 years, and 4 years by using ImmunoCAP (Phadia AB, Uppsala,

Sweden)^{E5} against the most common food and inhalant allergens.^{E6} Skin prick tests were performed with cat, dog, horse, birch, timothy grass, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, mugwort, *Alternaria alternata*, *C herbarium*, egg, milk, peanut, cod, wheat, rye, beef, pork, and soya bean allergen extracts (ALK-Abelló, Copenhagen, Denmark), as well as raw egg and milk.^{E7} Sensitization was defined as having any positive skin prick test response (>3 mm) or having any specific IgE level of greater than 0.35 kU/L.

Diagnosis of asthma

Recurrent wheeze was diagnosed from diary data as 5 wheezy episodes within 6 months or daily symptoms for 4 consecutive weeks.^{E3,E8} Asthma during the first 3 years of life was diagnosed according to international guidelines, as previously detailed,^{E3} and was based on a history of recurrent symptoms (as defined above) recorded in diaries and judged to be typical of asthma (eg, exercise-induced symptoms, prolonged nocturnal cough, persistent cough not caused by the common cold, and symptoms causing wakening at night), response to a 3-month course of inhaled corticosteroids, and a requirement for intermittent use of an inhaled β_2 -agonist to relieve dyspnea.

REFERENCES

- E1. Bisgaard H. The Copenhagen Prospective Study on Asthma in Childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study. *Ann Allergy Asthma Immunol* 2004;93:381-9.
- E2. Bisgaard H, Hermansen MN, Bønnelykke K, Stokholm J, Baty F, Skytt NL, et al. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *BMJ* 2010;341:c4978.
- E3. Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bønnelykke K, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357:1487-95.
- E4. American Society for Microbiology. Manual of clinical microbiology. 7th ed. Washington (DC): ASM Press; 1999; 1773.
- E5. Paganelli R, Ansotegui IJ, Sastre J, Lange CE, Roovers MH, de Groot H, et al. Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, UniCAP, in six European allergy clinics. *Allergy* 1998;53:763-8.
- E6. Bisgaard H, Simpson A, Palmer CNA, Bønnelykke K, McLean I, Mukhopadhyay S, et al. Gene-environment interaction in the onset of eczema in infancy: filaggrin loss-of-function mutations enhanced by neonatal cat exposure. *PLoS Med* 2008; 5:e131.
- E7. Bisgaard H, Li N, Bønnelykke K, Chawes BL, Skov T, Paludan-Müller G, et al. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol* 2011; 128:646-52, e1-5.
- E8. Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med* 2006; 354:1998-2005.

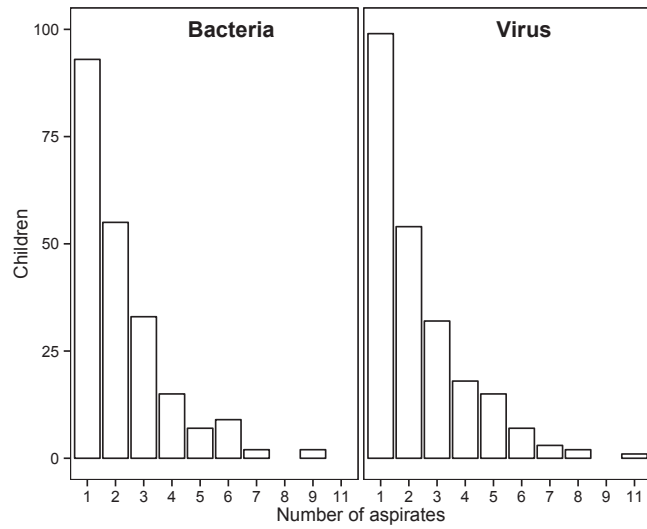


FIG E1. Distribution in numbers of pharyngeal aspirates obtained during wheezy episodes per child.

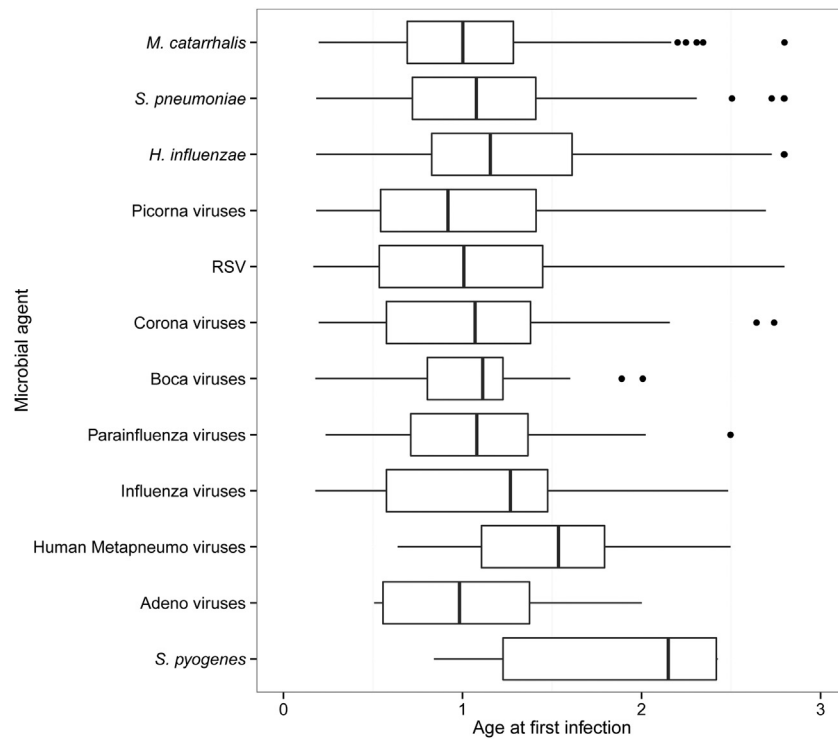


FIG E2. Mean age at first infection with various viral and bacterial agents.

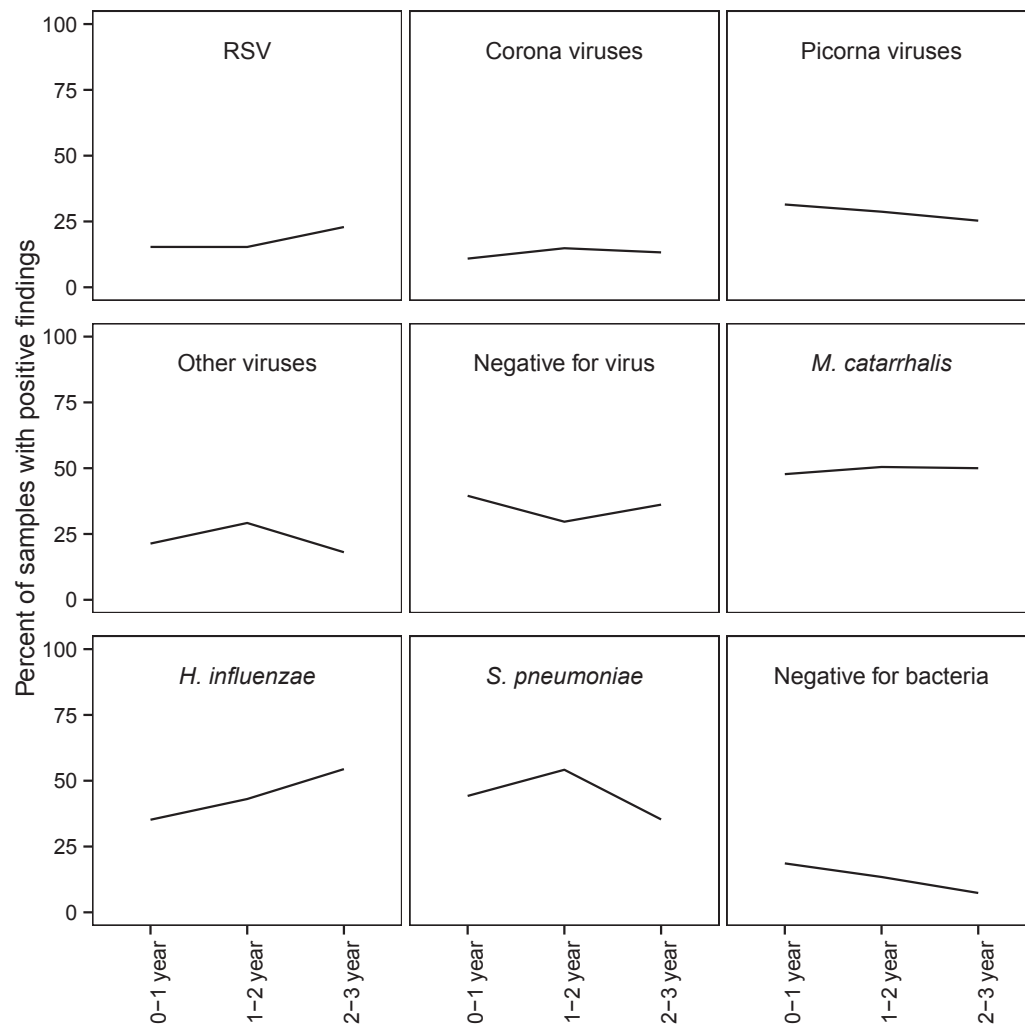


FIG E3. Viral and bacterial findings with wheezy episodes by year 1 to 3 of life. The *y-axis* shows the proportion of aspirates with relevant findings within each year, and the *x-axis* represents years 1 to 3 of life.

TABLE E1. Duration of wheezy episodes in young children in relation to viral infection, bacterial infection, or coinfection

	Median days (IQR)	No. of episodes	No. of children	Results from GEE models	
				Unadjusted IRR (95% CI), <i>P</i> value (n = 388)	Confounder-adjusted IRR* (95% CI), <i>P</i> value (n = 325)
Only virus	9 (5-22)	39	32	1.16 (0.72-1.88), .54	0.95 (0.53-1.70), .87
Only bacteria	11 (6-18)	122	88	1.32 (0.90-1.93), .15	1.15 (0.71-1.84), .58
Coinfection	10 (6-17)	211	134	1.23 (0.85-1.79), .28	1.05 (0.67-1.66), .82
No pathogens	10 (7-16)	16	16	Reference	Reference

GEE, Generalized estimating equations; *IQR*, interquartile range; *IRR*, incidence rate ratio.

*Sex; age at episode; father's asthma; mother's educational level; nicotine in the hair at age 1 year; sensitization at the ages of 6 months, 18 months, and 4 years; asthma from 0-3 years of age; and chromosome 17q21 variant.