Viral Etiology of Severe Pneumonia Among Kenyan Infants and Children

James A. Berkley, FRCPCH
Patrick Munywoki, MSc
Mwanajuma Ngama, HND
Sidi Kazungu, RN
John Abwao, HND
Anne Bett, HND
Ria Lassauniére, BSc
Tina Kresfelder, PhD
Patricia A. Cane, PhD
Marietjie Venter, PhD
J. Anthony G. Scott, FRCP
D. James Nokes, PhD

Context Pneumonia is the leading cause of childhood death in sub-Saharan Africa. Comparative estimates of the contribution of causative pathogens to the burden of disease are essential for targeted vaccine development.

Objective To determine the viral etiology of severe pneumonia among infants and children at a rural Kenyan hospital using comprehensive and sensitive molecular diagnostic techniques.

Design, Setting, and Participants Prospective observational and case-control study during 2007 in a rural Kenyan district hospital. Participants were children aged 1 day to 12 years, residing in a systematically enumerated catchment area, and who either were admitted to Kilifi District Hospital meeting World Health Organization clinical criteria for severe pneumonia or very severe pneumonia; (2) presented with mild upper respiratory tract infection but were not admitted; or (3) were well infants and children attending for immunization.

Main Outcome Measures The presence of respiratory viruses and the odds ratio for admission with severe disease.

Results Of 922 eligible admitted patients, 759 were sampled (82% [median age, 9 months]). One or more respiratory viruses were detected in 425 of the 759 sampled (56% [95% confidence interval {CI}, 52%-60%]). Respiratory syncytial virus (RSV) was detected in 260 participants (34% [95% CI, 31%-38%]) and other respiratory viruses were detected in 219 participants (29%; 95% CI, 26%-32%), the most common being *Human coronavirus 229E* (n=51 [6.7%]), influenza type A (n=44 [5.8%]), *Parainfluenza type 3* (n=29 [3.8%]), *Human adenovirus* (n=29 [3.8%]), and *Human metapneumovirus* (n=23 [3.0%]). Compared with well control participants, detection of RSV was associated with severe disease (5% in control participants; adjusted odds ratio, 6.11 [95% CI, 1.65-22.6]) while collectively, other respiratory viruses were not associated with severe disease (23% in control participants; adjusted odds ratio, 1.27 [95% CI, 0.64-2.52]).

Conclusion In a sample of Kenyan infants and children admitted with severe pneumonia to a rural hospital, RSV was the predominant viral pathogen.

JAMA. 2010;303(20):2051-2057 www.jama.com

PNEUMONIA IS THE LEADING CAUSE of childhood death in sub-Saharan Africa. The main means for controlling disease and death due to pneumonia are infant vaccination and case management. Thus, establishing the contribution to severe disease of individual pathogens and vaccine efficacy in infancy are essential to reducing the burden of disease.

Observational studies, including microbial cultures and vaccine probe studies, have identified *Streptococcus pneumoniae* and *Haemophilus influenzae* as the most common bacterial causes.[1,2] Both are preventable by currently available conjugate vaccines. Consequently, other bacteria, eg, *Mycobacterium tuberculosis*, and *Pneumocystis jiroveci,* and respiratory viruses are becoming of increased importance.[3] Vaccines currently exist or are in development for several respiratory viruses including respiratory syncytial virus (RSV), influenza type A (FLUAV), and *Human parainfluenza virus 3* (HPIV-3), including combinations.[4-6] In previous studies in sub-Saharan Africa, viral etiology of pneumonia has been examined by antigen detection, serology, and viral culture.[7-11]

The development of molecular methods has led to 2 advances in viral diagnosis: increased sensitivity and the discovery of new viruses of clinical importance. However, the high sensitivity of molecular diagnostics raises questions about specificity since viral nucleic acid sequences may be detected in healthy children and may persist after illness.[12] The detection of multiple respiratory viruses by molecular

Author Affiliations: Kenya Medical Research Institute (KEMRI), Centre for Geographic Medicine Research—Coast, Kilifi, Kenya (Drs Berkley, Scott, and Nokes, Messrs Munywoki and Abwao, and Mss Ngama, Kazungu, and Bett); Centre for Clinical Vaccinology & Tropical Medicine, University of Oxford, Oxford, England (Drs Berkley and Scott); Respiratory and Zoonotic Virus Programme, Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa (Ms Lassauniére and Drs Kresfelder and Venter); Centre for Infections, Health Protection Agency, Colindale, London, England (Dr Cane); Respiratory Virus Unit, National Institute for Communicable Diseases, Sandringham, South Africa (Dr Venter); and Department of Biological Sciences, University of Warwick, Coventry, England (Dr Nokes).
Corresponding Author: James A. Berkley, FRCPCH, PO Box 230, Kilifi, 80108, Kenya (jberkley@kilifi.kemri-wellcome.org).

methods in individual cases further underlines this etiological quandary.[13] To date, we have found no published studies reporting comprehensive viral etiology of pneumonia among children in sub-Saharan Africa using sensitive molecular diagnostic methods.

We aimed to determine viral etiology, incidence, and clinical features among infants and children meeting World Health Organization (WHO) clinical criteria for severe pneumonia or very severe pneumonia admitted to a rural Kenyan district hospital.

METHODS

Location

The study was conducted at Kilifi District Hospital in a rural area on the Kenyan coast. Humidity is high throughout the year and there are 2 annual rainy seasons, April through July and November through December.[14] The area is endemic for malaria, with declining transmission over the last 10 years. *H influenzae* type b conjugate vaccine, given with the diphtheria and tetanus toxoids and pertussis vaccine to infants aged 6, 10, and 14 weeks, was introduced in 2001. Coverage of these vaccines (third dose) and measles vaccine administered at 9 months of age are greater than 90%.[15] Conjugate pneumococcal vaccine had not been introduced at the time of this study.

The Collaborative Research Programme between the Kenya Medical Research Institute (KEMRI) and the Wellcome Trust provides clinical care on the hospital pediatric wards and a demographic surveillance system covering an area of approximately 900 km,[2] which is within 50 km north, 50 km south, and 30 km west of the hospital, and includes a population of approximately 240 000. The area was mapped in 2000 and every building was registered by global positioning system. The population register is updated for births, deaths, and migration events by household visits with 3 re-enumeration rounds each year.[16] All field-based data are checked, double-entered, and verified within 48 hours of acquisition. Since 2002, patients admitted to the hospital are individually identified on the population register. In 2007, approximately 65% of pediatric admissions were confirmed residents of the census area.

Participants and Clinical Methods

We included all children aged 1 day to 12 years residing in the census area and who were admitted to the pediatric wards between January 1, 2007, and December 31, 2007, and met WHO criteria for the clinical syndromes of severe pneumonia (cough or difficult breathing plus lower chest wall indrawing and no signs of very severe pneumonia) or very severe pneumonia (cough or difficult breathing plus at least 1 of hypoxia, defined as an oxygen saturation <90% by fingertip pulse oximetry [Covidien-Nellcor, Boulder, Colorado], inability to drink or breast feed, inability to sit, or impaired consciousness at admission,[17] including infants younger than 2 months of age).

Clinicians were trained in the recognition of the clinical signs used in the WHO criteria through teaching sessions that included videos, practical demonstrations, and additional bedside training on the wards. Clinical findings were individually recorded for each item of history or examination in a database during the admission assessment. Next, a standardized set of investigations were performed including complete blood cell count (Beckman Coulter Inc, Brea, California), blood film for malaria parasites (stained with Giemsa and read at ×1000 magnification), blood culture (Bactec PedsPlus [Becton Dickinson and Co, Franklin Lakes, New Jersey]) processed by standard methods,[18] and a nasal wash sample.[19] Nasal washes were conducted between 8 AM and 10 PM daily. Nasal wash was not performed on infants and children with severe respiratory or cardiovascular compromise.

Human immunodeficiency virus (HIV) testing was performed according to the Kenyan national policy for all pediatric hospital admissions using 2 rapid antibody tests: Determine (Inverness Medical Innovations Inc, Waltham, Massachusetts) and Unigold (Trinity Biotech, Bray, Ireland). Children and their families who tested HIV positive were given further counseling and referred to the hospital comprehensive HIV care clinic.

For this analysis, capillary refill time of 3 seconds or more was used as a marker of circulatory shock. Severe anemia was defined as a hemoglobin level of less than 5 g/dL. Severe malnutrition was defined as weight-for-height z score of less than –3 (National Centers for Health Statistics reference standards, Centers for Disease Control and Prevention, Atlanta, Georgia) or kwashiorkor.[17] Treatment for pneumonia and other conditions was according to current WHO guidelines.[17]

To estimate the association of respiratory viral infection with severe disease, we recruited 2 further sets of children resident in the census area by convenience sampling from May 1, 2007, to April 30, 2008: children with mild upper respiratory tract infection (URTI)—including symptoms of cough, runny or blocked nose, sore throat, or sneezing, being managed as outpatients and not meeting any criteria for pneumonia; and well infants and children—without any symptoms or signs of upper or lower respiratory infection attending for routine immunization at the hospital. After obtaining clinical history and examining these participants, a nasal wash specimen was taken.

Laboratory Methods

Nasal wash samples were stored at –80°C and batch analyzed at study end at the University of Pretoria, South Africa. Total viral nucleic acids were extracted from 200 µL of the respiratory specimens using the Magnapure LC Total Nucleic Acid Isolation Kit (Roche, Manheim, Germany), following manufacturer instructions. Complementary DNA was synthesized using Expand Reverse Transcriptase (Roche). Real-time polymerase chain reactions (PCRs) were performed using the LightCycler Fast Start DNA MasterPLUS HybProbe kit (Roche) for the following: *Human adenovirus* (HAdV), *Human para-*

influenza virus 1 (HPIV-1), *Human parainfluenza virus 2* (HPIV-2), HPIV-3, RSV, FLUAV, influenza type B, *Human metapneumovirus* (HMPV), *Human bocavirus* (HBoV), *Human coronavirus OC43* (HCoV-OC43), HCoV/Hong Kong, HCoV-229E, and HCoV-NL63. PCR amplicons for DNA sequencing were gel purified using the Wizard SV gel and PCR Clean-Up System (Promega, Madison, Wisconsin), according to the manufacturer's instructions. DNA sequencing was performed with specific primers using the ABI PRISM BigDye Terminator Cycle Sequencing Reaction kit (version 3.1) on an ABI PRISM 3130 DNA sequencer (Applied Biosystems, Foster City, California), following manufacturer instructions.

Statistical Analysis

Statistical analysis was performed using STATA 9.1 (Stata Corp, College Station, Texas). Eligibility and classification of the clinical syndromes of pneumonia were determined from the original record of each item of medical history and examination in the database.

Incidence rates were estimated using data from all eligible patient admissions known to be resident in the census area on the day of admission, and the midstudy (July 1, 2007) census population estimates interpolated from the linear equation determined by regressing population size ($\log_{10}$) for all enumeration rounds to the end of 2008 against the mid-date of each round.

To describe demographic and clinical features of cases of severe and very severe pneumonia, we compared those with and without a respiratory virus detected. Proportions were compared using the χ^2 and Fisher exact tests as appropriate. Continuous data were compared by *t* test or the Kruskal-Wallis rank sum test. Variables presented were selected a priori from more than 50 clinical variables collected. No adjustment was made for multiple comparisons because each variable represents a discrete test of a biologically plausible hypothesis.

To estimate the strength of association of detection of RSV and non-RSV viruses with severe disease, we compared patients admitted with severe and very severe pneumonia with control group participants comprising well infants and children (excluding those with signs of URTI) using a case-control design. Odds ratios (ORs) were calculated by multivariable logistic regression and presented models for RSV (including those with RSV plus an additional virus) and respiratory viruses not including RSV that were unadjusted (model 1); and adjusted for age and season in monthly intervals (model 2). To address a potential bias of underrepresentation of fatal cases, the (unadjusted) ORs for severe disease among all eligible admitted patients were modeled by imposing the proportions with RSV and other viruses detected among fatal and nonfatal sampled cases on all eligible admissions (model 3). All tests were 2-sided with a 5% significance level.

Sample size for the case group was determined by previous annual admissions, which would allow the prevalence of pathogens of 5% and 20% to be estimated with precision of ± 2% and ± 3%, respectively. We aimed to recruit 100 well control participants and 100 individuals with mild URTI to describe specificities of 95% and 85%, with a lower-side precision of –6% and –8%, respectively. Power for the case-control analysis was confirmed for the actual numbers sampled using the method described by Fliess.[20]

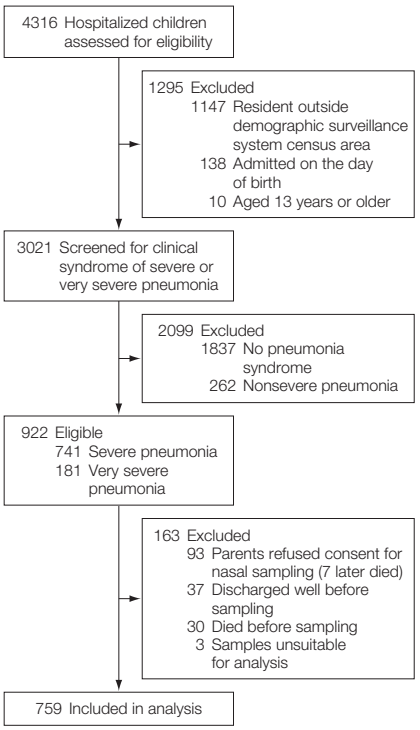
Ethical Approval

The study was approved by the Kenyan National Ethical Review Committee (SSC 815) and the Oxford Tropical Ethical Review Committee (011 06). Individual written informed consent was obtained from parents or guardians of all participants.

RESULTS

From January 1, 2007, through December 31, 2007, there were 922 eligible infants and children with severe pneumonia or very severe pneumonia admitted, and viral screening was conducted on 759 patients (82%) (FIGURE 1). Median age was 9.0 months (interquartile range [IQR], 3.0-20), 59% were male, and 52 had a positive HIV rapid antibody test (6.9%; 95% confidence interval [CI], 5.2%-8.9%). Children who were not sampled were more severely ill than those sampled (eTable 1 available at http://www.jama.com). Three-quarters of deaths among nonsampled admitted patients occurred within 24 hours of admission.

Figure 1. Participant Selection



One or more respiratory viruses were detected in 425 participants in the case group (56% [95% CI, 52%-60%]; TABLE 1). RSV was the most commonly detected virus, present in 260 admissions overall (34% [95% CI, 31%-38%]; eTable 2), and in 192 of 453 infants (42% [95% CI, 38%-47%]). RSV was strongly seasonal ($P < .001$), detected in more than 50% of pneumonia cases in January, February, and December, but in fewer than 5% of pneumonia cases from July through September (FIGURE 2). Other viruses showing clear seasonality were HMPV, HPIV-3, and FLUAV (eFigure).

Other respiratory viruses were detected in 219 admissions (29% [95%

CI, 26%-32%]; eTable 2 and eFigure). These included HCoV-229E (n=51 [6.7%]), FLUAV (n=44 [5.8%]), HPIV-3 (n=29 [3.8%]), HAdV (n=29 [3.8%]), HMPV (n=23 [3.0%]), HPIV-1 (2.4%), HBoV (2.1%), HCoV-OC43 (1.8%), HPIV-2 (1.3%), HCoV-NL63 (1.3%), influenza type B (0.1%), and HCoV/Hong Kong-1 (0.1%).

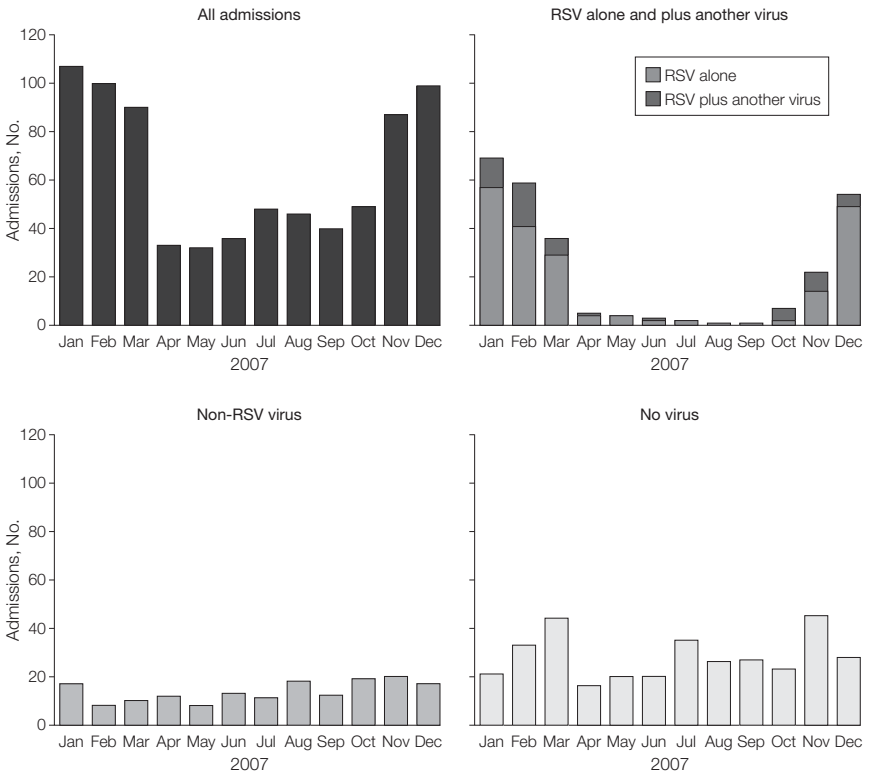
RSV plus 1 or more other respiratory viruses were detected in 54 cases (7%), representing 21% (95% CI, 16%-26%) of all RSV cases. These comprised HCoV-229E (14), FLUAV (10), HAdV (10), HPIV-3 (6), HBoV (6), HMPV (3), HPIV-1 (3), HPIV-2 (3), HCoV-NL63 (2), and HCoV-OC43 (1).

Table 1. Respiratory Viruses Detected

	No. (%)		
	Severe or Very Severe Pneumonia (n = 759)	Outpatient URTI (n = 96)	Well Control Participants (n = 57)
No virus	334 (44)	54 (56)	41 (72)
Any virus	425 (56)	42 (44)	16 (28)
1 Virus	351 (46)	36 (38)	15 (26)
2 Viruses	66 (8.7)	5 (5)	1 (2)
3 Viruses	8 (1.1)	1 (1)	0 (0)
RSV only	206 (27)	15 (16)	2 (4)
RSV plus another virus	54 (7.1)	2 (2)	1 (2)
Non-RSV virus	165 (22)	25 (26)	13 (23)

Abbreviations: RSV, respiratory synctial virus; URTI, upper respiratory tract infection.

Figure 2. Seasonal Pattern of RSV and Other Viruses Detected Among Infants and Children Admitted With Severe or Very Severe Pneumonia



RSV indicates respiratory synctytial virus.

The incidence of admission with severe pneumonia or very severe pneumonia was 4.8% per child per year in the first year of life, 1.5% per child per year in children younger than 5 years of age, and 0.1% among children who were 5 years of age or older (**TABLE 2**). The incidence of admission for any respiratory virus and with RSV was highest in the first year of life (3.0% per child per year and 2.0% per child per year, respectively).

Patients admitted with respiratory viruses detected were a median age of 7.5 months (IQR, 2.7-18) and were younger than those with no virus detected (median age, 11.3 months [IQR, 3.8-24]; $P < .001$). However, this effect was completely accounted for by RSV (**TABLE 3**). Very severe pneumonia was less common among patients admitted with respiratory viruses ($P < .001$). There was no association between the presence of wheezing or hypoxia and a respiratory virus being detected. Most participants in the case group with RSV did not have an admission diagnosis of bronchiolitis. Patients admitted with RSV were less severely ill and had fewer adverse risk factors than those admitted with no virus and were less likely to be prematurely born, in circulatory shock, severely malnourished, or to die. Of the 2 deaths among children with RSV, both had congenital heart disease. Patients admitted with respiratory viruses other than RSV were similar in all these respects to those admitted with no virus detected. Among 24 deaths, 8 (33%; 95% CI, 16%-55%) occurred in patients admitted with a virus detected.

Thirty-six patients admitted with severe or very severe pneumonia (4.7% [95% CI, 3.3%-6.5%]) were bacteremic, with 16 having a respiratory virus detected (44% [95% CI, 28%-62%]). Bacterial species were *Streptococcus pneumoniae* (12), *Escherichia coli* (9), nontyphoidal salmonella (3), *Staphylococcus aureus* (3), *Acinetobacter* species (3), β-*hemolytic streptococci* (3), *Enterobacter* species (2), and *H influenzae* (1).

Among 57 well infants and children (median age, 6.0 months [IQR, 3.3-11]) and 96 children with symptoms of mild URTI (median age, 13 months; IQR,

5.6-25), respiratory viruses were detected in 28% and 44%, respectively, and were less frequent than among admitted pneumonia cases ($P<.001$ and $P=.03$, respectively [Table 1]). These differences were due to RSV since overall, viruses other than RSV were as common in well infants and children and those with mild URTI as among admitted patients in the case group (Table 1). Among those with mild URTI, viruses detected were RSV (18%), HAdV (8%), FLUAV (7%), HBoV (5%), HMPV (3%), HCoV-229E (3%), HPIV-1 (2%), HCoV/Hong Kong-1 (2%), and HCoV-OC43 (2%); and viruses found in well infants and children were RSV (5%), HBoV (9%), HCoV-OC43 (7%), and less than 1% for each of HPIV-1, HPIV-3, HAdV, HCoV/Hong Kong-1, and HCoV-229E. FLUAV and HMPV were not detected among well infants (eTable 2).

Detection of RSV was associated with admission with severe disease (34%) when compared with well control participants (5% [OR, 9.38; 95% CI, 2.99-47.3]) using model 1. There was no evidence of an association between viruses other than RSV and severe disease, (22% in those admitted with severe or very severe pneumonia and 23% in control participants [OR, 0.94; 95% CI, 0.48-1.94] using model 1). Models adjusted for age and calendar month (model 2) showed no evidence of lack of fit (Hosmer-Lemeshow χ^2, 7.19; $P=.52$) and did not qualitatively alter this result (OR for RSV, 6.11 [95% CI, 1.65-22.6], and non-RSV viruses [OR, 1.27; 95% CI, 0.64-2.52]). ORs in model 3, including all eligible admissions, to address the potential bias of underrepresentation of fatal cases, did not differ significantly from the observed, unadjusted ORs (model 3 for RSV only, 8.99 [95% CI, 2.87-45.2], and for non-RSV viruses 0.88 [95% CI, 0.45-1.83], when compared with model 1).

COMMENT

We found that in the catchment area of Kilifi District Hospital on the Kenyan coast, the incidence of admission with clinical syndromes of severe or very severe pneumonia ranged from 4.8% in the first year of life to 0.1% among participants aged 5 years or older. RSV was

Table 2. Incidence of Admission With Severe Pneumonia or Very Severe Pneumonia

Denominator	Per 1000 Live Births, Aged <28 d (n = 9423)	Per 100 000 Children by Age Group, y					
		All <1 (n = 8837)	1-<2	2-<5	All <5 (n = 44 538)	5-<13	All <13 (n = 104 505)
Severe or very severe pneumonia	6.65	4798	1674	543	1522	99	681
Any respiratory virus	3.79	2993	871	213	862	36	380
Respiratory synctial virus	2.46	2038	455	85	535	15	233
Human coronavirus 229E	0.51	318	135	32	105	3	46
Influenza type A	0.31	244	97	32	82	15	39
Human parainfluenza virus 3	0.10	212	48	11	57	6	26
Human adenovirus	0.10	149	77	21	55	9	26
Human metapneumovirus	0.20	138	58	11	44	6	21

Table 3. Clinical Features of 759 Children Admitted With RSV and Other Respiratory Viruses[a]

Characteristic	No Virus (n = 334)	Any Virus (n = 425)	*P* Value[b]	RSV Only (n = 206)	*P* Value[b]	RSV Plus Another Virus (n = 54)	*P* Value[b]	Non-RSV Virus (n = 165)	*P* Value[b]
Age, median (IQR), mo	11.3 (3.8-24)	7.5 (2.7-18)	<.001	6.1 (2.5-13)	<.001	7.5 (2.7-16)	.01	10 (4.5-20)	.39
Inpatient stay, median (IQR), d	4 (2-6)	3 (2-5)	.17	3 (2-5)	.07	4 (3-6)	.80	4 (2-6)	.56
Very severe pneumonia	73 (22)	53 (13)	.001	22 (11)	.001	8 (15)	.24	23 (14)	.04
Wheezing	50 (15)	59 (14)	.67	30 (15)	.90	8 (15)	.98	2 (13)	.50
Hypoxia	40 (13)	40 (9)	.13	18 (8.7)	.14	8 (15)	.70	14 (8.5)	.14
Capillary refill ≥3 seconds	16 (4.8)	10 (2.4)	.07	1 (0.5)	.004	1 (1.9)	.49	8 (4.9)	.98
Severe anemia	15 (4.6)	12 (2.9)	.23	3 (1.5)	.08	1 (1.9)	.71	8 (4.9)	.85
History of prematurity	17 (7.8)	14 (4.1)	.07	5 (2.9)	.05	2 (4.4)	.54	7 (5.7)	.49
Congenital heart disease	12 (3.6)	5 (1.2)	.05	2 (1.0)	.09	0	.23	3 (1.8)	.40
Human immunodeficiency virus	26 (8.0)	26 (6.2)	.35	8 (4.0)	.07	4 (7.6)	1	14 (8.6)	.82
Severe malnutrition	26 (7.8)	18 (4.2)	.04	5 (2.4)	.009	2 (3.7)	.40	11 (6.7)	.65
Bacteremia	20 (6.0)	16 (3.8)	.15	5 (2.4)	.06	2 (3.7)	.75	9 (5.5)	.81
Death	16 (4.8)	8 (1.9)	.02	2 (1.0)	.02	0	.14	6 (3.6)	.56

Abbreviations: IQR, interquartile range; RSV, respiratory synctytial virus.
[a] Values for characteristics are shown as No (%) unless otherwise indicated as median IQR or as *P* values.
[b] *P* values are based on comparisons with children with no virus.

detected in one-third of cases overall and in almost half of infant cases. The seasonality of severe pneumonia was almost entirely determined by RSV. No other virus was identified in more than 7% of admitted infants or children, and these participants in the case group were clinically similar to those in whom no virus was detected. Viruses other than RSV were as common among well infants and children and those with mild URTI as among those with severe disease. These findings suggest that non-RSV viruses make only a minor contribution to the burden of severe clinical pneumonia in this setting.

In a previous study in the same enumerated population of Kilifi (2002 through 2007), RSV was detected by immunofluorescence in 15% of severe pneumonia or very severe pneumonia admissions of children younger than 5 years of age, increasing to 27% during epidemic periods. Incidence from 2006 to 2007 was estimated at 0.99% and 0.27% per child/year in infants and children younger than 5 years of age, respectively.[14] In the present study, we found approximately twice the incidence. It is known that real-time PCR is more sensitive than traditional diagnostics. In a study by Kuypers et al,[21] RSV detected by both immunofluorescence and PCR had a mean viral load of 6.1×10^7 copies per mL, whereas RSV detected by PCR only had a lower mean viral load of 4.1×10^4 copies per mL. An immunofluorescence assay detected only 19% of RSV with viral loads of less than 10^6 copies per mL.

We considered whether our findings could be due to detection of persistent viral RNA or false-positive laboratory results, but believe this is unlikely. First, considering our well control participants, if an assumption is made that all of the RSV detected are false positives due to persistence or laboratory error, then our test specificity is 95% (95% CI 85% to 99%). This is similar to data reported for immunofluorescence. Such specificity would not significantly alter the interpretation of our findings among case participants. This is further supported by an OR suggesting that detection of RSV was strongly associated with severe disease. To compare these findings with those from traditional methods, we examined published data from 8 previous studies in developing countries, using immunofluorescence, serology, or viral culture.[7,8,11,22-27] Overall, RSV was detected in 746 of 3463 (21.5%) case participants and in 29 of 1203 (2.4%) control participants, giving a pooled (fixed-effects) OR for disease (unadjusted for age and season) of 11.1 (95% CI, 7.69-16.0), which is close to the unadjusted OR in our study.

High prevalence of RSV in children admitted with severe clinical pneumonia (34%), in contrast with prevalence in well control participants (5%), further supports that RSV vaccination may offer considerable public health benefit. Although the development of a vaccine for the key target age group of infants (<2 months) has focused on live virus vaccines, none has achieved requisite levels of both safety and immunogenicity.[4] A combined HPIV-3/RSV live-attenuated vaccine (Medi-534 [MedImmune LLC, Gaithersburg, Maryland]) for use in young infants (≤2 months) and in older infants and young children (6-<24 months) is currently undergoing phase 1 and 2 clinical trials.[28] Ideally, such trials should be accompanied by modeling studies of the potential consequences on effectiveness of delayed delivery to older infants[29,30] in whom vaccine safety and immunogenicity are demonstrably improved.[5,31]

Of equal interest to the high prevalence of participants in the case group with RSV detected, is the low proportion of participants in the case group with any of the other respiratory viruses. The real-time PCR assay that we used has previously been shown to be sensitive for these viruses.[32] It is possible that the use of nasal washings targets a site within the upper respiratory tract that is preferred by RSV over any other virus. However, to our knowledge, there are no definitive studies in the literature that clearly identify selective bias in the range of respiratory viruses detectable in different parts of the upper respiratory tract using molecular diagnostics.

Influenza and HPIV-3 have been the most frequently detected viruses, other than RSV, in studies using traditional diagnostics in sub-Saharan Africa. We found that HCoV-229E and HMPV were the most commonly detected non-RSV viruses in case participants. However, these viruses appear to be common in the community (control group participants) causing URTI, and some are present in well control participants. In a nonepidemic period, we estimate that as much as approximately 6% of severe disease is associated with FLUAV. There appears to be little potential to prevent severe disease through vaccination against HPIV-3.

Strengths of our study include systematic sampling of well-characterized hospitalized children from a well-established, enumerated population base, and detection of a comprehensive set of respiratory viruses.

Our study has several limitations. As in similar studies, our sampling missed most deaths because nasal washing cannot be conducted in the sickest children. However, our sensitivity analysis (model 3) suggests this is unlikely to significantly alter the main findings. We did not screen for rhinovirus and there were a lack of radiographic data. Our data reflect a single site and findings in other ecological settings may differ. We applied WHO guidelines carefully and there was good availability of clinical staff, oxygen, and drugs. Case fatality may differ in locations where resources are more constrained. Our well control participants were limited in number since we did not achieve our target sample size due to factors outside of our control. Additionally, our well control participants were a convenience sample and not matched to exactly the same calendar year. Small numbers resulted in wide CIs in some subgroups. However, power was sufficient (>99%) for our case-control analysis of RSV. It is likely that not all cases of severe pneumonia in the community were admitted to hospital, thus our incidence estimates are the minimum. There is a demonstrable decay with increased distance from the hospital in the incidences of all childhood ad-

missions and of admissions with detectable RSV.[14] A study spanning only 1 year and from a single location cannot expect to account for likely variation in the occurrence of respiratory viruses from year to year.

In summary, our study of the occurrence of respiratory viruses in children admitted with clinical syndromes of severe or very severe pneumonia to a rural district hospital in coastal Kenya has identified more than 50% of case participants with a detectable virus in whom RSV was clearly predominant. We estimate that the prevention of RSV-associated severe pneumonia might reduce all-cause clinically severe or very severe pneumonia admissions to the Kilifi District Hospital by one-third. This contrasts with no evidence to suggest a marked effect on such admissions would occur from the prevention of any other respiratory virus, with the possible exception of FLUAV. Further molecular-based studies of respiratory virus etiology of severe pneumonia over longer periods and in multiple settings in sub-Saharan Africa are needed.

Author Contributions: Dr Berkley had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Berkley, Cane, Scott, Nokes.
Acquisition of data: Berkley, Munywoki, Ngama, Kazungu, Abwao, Bett, Lassauniére, Kresfelder, Venter.
Analysis and interpretation of data: Berkley, Munywoki, Scott, Nokes.
Drafting of the manuscript: Berkley, Ngama, Kazungu.
Critical revision of the manuscript for important intellectual content: Munywoki, Abwao, Bett, Lassauniére, Kresfelder, Cane, Venter, Scott, Nokes.
Statistical analysis: Berkley, Scott.
Obtained funding: Berkley, Scott, Nokes.
Administrative, technical, or material support: Munywoki, Abwao, Bett, Lassauniére, Kresfelder, Cane.
Study supervision: Munywoki, Venter, Scott, Nokes.
Financial Disclosures: None reported.
Funding/Support: The study was funded by a project grant (081186) from the Wellcome Trust (Great Britain). Drs Berkley, Scott, and Nokes report receipt of support by fellowships from the Wellcome Trust (Great Britain).
Role of the Sponsor: The Wellcome Trust had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.
Online-Only Material: The eFigure and eTables 1 and 2 are available at http://www.jama.com.
Additional Contributions: We are grateful to the staff and patients of the pediatric wards and outpatient department of Kilifi District Hospital, and to the clinical, Information and Communication Technology, and laboratory staff of the KEMRI/Wellcome Trust Research Programme who contributed to this study. We thank Norbert Peshu, MBChB, MPH, Director of the Centre for Geographic Medicine Research—Coast, and Iqbal Khandwalla, MBChB, MMed, Medical Superintendent, Kilifi District Hospital, for their oversight and making facilities available for this research. No compensation was received for participation in this research. This article is published with permission of the director of KEMRI.

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