

Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study



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Summary

Background Pneumonia is a leading cause of mortality and morbidity in children globally. The cause of pneumonia after introduction of the 13-valent pneumococcal conjugate vaccine (PCV13) has not been well studied in low-income and middle-income countries, and most data are from cross-sectional studies of children admitted to hospital. We aimed to longitudinally investigate the incidence and causes of childhood pneumonia in a South African birth cohort.

Methods We did a nested case-control study of children in the Drakenstein Child Health Study who developed pneumonia from May 29, 2012, to Dec 1, 2014. Children received immunisations including acellular pertussis vaccine and PCV13. A nested subgroup had nasopharyngeal swabs collected every 2 weeks throughout infancy. We identified pneumonia episodes and collected blood, nasopharyngeal swabs, and induced sputum specimens. We used multiplex real-time PCR to detect pathogens in nasopharyngeal swabs and induced sputum of pneumonia cases and in nasopharyngeal swabs of age-matched and site-matched controls. To show associations between organisms and pneumonia we used conditional logistic regression; results are presented as odds ratios (ORs) with 95% CIs.

Findings 314 pneumonia cases occurred (incidence of 0·27 episodes per child-year, 95% CI 0·24–0·31; median age 5 months [IQR 3–9]) in 967 children during 1145 child-years of follow-up. 60 (21%) cases of pneumonia were severe (incidence 0·05 episodes per child-year [95% CI 0·04–0·07]) with a case fatality ratio of 1% (three deaths). A median of five organisms (IQR 4–6) were detected in cases and controls with nasopharyngeal swabs, and a median of six organisms (4–7) recorded in induced sputum ($p=0\cdot48$ compared with nasopharyngeal swabs). *Bordetella pertussis* (OR 11·08, 95% CI 1·33–92·54), respiratory syncytial virus (8·05, 4·21–15·38), or influenza virus (4·13, 2·06–8·26) were most strongly associated with pneumonia; bocavirus, adenovirus, parainfluenza virus, *Haemophilus influenzae*, and cytomegalovirus were also associated with pneumonia. In cases, testing of induced sputum in addition to nasopharyngeal swabs provided incremental yield for detection of *B pertussis* and several viruses.

Interpretation Pneumonia remains common in this highly vaccinated population. Respiratory syncytial virus was the most frequently detected pathogen associated with pneumonia; influenza virus and *B pertussis* were also strongly associated with pneumonia. Testing of induced sputum increases the yield for detection of several organisms. New vaccines and strategies are needed to address the burden of childhood pneumonia.

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Introduction

The incidence of childhood pneumonia has declined in the past decade, contributing to a substantial reduction in childhood mortality.¹ However, pneumonia remains the major cause of death in children outside the neonatal period, accounting for about 17% or 1 million deaths annually, with the burden heavily skewed to low-income and middle-income countries.¹ Reductions in pneumonia-associated mortality have been achieved through improved strategies to prevent and treat childhood pneumonia.²

New vaccines, particularly pneumococcal conjugate vaccine (PCV) and *Haemophilus influenzae* type b vaccine, have been a major advance in reduction of incidence and severity of childhood pneumonia.^{1–6} After PCV introduction in national immunisation programmes, reductions in the incidence and severity

of childhood pneumonia have been reported in high-income countries^{4,6} and in Latin America.⁵ However, the effect of these programmes on childhood pneumonia and child health in Africa, where a large burden of pneumonia exists, is not well studied outside of vaccine trials. Identification of pneumonia causes in the context of robust immunisation programmes is an ongoing priority for child health. Case-control studies undertaken in high-income countries have reported viral pathogens, particularly respiratory syncytial virus (RSV), to be a major cause of pneumonia in children vaccinated with 13-valent PCV (PCV13),^{7,8} but data are few from low-income and middle-income countries. Furthermore most causal data comes from cross-sectional samples of pneumonia cases admitted to hospital, but cause might vary with patient age or severity of disease.

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Research in context

Literature review

We searched PubMed for articles published in English from Jan 1, 2000, to Aug 31, 2015, with the search terms “child”, “pneumonia”, “aetiology/etiology”, and “pneumococcal conjugate vaccine”. We identified case-only, cross-sectional, or case-control studies predominantly from high-income countries. These studies reported reductions in admissions to hospital for pneumonia during childhood after introduction of pneumococcal conjugate vaccination (PCV) with viral pathogens commonly detected. However, scarce comprehensive causal data are available after 13-valent PCV (PCV13) introduction, especially in low-income and middle-income countries where the predominant burden of pneumonia occurs. Furthermore, available data focus on cases admitted to hospitals, are cross-sectional, seldom include comparison with a carefully selected control group, and rarely investigate comprehensively for potential bacterial and viral pathogens in different specimens.

Added value of this study

With a unique design we did a nested case-control analysis of organisms associated with pneumonia in an African birth cohort study, using state-of-the-art diagnostic techniques.

Vaccination coverage, including for PCV13, was high. The incidence of pneumonia was high, especially for children in the first 6 months of life with about a third of cases requiring admission to hospital. Several organisms were identified at the time of pneumonia onset. Respiratory syncytial virus was the predominant pathogen and occurred early in infancy. Other organisms strongly associated with pneumonia were *Bordetella pertussis* and influenza virus, whereas bocavirus, adenovirus, parainfluenza viruses, *Haemophilus influenzae*, and cytomegalovirus were less strongly associated with pneumonia. Testing of both induced sputum and nasopharyngeal specimens identified the presence of significantly more potential pathogens, including *B pertussis* and some viruses compared with testing only nasopharyngeal specimens.

Implications of the available evidence

Childhood pneumonia remains an important public health problem despite the introduction of PCV13. Several organisms might cause this disease. An induced sputum specimen adds value to the diagnostic work-up even in young infants. New vaccines and novel strategies to prevent pneumonia, such as immunisation of pregnant women against respiratory syncytial virus, are needed.

Development of improved methods for specimen collection in children and advances in molecular diagnostics have strengthened the ability to identify potential pathogens.⁹ Sputum induction has been reported to be feasible in infants and effective for microbiological confirmation of childhood tuberculosis,¹⁰ but few studies have assessed its use in childhood pneumonia.¹¹ Better specimens and increasingly sensitive detection methods allow for identification of a broad range of pathogens and for comparison of cause by disease severity.

In 2015, we reported a high incidence of pneumonia and severe disease in children enrolled in the Drakenstein Child Health Study,¹² a South African birth cohort study, despite high immunisation coverage including PCV13. In this study, we aimed to longitudinally investigate the causes of pneumonia in this cohort using a novel design.

Methods

Study design and participants

We did a nested case-control study of children included in the Drakenstein Child Health Study¹² who developed pneumonia from May 29, 2012, to Dec 1, 2014. The Drakenstein Child Health Study¹² was undertaken at two public, primary health-care clinics located about 2 km apart in Paarl, a periurban area in South Africa. One clinic (TC Newman) served a mixed-race population and the second clinic (Mbekweni) a black African population. Pregnant women aged 18 years or older, at 20–28 weeks' gestation, attending one of the two clinics for antenatal care, and remaining in the area for at least 1 year were

enrolled. Ethics approval was obtained from the University of Cape Town Faculty of Health Sciences Research Ethics Committee, and the Provincial Research committee approved the study. Mothers provided written informed consent at enrolment and provided consent after the first year.

All births occurred at Paarl hospital. Follow-up of children was done from May 29, 2012, through early childhood¹³ and paralleled routine child health visits at 6, 10, and 14 weeks and 6, 9, 12, 18, 30, and 42 months. An additional study visit was done at 6–10 weeks at Paarl hospital.

All children were given primary health care and immunisations at the two clinics including four doses of a five vaccine combination (diphtheria, tetanus, acellular pertussis, *H influenzae* type b, and inactivated polio vaccine) at 6, 10, and 14 weeks and 18 months; the measles vaccine at 9 months and 18 months; and the PCV13 at 6 weeks, 14 weeks, and 9 months.

Pneumonia surveillance and risk factors

Continuous pneumonia surveillance was implemented at all local clinics and at Paarl hospital. Mothers were given a mobile phone number if they needed to contact the study team at any time. Mothers were counselled regarding key respiratory symptoms and advised to attend or contact study staff whenever a child developed cough or difficulty breathing. Primary health nurses and study staff were trained to recognise WHO-defined pneumonia or severe pneumonia.¹⁴ Study staff reviewed patient records at catchment clinics (Phola Park, Thokoza;

	All children (n=967)	Cases (n=284)*	Controls (n=418)*†	Odds ratio (95% CI)
Sociodemographics				
Age (months)	11 (3–19)	5 (3–9)	5 (2–8)	..
Boys	509 (53%)	192 (68%)	228 (55%)	1.67 (1.21–2.31)
Household income per month (South African Rand [ZAR])				
<R1000	397 (41%)	131 (46%)	171 (41%)	1.00 (reference)
R1000–5000	428 (44%)	107 (38%)	177 (42%)	0.86 (0.61–1.21)
>R5000–10 000	118 (12%)	35 (12%)	48 (11%)	0.93 (0.57–1.53)
>R10 000–15 000	17 (2%)	5 (2%)	12 (3%)	0.71 (0.23–2.19)
Socioeconomic status quartile				
Highest (first quartile)	255 (26%)	109 (26%)	49 (17%)	1.00 (reference)
Moderately high (second quartile)	241 (25%)	106 (25%)	62 (22%)	1.30 (0.83–2.15)
Moderately low (third quartile)	273 (28%)	111 (27%)	96 (34%)	1.90 (1.23–2.98)
Lowest (fourth quartile)	198 (20%)	92 (22%)	77 (27%)	2.00 (1.21–3.17)
Maternal or household characteristics				
Maternal passive smoke exposure	374 (39%)	104 (37%)	158 (38%)	1.04 (0.69–1.56)
Maternal smoking	242 (25%)	107 (38%)	138 (33%)	1.52 (1.00–2.33)
Maternal HIV infection	198 (20%)	64 (23%)	69 (17%)	1.62 (1.02–2.56)
Number of family or household smokers				
0	339 (35%)	98 (35%)	174 (42%)	1.00 (reference)
1	208 (22%)	53 (19%)	98 (23%)	0.88 (0.55–1.41)
2	127 (13%)	47 (17%)	50 (12%)	1.78 (1.05–3.01)
≥3	112 (12%)	42 (15%)	58 (15%)	1.55 (0.86–2.80)
Birth characteristics				
Gestation (weeks)	39 (37–40)	39 (37–40)	39 (38–40)	0.93 (0.87–0.98)
Premature (<37 weeks' gestation)	249 (26%)	91 (32%)	108 (26%)	1.32 (0.94–1.86)
Birthweight (kg)‡	3.1 (2.7–3.4)	2.9 (2.6–3.3)	2.9 (2.6–3.4)	0.68 (0.52–0.89)
Weight-for-age Z score at birth (95% CI)‡	−0.53 (−1.31 to 0.08)	−0.78 (−1.44 to −0.05)	−0.68 (−1.36 to −0.07)	0.94 (0.81–1.10)
Height-for-age Z score at birth (95% CI)‡	0.06 (−0.82 to 0.95)	−0.23 (−1.16 to 1.01)	−0.08 (−0.85 to 0.87)	0.95 (0.85–1.06)
Feeding choice				
Initiated breastfeeding	762 (79%)	231 (81%)	369 (88%)	0.55 (0.33–0.91)
Infants at age 6–10 weeks				
Exclusive breastfeeding	342/750 (46%)	94/278 (33%)	180/414 (43%)	1.00 (reference)
Mixed feeding	237/750 (32%)	83/278 (29%)	122/414 (29%)	1.35 (0.90–2.01)
Exclusive formula feeding	171/750 (23%)	63/278 (22%)	78/414 (19%)	1.53 (0.93–2.49)
Infants at age 6 months				
Exclusive breastfeeding (age 6 months)	165/644 (26%)	53/280 (19%)	83/414 (20%)	1.00 (reference)
Mixed feeding (age 6 months)	152/644 (24%)	50/280 (18%)	95/414 (23%)	0.85 (0.50–1.43)
Exclusive formula feeding (age 6 months)	327/644 (51%)	159/280 (56%)	206/414 (50%)	1.39 (0.91–2.12)

(Table 1 continues on next page)

JJ du Preez, Paarl; Klein Nederburg, Paarl, South Africa) and Paarl hospital, and performed surveillance for any missed pneumonia episode. All admissions to hospital were at Paarl hospital, the only hospital serving this population. Children were followed throughout their duration in hospital, and at 2 days and 6 weeks after discharge or after an ambulatory episode.

Longitudinal measurement of risk factors (nutrition, environment, vaccinations received, and child and maternal factors) was done at study visits and at case presentation. Infant anthropometry and maternal smoking or passive smoke exposure were measured by urine cotinine longitudinally and at case presentation.¹⁵

Nasopharyngeal swabs were collected every 2 weeks for the first year in a subgroup (intensive cohort); enrolment in the intensive cohort was at the participant's discretion. All children had nasopharyngeal swabs taken every 6 months. A chest radiograph was done in infants with pneumonia who had been admitted to hospital. Laboratory staff were masked to case-control status.

Case-control design

Cases were any episode of pneumonia, irrespective of severity, excluding congenital pneumonia (defined as presentation before postnatal discharge). Controls were incidence-density matched to cases (1–2:1) by birth date

	All children (n=967)	Cases (n=284)*	Controls (n=418)*†	Odds ratio (95% CI)
(Continued from previous page)				
Vaccinations received				
1st dose (EPI at 6 weeks)				
Received on time	690/790 (87%)	242/283 (86%)	381/415 (92%)	1.00 (reference)
Received 2 weeks late	55/790 (7%)	37/283 (13%)	32/415 (8%)	1.95 (1.17–3.25)
2nd dose (EPI at 10 weeks)				
Received on time	594/745 (80%)	213/275 (77%)	340/411 (83%)	1.00 (reference)
Received 2 weeks late	105/745 (14%)	59/275 (21%)	68/411 (17%)	1.52 (1.01–2.28)
3rd dose (EPI at 14 weeks)				
Received on time	495/719 (69%)	171/274 (62%)	292/408 (72%)	1.00 (reference)
Received 2 weeks late	165/719 (23%)	91/274 (33%)	104/408 (25%)	1.59 (1.12–2.26)
4th dose (EPI at 9 months)				
Received on time	387/539 (72%)	147/200 (74%)	249/334 (75%)	1.00 (reference)
Received 2 weeks late	88/539 (16%)	45/200 (23%)	63/334 (19%)	1.46 (0.91–2.35)

Data are median (IQR), n (%), or N. EPI=Expanded Program on Immunization. *Only 702 children included in case-control analysis. †Controls could be sampled more than once.
‡Changes in the relative odds of pneumonia for a 1-week increase in gestation, a 1 Z score change in weight-for-age or height-for-age Z scores or a 0.1 kg change in birthweight.

Table 1: Baseline characteristics of study population

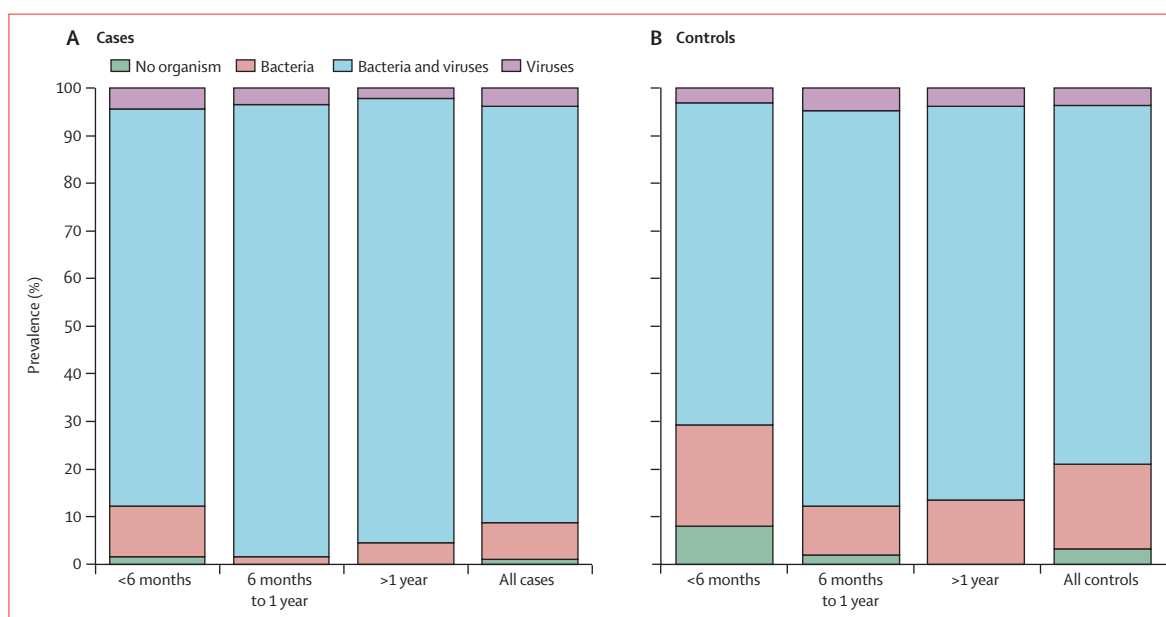


Figure: Organism prevalence by age and type in pneumonia cases and matched* controls from nasopharyngeal samples

*Controls were matched by age and site of enrolment.

(to within 2 weeks), age of presentation (to within 2 weeks), and site of enrolment. By design, controls could be sampled more than once, but this occurred only infrequently. We did separate analyses of cases compared with asymptomatic controls and with controls with symptoms of upper respiratory tract infection (ie, cough, runny or blocked nose, or sore throat).

Specimen collection and analysis

For every case, two nasopharyngeal swabs (FLOQSwabs, Copan Diagnostics, Murrieta, CA, USA) and an

induced sputum specimen were obtained.¹⁰ The first nasopharyngeal swab taken was immediately transferred into nucleic acid preservation medium (PrimeStore, Longhorn Vaccines and Diagnostics, San Antonio, TX, USA), the second swab was placed into 1 mL of skimmed milk-tryptone-glucose-glycerol (STGG) transport medium. Swabs were transported on ice to the laboratory and frozen at -80°C for batch testing. The swab in STGG was cultured for bacteria; total nucleic acid was extracted from the swab in nucleic acid preservation medium with mechanical lysis on a TissueLyzer LT (Qiagen, Hilden, Germany) followed

by extraction with the QIA Symphony Virus/Bacteria Mini Kit (Qiagen, Hilden, Germany). We did quantitative, multiplex, real-time PCR (qPCR) with FTDResp33 (Fast-Track Diagnostics, Esch-sur-Alzette, Luxembourg) to identify up to 33 potential organisms of respiratory viruses (influenza A, B, and C; parainfluenza 1, 2, 3, and 4; coronaviruses NL63, 229E, OC43, HKU1; human metapneumoviruses A and B; rhinovirus; respiratory syncytial viruses A and B; adenovirus; enterovirus; parechovirus; bocavirus; and cytomegalovirus), fungi (*Pneumocystis jirovecii*), and bacteria (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Streptococcus pneumoniae*, *H influenzae* type b, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Bordetella pertussis*, *Klebsiella pneumoniae*, *Legionella* species, salmonella species, and *H influenzae*). *K pneumoniae* and *Legionella* spp were omitted from this analysis because of difficulties with assay specificity. Standard curves were derived with plasmid standards supplied by the manufacturer for each organism.

Every induced sputum specimen was transported to the laboratory on ice and split into two aliquots. The first underwent nucleic acid extraction and testing with FTDResp33, as previously stated; the second was cultured for bacteria. A blood culture for bacteria was obtained in cases admitted to hospital.

For control children, two nasopharyngeal swabs were collected at each visit and stored for later retrieval and processing as described for case swabs. Laboratory staff were masked to case-control status.

Statistical analysis

Weight-for-age and height-for-age Z scores were derived from WHO child growth standards.¹⁶ Socioeconomic status comprised a composite of asset ownership, household income, employment, and education.¹³ We compared data from cases and controls with conditional logistic regression. Dependent variables of interest were organisms from FTDResp33, analysed as binary (present or absent) and continuous (log copies or specimen) values. Model building examined potential confounding factors identified a priori from demographic and clinical measures of child's sex, in-utero HIV exposure, maternal age, maternal smoking, and socioeconomic status; because no clear confounding factors were consistently identified based on appreciable changes in the point estimate for pathogen-pneumonia associations, models presented account for matching factors only. Because children might have had more than one episode of pneumonia (and thus participate in more than one case-control set), we also examined mixed-effects models with children and case-control sets as random or fixed effects, and also conditional logistic regression models restricted to first case episodes. We based co-organism analysis on matched case-control pairs, investigating the presence or absence of two organisms at a time. Of 16 possible response patterns, seven involved co-occurrence, with the less than one, one,

or more than one pattern of co-occurrence in both cases and controls not adding information that differentiated cases from controls. The observed frequencies of the remaining six response patterns were compared with their expected values based on the hypothesis of random co-occurrence with a Pearson χ^2 test. Multiple p values for different organism pairs were corrected with use of the Benjamini-Hochberg correction for false discovery rate. We did subsidiary analyses to stratify case-control comparisons on pneumonia severity and control children's symptoms. Throughout, regression diagnostics followed standard procedures, and all statistical tests were two-sided

	Prevalence of organism			Quantification of organism (median [IQR]; log ₁₀ copies per mL)	
	Case (N=284)	Control (N=412)	Odds ratio (95% CI)	Case	Control
Bacteria					
<i>Bordetella pertussis</i>	6 (2%)	1 (0%)	11.08 (1.33–92.54)	7.38 (4.95–9.84)	8.36 (8.36–8.36)
<i>Haemophilus influenzae</i> type b	4 (1%)	5 (1%)	1.08 (0.28–4.10)	5.32 (4.18–6.14)	5.87 (5.26–6.22)
<i>Mycoplasma pneumoniae</i>	10 (4%)	14 (3%)	1.20 (0.54–2.78)	4.61 (3.73–5.16)	5.22 (4.21–6.61)
<i>Staphylococcus aureus</i>	81 (28%)	142 (35%)	0.70 (0.48–1.02)	4.59 (3.47–5.57)	5.12 (3.71–6.42)
<i>H influenzae</i>	152 (54%)	164 (40%)	1.67 (1.20–2.30)	6.57 (5.53–7.31)	6.53 (5.75–7.26)
<i>Streptococcus pneumoniae</i>	168 (60%)	237 (58%)	1.07 (0.76–1.48)	6.07 (5.31–6.62)	6.03 (5.08–6.65)
<i>Moraxella catarrhalis</i>	214 (75%)	292 (71%)	1.19 (0.82–1.74)	6.58 (5.79–7.20)	7.03 (6.40–7.50)
Viruses					
Respiratory syncytial virus	66 (23%)	17 (4%)	8.05 (4.21–15.38)	6.77 (5.75–7.68)	4.37 (3.55–5.05)
Influenza (types A, B, and C)	32 (11%)	11 (3%)	4.13 (2.06–8.26)	3.84 (2.62–5.29)	4.38 (2.73–6.34)
Parainfluenza (types 1, 2, 3, and 4)	35 (12%)	26 (6%)	2.03 (1.20–3.42)	5.44 (3.86–7.08)	4.29 (3.44–5.37)
Adenovirus	53 (19%)	41 (10%)	2.15 (1.31–3.53)	5.38 (3.54–6.70)	5.16 (4.06–5.74)
Metapneumovirus	29 (10%)	44 (11%)	1.12 (0.67–1.88)	5.57 (4.56–6.50)	4.98 (4.33–5.72)
Bocavirus	37 (13%)	32 (8%)	2.29 (1.25–4.17)	4.34 (3.79–5.22)	4.23 (3.43–5.27)
Cytomegalovirus	151 (53%)	177 (43%)	1.57 (1.11–2.21)	4.98 (4.10–5.62)	4.99 (4.41–5.60)
Coronavirus (NL63, 229E, OC43, and HKU1)	33 (12%)	43 (10%)	1.20 (0.75–1.97)	6.58 (4.22–7.74)	5.38 (4.07–7.21)
Enterovirus	37 (13%)	57 (14%)	0.93 (0.58–1.49)	3.75 (2.98–5.31)	4.02 (3.45–5.27)
Rhinovirus	100 (35%)	161 (39%)	0.87 (0.63–1.20)	4.12 (3.43–4.91)	4.38 (3.59–5.13)
Fungi					
<i>Pneumocystis jirovecii</i>	44 (16%)	122 (30%)	0.35 (0.22–0.55)	4.10 (3.69–4.78)	4.08 (3.69–4.78)

Table 2: Organism-specific prevalence and quantification of load in cases and controls from nasopharyngeal samples

	RSV (n=79)	Influenza (n=40)	Adenovirus (n=77)	Parainfluenza (n=52)	Bocavirus (n=49)
Age (months)	4.1 (2.2–7.8)	4.4 (2.3–9.1)	9.7 (5.6–13.4)	6.1 (4.2–11.0)	11.3 (6.5–13.4)
Boys	41 (52%)	26 (65%)	48 (62%)	36 (69%)	33 (67%)
Prematurity	20 (25%)	12 (30%)	21 (27%)	19 (37%)	18 (37%)
Maternal antepartum smoking exposure	54 (68%)	30 (75%)	57 (74%)	40 (77%)	36 (73%)
Maternal HIV infection	13 (16%)	10 (25%)	15 (19%)	11 (21%)	12 (24%)
Season when acquired viral- associated pneumonia					
Summer (December to February)	0	4 (10%)	15 (19%)	2 (4%)	9 (18%)
Autumn (March to May)	22 (28%)	4 (10%)	10 (13%)	4 (8%)	6 (12%)
Winter (June to August)	49 (62%)	24 (60%)	20 (26%)	22 (42%)	19 (39%)
Spring (September to November)	8 (10%)	8 (20%)	32 (42%)	24 (46%)	15 (31%)
Cough	76 (96%)	40 (100%)	74 (96%)	50 (98%)	45 (92%)
Wheeze	46 (58%)	15 (38%)	30 (39%)	22 (43%)	19 (39%)
Respiratory rate per min	60 (52–64)	55 (49–62)	53 (46–58)	56 (50–62)	52 (46–60)
Fever	50 (63%)	25 (63%)	46 (61%)	37 (70%)	32 (68%)
Lower chest wall indrawing	49 (62%)	19 (49%)	30 (40%)	26 (49%)	17 (36%)
Cough duration (days)	3 (2–4)	3 (2–5)	3 (2–4)	3 (2–5)	3 (2–4)
Wheeze duration (days)	2 (2–3)	3 (1–3)	2 (2–4)	3 (2–7)	2.5 (2–4)
Admitted to hospital	33 (42%)	13 (33%)	21 (27%)	15 (29%)	9 (18%)
Severe pneumonia	20 (25%)	8 (20%)	10 (13%)	10 (19%)	7 (14%)
Oxygen given	23 (29%)	5 (13%)	6 (8%)	8 (15%)	4 (8%)
Number of deaths	0	1 (<1%)	0	1 (<1%)	0
Number before 1st PCV	12 (15%)	5 (13%)	5 (6%)	10 (19%)	2 (4%)
Number before 2nd PCV	39 (49%)	18 (45%)	16 (21%)	17 (33%)	6 (12%)

Data are n (%) or median (IQR). RSV=respiratory syncytial virus. PCV=pneumococcal conjugate vaccine.

Table 3: Clinical characteristics of 284 children with viral-associated pneumonia

See Online for appendix at $\alpha=0.05$. We used Stata (version 13.0) and R (version 3.2.2) for data analyses.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data and had final responsibility for the decision to submit for publication.

Results

Table 1 shows characteristics of cases and controls. 967 children accrued 1145 child-years of follow-up. 719 (74%) children were enrolled in the intensive cohort (with nasopharyngeal swabs collected every 2 weeks for the first year). Cohort retention was high with only 130 (13%) of 967 children lost to follow-up.

Tobacco smoke exposure was common (table 1). 242 (25%) mothers smoked during pregnancy and 374 (39%) had passive smoke exposure. Although 198 (20%) mothers had HIV infection, only two children had HIV infection (one case, one control). Although

762 (79%) mothers initiated breastfeeding, only 342 (46%) were exclusively breastfeeding by 6–10 weeks, and 165 (26%) by 6 months (table 1). Immunisation coverage was high at 6, 10, and 14 weeks and 9 months (table 1). However, vaccines were delayed by more than 2 weeks in 55 (7%) of 790 children at 6 weeks, 105 (14%) of 745 children at 10 weeks, 165 (23%) of 719 children at 14 weeks, and 88 (16%) of 539 children at 9 months (table 1).

24 infants had congenital pneumonia and were excluded from this present analysis. There were 314 cases of pneumonia, an incidence of 0.27 episodes per child-year (95% CI 0.24–0.31) with similar incidences at each clinic. 30 pneumonia cases were excluded with incomplete specimen collection. The highest incidence was in children aged 1–6 months (0.54 episodes per child-year [95% CI 0.46–0.62]) and in winter (0.51 episodes per child-year [0.43–0.61]; appendix p 1). Male sex, low socioeconomic status, HIV exposure, lack of breastfeeding, more than two household smokers, lower birthweight, and delayed vaccinations were associated with an increased risk of pneumonia (table 1). In multivariate analysis, only male sex, birthweight, and socioeconomic status were significantly associated with pneumonia (data not shown). Most cases (205 [72%] of 284) of pneumonia were first episodes and 79 cases (28%) were repeat episodes, of which most (51 [65%]) were second events.

60 (21%) pneumonia cases were severe (incidence 0.05 episodes per child-year [95% CI 0.04–0.07; appendix p 2). Almost a third of children (92 [32%]) were admitted to hospital, and 45 children (16%) received oxygen therapy (appendix p 2). Median age of children with pneumonia was 5 months (IQR 3–9), whereas the median age of children with severe pneumonia was 2 months (1–4; $p=0.001$; appendix p 2). Most cases (237 [84%]) received antibiotics (87 [95%] children admitted to hospital and 150 [78%] in ambulatory care). Chest radiographs, available for 80 (87%) of 92 children with pneumonia who were admitted to hospital, had radiological changes of pneumonia in 60 (75%). Median duration of hospital stay was 3 days (IQR 2–5; appendix p 2). Of the children admitted to hospital, there were three deaths due to pneumonia, a case fatality rate of 1%. Two control children died—one from liver failure and one from sudden infant death syndrome.

Blood cultures were available from 90 (98%) of 92 children with pneumonia admitted to hospital; cultures were only positive and grew *S pneumoniae* from an infant aged 1 month who had not received PCV13.

A total of 284 pneumonia cases and 418 age-matched and site-matched controls (selected from the 719 intensive cohort who had nasopharyngeal swabs taken every 2 weeks) were included in case-control analysis from nasopharyngeal swabs. A median of five organisms (IQR 4–6) were reported in cases and controls. An organism was detected in about 97% of swabs from cases or

controls; both viruses and bacteria were detected in 208 (87%) of 284 cases and 307 (73%) of controls ($p=0.0001$), whereas only a few swabs from children had either bacteria or viruses alone (figure). A higher proportion of controls had only bacteria (73 [18%] in 418 controls, 22 [8%] in 284 cases; $p=0.0002$) or no organism (13 [3%] in 418 controls, three [1%] in cases; $p=0.082$) shown in the nasopharyngeal swabs.

Bacteria associated with pneumonia were *B pertussis* and *H influenzae* (table 2). Several viruses were associated with pneumonia; RSV was the most strongly associated followed by influenza virus, bocavirus, adenovirus, and parainfluenza. Cases were less likely to have *P jirovecii* than controls (table 2). These results did not vary appreciably under different modelling approaches (eg, the association of RSV with pneumonia varied from OR 7.52 to 13.65 with different models; appendix p 3). Analyses adjusted for child and maternal factors showed similar results (appendix p 4). No difference was reported in quantitative organism load between cases and controls except for RSV and parainfluenza virus (with higher loads in cases) and *S aureus* and *M catarrhalis* (with lower loads in cases; table 2). The associations were strongest when comparisons were restricted to asymptomatic controls, compared with those with upper respiratory tract infection symptoms (appendix p 5). *C pneumoniae* was not detected.

These organism-specific associations persisted when cases were stratified by disease severity; however, RSV was more strongly associated with severe pneumonia (OR 25.30, 95% CI 3.30–191.61) than with pneumonia (appendix pp 6–7). In severe pneumonia, quantitative load of *S aureus* and of *M catarrhalis* was significantly lower in cases than controls, but no other significant differences were reported (appendix p 7).

Of viral organisms, significant differences were noted in the age of cases, seasonal distribution, and clinical features (table 3). RSV or influenza were detected in younger children than in those with parainfluenza, adenovirus, or bocavirus (table 3). Age-specific incidence of RSV was highest for ages 1–6 months (0.15 episodes per child-year [95% CI 0.11–0.20]); incidence in those aged younger than 1 month was 0.04 episodes per child-year (0.01–0.11), 0.05 episodes per child-year (0.03–0.09) in infants aged 6–12 months, and 0.03 episodes per child-year (0.01–0.06) in those aged 12–18 months. RSV, influenza virus, and bocavirus were more common in winter, whereas adenovirus and parainfluenza virus occurred predominantly in spring (table 3). RSV was associated with a higher prevalence of wheezing, greater prevalence of lower chest wall indrawing, and a higher median respiratory rate compared with other viruses (table 3).

Despite several organisms being present in both cases and controls, patterns of co-prevalence (of any two organisms) were similar. No significant differences were noted between cases and controls in co-prevalence of any two organisms (appendix pp 8–10).

	Only nasopharyngeal swabs	Nasopharyngeal swabs and induced sputum both positive	Only induced sputum	Proportion of cases detected by induced sputum only
Bacteria				
<i>Bordetella pertussis</i>	0	4	12	75%
<i>Haemophilus influenzae</i> type b	1	3	15	79%
<i>Mycoplasma pneumoniae</i>	3	4	8	53%
<i>Staphylococcus aureus</i>	23	49	30	29%
<i>H influenzae</i>	10	129	10	7%
<i>Streptococcus pneumoniae</i>	12	140	11	7%
<i>Moraxella catarrhalis</i>	15	178	9	4%
Viruses				
Respiratory syncytial virus	7	53	12	17%
Influenza (types A, B, and C)	17	15	8	20%
Parainfluenza (types 1, 2, 3, and 4)	17	18	17	33%
Adenovirus	10	41	24	32%
Metapneumovirus	13	14	7	21%
Bocavirus	7	28	12	26%
Cytomegalovirus	14	121	18	12%
Coronavirus (NL63, 229E, OC43, and HKU1)	13	20	8	20%
Enterovirus	15	19	15	31%
Rhinovirus	30	51	33	29%
Fungi				
<i>Pneumocystis jirovecii</i>	16	25	21	34%

*Specimens included for cases where both nasopharyngeal swabs and induced sputum samples were available.

Table 4: Proportion of total cases of microorganisms detected by induced sputum and nasopharyngeal swabs in children with pneumonia*

In 251 (88%) cases, a matched nasopharyngeal swab and induced sputum sample were obtained; a median of six organisms (IQR 4–7) were detected on induced sputum compared with five (4–7) on nasopharyngeal swabs ($p=0.48$). There was an increased yield from testing induced sputum for several organisms and all viruses, most notably for *B pertussis* (12 of 16 cases on induced sputum), *H influenzae* type b (15 of 19 cases on induced sputum), *M pneumoniae* (8 of 15 cases on induced sputum; table 4).

Discussion

This study showed that pneumonia is a major cause of illness and admissions to hospital, particularly in the first 6 months of life, despite excellent immunisation coverage including PCV13. Several viruses, most strikingly RSV, were strongly associated with pneumonia. *B pertussis* was strongly associated with pneumonia, but occurred in a small number of children; *H influenzae* was less strongly associated with pneumonia but was common. Induced sputum samples provided an increased yield for detection of potential pathogens compared with nasopharyngeal swabs. Despite high severity of disease, we recorded a low case fatality attesting to good access to care and a strong primary health-care programme.

Several potential pathogens were identified in children with pneumonia, adding to evidence that childhood pneumonia and severe disease might often not be due to a single organism.^{7,8} RSV, the most identified organism, was associated with severe disease but not with high mortality, which is consistent with global and African data preceding use of PCV that reported RSV to be the most common pathogen in children with lower respiratory tract infection (LRTI).^{17–19} These results are similar to those from a 2015 meta-analysis²⁰ of case-control studies in childhood in which RSV was most strongly associated with LRTI, with influenza, parainfluenza, and human metapneumovirus also important causative organisms.²⁰ With increasing uptake of PCV in low-income and middle-income countries, the proportion of LRTI due to viruses—particularly RSV—could be expected to increase.²¹ In South Africa, in view of these incidence rates, about 270 000 pneumonia cases might be expected in infants annually, with almost 32 400 cases due to RSV. In our study, many children had wheezing associated with RSV, raising the question of whether these were cases of pneumonia or of bronchiolitis. In our study we applied WHO pneumonia definitions because they are widely used for their high sensitivity. Furthermore, pneumonia and bronchiolitis are a spectrum of LRTI, representing different manifestations of infection or host response. Of note, RSV occurred in young infants with the peak incidence in those younger 6 months, as has been previously reported.¹⁷ Maternal immunisation against RSV during late pregnancy might therefore be an attractive novel strategy to prevent disease in young infants.²²

Bacterial–viral co-infections have been reported to be important, especially in severe pneumonia.²³ In an African PCV9 trial,²⁴ immunisation reduced hospital admissions for viral LRTI by a third, suggesting that pneumococcal–viral co-infection leads to severe pneumonia. Another study²³ reported that an increasing number of co-infections was associated with more severe disease and higher mortality in children admitted to hospital with LRTI. However in our study, the only bacteria associated with pneumonia were *B pertussis* and *H influenzae*. *B pertussis* occurred in young infants and mostly before completion of the three immunisations; immunisation of pregnant women with *B pertussis* might be effective to prevent this burden.²⁵ The observed association between *H influenzae* and pneumonia supports recent evidence suggesting that non-typeable *H influenzae* might be an important pathogen in pneumonia.²⁶ Although the strength of the association was relatively weak, since *H influenzae* was identified in more than half of cases the contribution of this organism to pneumonia might be substantial. The absence of an association of other bacteria with pneumonia could reflect several possibilities. High PCV13 coverage reduces pneumonia incidence, as described in other settings.^{4,6,27} In a USA

case-control study⁷ of children admitted to hospital for pneumonia who had received PCV, more than two-thirds were reported to be due to viral causes (with RSV predominating) whereas only 7% of children had bacterial–viral co-infection. Additionally, bacterial–viral co-infection has been predominantly associated with severe or very severe pneumonia,^{23,24} but in our study most cases were non-severe pneumonia. Finally, bacterial causes might be especially difficult to ascribe because of high rates of colonisation. Specimens from the upper respiratory tract might be of little use to establish bacterial causes. Research is needed to understand interactions between organisms and the role of several potential pathogens.

Defining the aetiology of pneumonia might be challenging. To obtain a representative sample from the lower respiratory tract might be difficult in young children and contamination with colonising organisms, such as *S pneumoniae*, could occur.¹¹ This contamination might lead to an underestimation of the contribution of bacteria causing pneumonia. Similarly, although new molecular diagnostics enable detection of many respiratory viruses and less easily culturable bacteria, distinguishing live pathogenic organisms from those colonising or infecting the upper respiratory tract or residual nucleic acid from previous infections is difficult.⁹ Taking samples from the upper respiratory tract, therefore, restricts inferences of causality regarding lung disease. Furthermore, multiplex PCR identifies a broad range of potential pathogens, but the sensitivity for individual pathogens might be lower than a singleplex PCR. Sample quality is another key determinant of the accuracy of a diagnostic test, but we could not verify the quality of samples, or normalise quantitative results to a host target. A strength of this study is the use of different or several specimens, which increases detection of organisms. In a pre-PCV Kenyan study¹⁸ of children admitted to hospital with pneumonia, culture or PCR of blood, induced sputum, oropharyngeal, and nasopharyngeal specimens led to incremental detection of organisms. However, with a case-control analysis of nasopharyngeal swabs, RSV was the only significantly associated pathogen.¹⁸ Similarly, although our study was able to detect several potential pathogens through intensive specimen collection and use of sensitive assays, the case-control analysis showed significant associations with pneumonia for only *B pertussis*, *H influenzae*, RSV, influenza virus, parainfluenza, adenovirus, bocavirus, and cytomegalovirus.

This study is, to our knowledge, one of the first to show that use of induced sputum samples might improve diagnostic yield, especially for specific organisms. Of note, induced sputum was obtainable in young infants and in those with severe pneumonia. Although the value of induced sputum for microbiological confirmation of tuberculosis in children has been shown in large studies,¹⁰ in our study its use showed a substantial incremental yield

for *B pertussis* and for some viruses associated with pneumonia. A Kenyan study¹⁸ found induced sputum increased the yield of potential bacterial pathogens compared with blood culture, and detected a virus in more than half of children admitted to hospital with pneumonia. However, there was discordance between results from induced sputum and blood culture samples.¹⁸ A Finnish study²⁸ reported that a potential bacterial or viral pathogen was detected by PCR in 97% of children admitted to hospital with pneumonia with an adequate induced sputum sample. Accurate detection of a pathogen—eg, *B pertussis*—enables timely appropriate treatment, infection control, and could help to reduce morbidity. Use of induced sputum samples should be further investigated for causal diagnosis of childhood pneumonia.

We found quantitative analysis of organism load unhelpful for distinguishing colonising from pathogenic organisms, except for RSV. However, since RSV was strongly associated with pneumonia its quantification did not provide additional information. Our study highlights the need for better diagnostics in childhood pneumonia to distinguish colonisation from pathogenic organisms, particularly for potential bacterial pathogens. Until more specific tests for bacterial causes are available, no changes can be made to WHO's recommended empirical therapy with antibiotics.¹⁴

Several features of our study strengthen our results. First, selection of cases and controls from a large prospective cohort allows inferences that extend beyond those afforded by case-only designs, while minimising for selection biases in previous studies of pneumonia causes. Second, investigation of causes by pneumonia severity (including ambulatory cases and cases admitted to hospital) and control symptomatology allows more definitive examination of pathogen associations. However, our study is still limited by absence of data for longitudinal nasopharyngeal colonisation to attribute causes at the time of pneumonia onset. Longitudinal analyses are underway to address this issue and to further study co-pathogens. Although we might have missed some cases of pneumonia, the pneumonia surveillance systems, longitudinal review of patient records, and excellent cohort retention ensured that this was extremely rare. These results might not be generalisable to low-income and middle-income countries with lower levels of immunisation coverage or in countries where *H influenzae* type b or PCV are not included in immunisation programmes. However, the uptake of *H influenzae* type b and PCV globally including in Africa has increased,²⁹ so these results are likely to be widely applicable in low-income and middle-income countries. Our study provides important new information on the association between specific organisms and pneumonia after children have received PCV in a middle-income country, but also highlights the complexities in ascribing causality and in defining the role of several organisms in childhood pneumonia.

Contributors

HJZ is the principal investigator of the Drakenstein Child Health Study, and conceived and designed the study. HJZ, LM, and MPN obtained funding. LM led the epidemiological and analytical aspects of this study. MPN is the lead microbiologist. WB is the project manager and provided operational oversight. AS is the study clinician and coordinator. LM and SG-L did data analyses. HJZ drafted this manuscript. All authors reviewed, contributed to, and approved the final version.

Declaration of interests

All authors report grants from the Bill & Melinda Gates Foundation, Medical Research Council South Africa, National Research Foundation South Africa, National Institutes of Health, and H3Africa, during the conduct of the study. HJZ reports personal fees from Pfizer and Abbvie, outside of the submitted work. The other authors declare no competing interests.

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