Is Higher Viral Load in the Upper Respiratory Tract Associated With Severe Pneumonia? Findings From the PERCH Study

Daniel R. Feikin,1,2 Wei Fu,1,2 Daniel E. Park,3 Qiyuan Shi,1 Melissa M. Higdon,1 Henry C. Baggett,5,6 W. Abdullah Brooks,18 Maria Deloria Knoll,1 Laura L. Hammitt,3,4 Stephen R. C. Howie,1,10,11 Karen L. Kotloff,1,10 Orin S. Levine,1,3,4 Shabir A. Madhi,1,15 J. Anthony G. Scott,10 Donald M. Thea,1
Peter V. Adimé,5 Martin Antonio,10,11,12 Juliet D. Awoori,13 Vicky L. Baillie,15,16 Andrea N. DeLuca,18 Amanda J. Driscoll,1 Bernard E. Ebruke,1 Doli Goswami,1 Ruth A. Karron,1 Mengying Li,12 Susan C. Morphet,16,17 John Mwaba,16,20 James Mwansa,20,21 Christine Prosperi,1 Pongpun Sawatwong,5 Samba O. Sow,27 Milagritos D. Tapia,1 Toni Whistler,1,2 Khalequ Zaman,1 Scott L. Zeger,18 Katherine L. O’Brien,1 and David R. Murdoch22,24, for the PERCH Study Group

1Department of International Health, International Vaccine Access Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; 2Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; 3Department of Rheumatology, Johns Hopkins School of Medicine, Baltimore, Maryland; 4Milken Institute School of Public Health, Department of Epidemiology and Biostatistics, George Washington University, District of Columbia; 5Global Disease Detection Center, Thailand Ministry of Public Health–US Centers for Disease Control and Prevention Collaboration, Nonthaburi; 6Counties Manukau District Health Board, Auckland, New Zealand; 7Study Group Health, Baltimore, Maryland; and 8Center for Vaccine Development, Institute of Global Health, University of Maryland School of Medicine, Baltimore.

DOI: 10.1093/cid/cix148

© The Author 2017. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. The etiologic inference of identifying a pathogen in the upper respiratory tract (URT) of children with pneumonia is unclear. To determine if viral load could provide evidence of causality of pneumonia, we compared viral load in the URT of children with severe pneumonia to viral load in children without pneumonia.

Methods. In the 9 developing country sites, nasopharyngeal/oropharyngeal swabs from children with and without pneumonia were tested using quantitative real-time polymerase chain reaction for 17 viruses. The association of viral load with case status was evaluated using logistic regression. Receiver operating characteristic (ROC) curves were constructed to determine optimal discriminatory viral load cutoffs. Viral load density distributions were plotted.

Results. The mean viral load was higher in cases than controls for 7 viruses. However, there was substantial overlap in viral load of different viruses in URT specimens. The etiologic inference of identifying a pathogen in the URT can better distinguish its causative role in pneumonia from their logistical ease of collection [1, 2]. However, detection of viruses in URT specimens has low specificity as this finding might simply reflect an URT infection without lower respiratory tract involvement or coincidental asymptomatic or past infection [2–4].

A possible solution to the lack of specificity of simply detecting the presence or absence of a virus in the URT of pneumonia patients is to determine whether the density of a virus in the URT can better distinguish its causative role in pneumonia. There are reports that a higher pathogen load in the URT is associated with pneumonia, for both Streptococcus pneumoniae
and some respiratory viruses [5–8]. In addition, for some viruses, higher viral load in the URT has been associated with worse outcomes [7, 9–11].

In this analysis, we describe viral load in nasopharyngeal/oropharyngeal (NP/OP) specimens from cases and community controls from a large multicountry childhood pneumonia study (Pneumonia Etiology Research for Child Health [PERCH]), as well as demographic and clinical characteristics associated with higher viral load and disease severity. An overarching aim was to explore whether the incorporation of viral load data into the main PERCH etiology analysis might improve the assignment of the etiology of pneumonia cases.

METHODS

The PERCH study design and enrollment strategy has been previously described [12]. In brief, PERCH is a case-control study of the etiology of World Health Organization (WHO)–defined severe and very severe pneumonia among hospitalized children aged 1–59 months and age frequency-matched community controls. Enrollment took place during August 2011–January 2014 for 24 months at each of 9 study sites located in 7 countries—Dhaka and Matlab, Bangladesh; Basse, The Gambia; Kilifi, Kenya; Bamako, Mali; Soweto, South Africa; Nakhon Phanom and Sa Kaeo, Thailand; and Lusaka, Zambia [13].

Case and Control Definitions

For this analysis, we included only cases with evidence of pneumonia on chest radiograph, defined as consolidation and/or any other infiltrate assessed according to the WHO radiological pneumonia criteria [14]. A control participant was considered to have a respiratory tract illness (RTI) if cough or runny nose was reported. RTI was also considered present if a child had (1) at least 1 of ear discharge, wheezing, or difficulty breathing and (2) either a measured temperature of ≥38.0°C within the previous 48 hours or a history of sore throat. Controls who did not meet the definition of RTI are referred to as non-RTI controls.

Specimen Collection and Laboratory Testing

Nasopharyngeal and oropharyngeal swabs were collected from PERCH cases and controls at enrollment. Nasopharyngeal specimens were collected by inserting flocked swabs (Copan ETC) into the posterior nasopharynx and rotating 180° for 2–3 seconds. Oropharyngeal specimens were then collected by rubbing Rayon swabs (Fisher Scientific) over both tonsillar pillars and the posterior oropharynx for several seconds. Following collection, swabs were placed together in the same 3-mL vial of universal transport media (Copan) and processed within 24 hours of collection. Specimens were left at room temperature for no more than 2 hours or at 4°C for no more than 24 hours, before freezing at –70°C.

All specimens were tested in-country using a standardized methodology, and details are described elsewhere [15]. Specimens were evaluated using the Fast-track Diagnostics Respiratory Pathogens 33 test (FTD Resp 33, Fast-track Diagnostics, Sliema, Malta), a 33-target, 8-multiplex real-time polymerase chain reaction (PCR) platform for the detection of viruses, bacteria, and fungi. The 18 viruses or virus classes included influenza A, B, and C viruses; parainfluenza virus (PIV) types 1, 2, 3, and 4; coronaviruses NL63, 229E, OC43, and HKU1; human metapneumovirus (HMPV) A and B (A and B not differentiated); rhinovirus; respiratory syncytial virus (RSV) A and B (A and B not differentiated); adenovirus; enterovirus and parechovirus (not differentiated); human bocavirus (HBOV); and cytomegalovirus. Cytomegalovirus is not included in this analysis but is discussed in a separate publication of the pathogen load of pathogens commonly detected in both cases and controls [16].

Some sites (Bangladesh, The Gambia, Mali, South Africa) collected lung aspirates from children with consolidation on chest radiograph who met clinical and radiologic criteria for the

| Characteristic | CXR+Cases* (n = 1733) | All Controls (n = 4986) | χ² PValueb |
|---------------|------------------------|------------------------|------------|
| Site          |                        |                        |            |
| Kenya         | 282 (16.3)             | 855 (17.2)             | <.001      |
| The Gambia    | 273 (15.8)             | 624 (12.9)             |            |
| Mali          | 239 (13.8)             | 724 (14.5)             |            |
| Zambia        | 189 (10.9)             | 556 (10.7)             |            |
| South Africa  | 433 (25.0)             | 823 (16.5)             |            |
| Thailand      | 98 (5.7)               | 657 (13.2)             |            |
| Bangladesh    | 219 (12.6)             | 768 (15.4)             |            |
| Age           |                        |                        |            |
| 1–5 mo        | 680 (39.2)             | 1556 (31.2)            | <.001      |
| 6–11 mo       | 415 (24.0)             | 1187 (23.8)            |            |
| 12–23 mo      | 424 (24.5)             | 1235 (24.8)            |            |
| 24–59 mo      | 214 (12.4)             | 1009 (20.2)            |            |
| Female sex    | 756 (43.6)             | 2477 (49.7)            | <.001      |
| Prior antibiotic usec | 597 (42.4) | 84 (1.7) | <.001 |
| Respiratory tract illnessd | NA | 1185 (23.8) | NA |
| No. of viruses detected | | | |
| 0 viruses     | 180 (10.4)             | 1048 (21.0)            |            |
| 1 virus       | 628 (36.2)             | 1928 (38.7)            | <.001      |
| 2 viruses     | 616 (35.6)             | 1420 (28.5)            |            |
| ≥3 viruses    | 309 (17.8)             | 590 (11.8)             |            |

Data are presented as No. (%), unless otherwise indicated.

Abbreviations: CXR+ chest radiograph positive; NA, not applicable.

⁎CXR+ defined as having radiographic evidence of pneumonia.

Combining distribution of characteristics between CXR+ cases and controls. Bolded values are significant (P < .05).

Prior antibiotic use: administered antibiotics at the study facility prior to the collection of specimens (cases only), antibiotics at a referral facility (cases only), or positive serum bioassay (cases and controls).

See Methods for respiratory tract illness definition.
procedure [17]. Lung aspirate specimens were tested for viral targets using the same method described for NP/OP specimens.

### Statistical Analysis

Human immunodeficiency virus (HIV)–positive cases were excluded from analyses unless stated otherwise. PCR quantification was log\(_{10}\)-transformed. Demographic characteristics of cases and controls were compared using the \(\chi^2\) test. All controls, both RTI and non-RTI, were included in the main analysis. All analyses of viral load were restricted to children positive for each virus. Among children positive for each virus, \(t\) tests adjusted for site and age were performed to compare mean cycle threshold (Ct) values between cases and controls. For each virus, a trend analysis, using simple linear regression, was performed to test if viral density increased with age for cases and for controls. Among cases only, mean Ct values were also compared by days since onset, severity, vital status, and HIV status. Multivariable logistic regression, adjusting for age and site, was performed to compare odds of being a case for each 3.4-unit drop in Ct value, which was approximately equivalent to a 1 log\(_{10}\) increase in viral copies/mL; Ct values instead of viral density were used for regression because viral density was only accurate within the linear range of the assay (10\(^5\)–10\(^8\) copies/mL). Kernel density distribution plots were created to show distributions of viral density by case/control status. Receiver operating characteristic (ROC) curves and the corresponding area under the curve (AUC) were generated to investigate the performance of viral load in determining case status among children positive by NP/OP PCR for each virus, and the Youden index was calculated to determine the optimized diagnostic cutoffs to differentiate cases and controls [18]. To guard against bias in the estimates of sensitivity due to having a small number of some viruses detected among cases, the Youden index was calculated using leave-one-out cross-validation where sample size was sufficient [19]. Redefining positivity using the optimal cutpoints, we calculated odds ratios associated with case status for children above vs below the optimal cutpoint including negatives. The proportion of radiographically confirmed cases attributable to each virus [population attributable fraction; population prevalence \(\times (1 – 1 / OR)\)] was calculated using 2 methods: (1) any positive vs negative and (2) positive above vs below the optimal cutpoint, the former method being optimal for laboratory sensitivity and the latter for a balance of epidemiological sensitivity and specificity. All analyses were performed using SAS software version 9.4 (SAS Institute, Cary North Carolina) and R Statistical Software 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). All \(P\) values are 2 sided.

### Table 2. Mean Nasopharyngeal/Oropharyngeal Polymerase Chain Reaction Cycle Threshold Values for Chest Radiograph–Positive Cases and Controls and Odds Ratios for Viral Load Being Predictive of Case Status—Pneumonia Etiology Research for Child Health (PERCH) Study, August 2011–January 2014

| Virus | CXR+ Cases (n = 1733) | All Controls (n = 4986) | Poly | All Controls (n = 4986) |
|-------|----------------------|------------------------|------|------------------------|
| No. (%) | Ct Value Mean (95% CI) | No. (%) | Ct Value Mean (95% CI) | OR per 1 Log\(_{10}\) Increase, Copies/mL (95% CI) |
| Adenovirus | 164 (9.5) | 277 (26.8–28.5) | 594 (11.9) | 29.5 (29.2–29.8) | <.001 | 1.27 (1.10–1.46) |
| Coronavirus 229 | 18 (1.1) | 31.1 (28.6–33.5) | 54 (1.1) | 30.2 (28.3–32.0) | .58 | 0.89 (0.62–1.26) |
| Coronavirus 43 | 38 (2.2) | 26.4 (24.4–28.5) | 192 (3.9) | 28.0 (27.1–28.8) | .30 | 1.13 (0.91–1.39) |
| Coronavirus 63 | 36 (2.1) | 27.0 (25.3–28.7) | 158 (3.2) | 28.5 (27.9–29.3) | .26 | 1.18 (0.90–1.55) |
| Coronavirus HKU | 37 (2.2) | 29.2 (27.0–31.4) | 111 (2.2) | 27.7 (26.5–28.9) | .40 | 0.91 (0.74–1.13) |
| Influenza A | 62 (3.6) | 28.5 (27.9–29.4) | 57 (1.2) | 29.8 (28.4–31.2) | .31 | 1.21 (0.85–1.72) |
| Influenza B | 18 (1.1) | 27.6 (25.7–29.5) | 29 (0.6) | 28.5 (26.7–30.3) | .82 | 1.07 (0.63–1.83) |
| Influenza C | 10 (0.6) | 28.1 (24.8–31.4) | 29 (0.6) | 27.3 (25.3–29.3) | .14 | 0.44 (0.17–1.15) |
| HBoV | 231 (13.4) | 30.5 (29.6–31.3) | 660 (13.3) | 31.7 (31.3–32.1) | .007 | 1.13 (1.03–1.24) |
| HMPV A/B | 185 (10.8) | 28.1 (21.6–28.7) | 206 (4.1) | 28.9 (28.2–29.5) | .02 | 1.23 (1.03–1.46) |
| Parainfluenza 1 | 89 (5.2) | 26.1 (24.9–27.2) | 49 (1.0) | 29.4 (27.6–31.2) | .008 | 1.37 (1.08–1.74) |
| Parainfluenza 2 | 23 (1.3) | 34.0 (31.7–36.3) | 53 (1.1) | 35.1 (33.9–36.3) | .70 | 1.10 (0.71–1.69) |
| Parainfluenza 3 | 104 (6.1) | 25.0 (24.0–25.9) | 142 (2.9) | 29.0 (28.0–30.0) | <.001 | 1.47 (1.22–1.77) |
| Parainfluenza 4 | 44 (2.6) | 31.7 (30.3–33.1) | 86 (1.7) | 32.2 (31.3–33.1) | .88 | 0.98 (0.73–1.31) |
| PV/EV | 131 (7.6) | 30.1 (29.5–30.8) | 423 (8.5) | 30.4 (30.0–30.7) | .45 | 1.08 (0.89–1.31) |
| Rhinovirus | 365 (21.2) | 31.7 (31.3–32.0) | 1056 (21.2) | 32.4 (32.3–32.6) | .003 | 1.21 (1.08–1.35) |
| RSV | 461 (26.8) | 22.2 (21.8–22.5) | 140 (2.8) | 27.0 (26.1–28.0) | <.001 | 2.02 (1.71–2.37) |

Abbreviations: CI, confidence interval; Ct, cycle threshold; CXR+, chest radiograph positive; HBOV, human bocavirus; HMPV, human metapneumovirus; OR, odds ratio; PV/EV, parechovirus/enterovirus; RSV, respiratory syncytial virus.

*Coronavirus defined as having radiographic evidence of pneumonia.

No. (%) positive in the nasopharynx/oropharynx among those with available results for the given virus.

Among those with a positive density.

Comparing mean cycle threshold value of CXR+ cases vs all controls using linear regression adjusting for age and site. Bolded values are significant \((P < .05)\).

#CID 2017:64 (Suppl 3) #S339

Viral Load and Severe Pneumonia in PERCH
Ethical Considerations

The PERCH study protocol was approved by the institutional review board or ethical review committee at each of the study site institutions and at the Johns Hopkins Bloomberg School of Public Health. Parents or guardians of all participants provided written informed consent.

RESULTS

Of 1935 radiographically confirmed cases and 5325 controls in PERCH, 1733 cases (1227 severe, 506 very severe) and 4986 controls (1185 RTI and 3801 non-RTI) were not known to be HIV infected and had viral density results available for analysis (Table 1).

Overall, 89.6% of cases and 79.0% of controls had at least 1 virus detected, and 53.4% and 40.3%, respectively, had ≥2 viruses detected (Table 1). Among the 17 viruses tested, RSV was the most commonly detected among cases (27%) but was uncommon among controls (3%) (Table 2). Rhinovirus was the next most commonly detected virus in cases but was present at a similar frequency among controls (21% for both).

Analysis of Viral Load Among Cases and Controls

RSV had the highest mean viral load among cases (7.3 log copies/mL; Figure 1); no viruses other than RSV had a mean viral load >6 log copies/mL. Among controls, no viruses had a mean viral load >6 log copies/mL. Eight viruses among cases (RSV, influenza C, PIV1, PIV3, PIV4, coronavirus 43, coronavirus 63, and HMPV) had mean viral loads >5 log copies/mL vs 5 viruses among controls (RSV, influenza C, PIV4, coronavirus 43, and coronavirus 63). There were 7 viruses that had significantly higher mean viral density among cases after adjusting for site and age—adenovirus, HBOV, HMPV, PIV1, PIV3, rhinovirus, and RSV. After adjusting for age and site, there was a significant increase in the odds of being a case (vs a control) for each 3.4-unit drop in Ct value (approximately 1 log increase in copies/mL) for the same 7 viruses, ranging from a 13% increased odds for HBOV to a 102% increased odds for RSV (Table 2).

Viral load was similar between RTI and non-RTI controls for most viruses with the exception of RSV, where the mean viral load was significantly higher for RTI controls (Supplementary Figure 1).

Despite the differences in viral load between cases and controls noted above, there was substantial overlap in the viral density distribution between cases and controls in which virus was detected, as shown in the box plots and kernel density distribution plots (Figures 1 and 2). Kernel density distribution plots were examined for a bimodal distribution with a smaller subset...
Figure 2. Kernel density distribution plots comparing nasopharyngeal/oropharyngeal (NP/OP) viral load among chest radiograph–positive (CXR+) cases and all controls for each viral polymerase chain reaction (PCR) target—Pneumonia Etiology Research for Child Health (PERCH) study, August 2011–January 2014. Tick marks across the top of each plot indicate viral load of each individual (first row of black ticks for cases and second row of gray ticks for controls). Dashed curves indicate areas outside the linear range of the assay for calculation of viral load from cycle threshold values. Dotted dashed vertical lines indicate optimal cutpoint distinguishing cases and controls calculated using Youden index. Black arrows in adenovirus and human metapneumovirus plots indicate NP/OP viral load of cases whose lung aspirate specimen was available and PCR positive for that virus. Abbreviations: Adeno, adenovirus; Boca, human bocavirus; CXR, chest radiograph; Flu, influenza virus; HCoV, human coronavirus; HMPV, human metapneumovirus; Para, parainfluenza virus; PV/EV, parechovirus/enterovirus; Rhino, rhinovirus; RSV, respiratory syncytial virus.
of cases having viruses at a higher viral load that might be indicative of those with pneumonia due to that virus. Adenovirus, coronavirus 229, and PIV1–3 had a suggestion of a bimodal distribution among cases. The NP/OP viral load for the 2 cases with viruses detected in PCR of lung aspirates (ie, HMPV and adenovirus) fell within the distribution of viral loads for other cases, as well as controls, positive for that virus (Figure 2).

When constructing ROC curves, no virus had an AUC >0.8 (Table 3). RSV had the highest AUC at 0.76, with only 3 other viruses having an AUC between 0.6 and 0.7 (influenza A, PIV1, and PIV3). Despite the low values for the AUC, when redefining positive for a virus as those with viral loads above the ROC optimal cutpoint value as determined by the Youden index, the odds ratio for predicting case status increased substantially for some viruses, approximately doubling for adenovirus, coronavirus 63, PIV2, and RSV (Table 4). Although the odds ratios increased, the population attributable fraction for most viruses did not change substantially, or even decreased (eg, influenza A, RSV) due to the lower frequency of cases with densities above the optimal cutpoint (Figure 3). This is because while the odds ratios are higher at the higher density cutoff, the prevalence of cases above the higher threshold was lower, and thus the proportion of PERCH cases assigned to the virus would not change appreciably by using the higher cutoff.

### Table 3. Receiver Operating Characteristic Areas Under the Curve, Optimal Nasopharyngeal/Oropharyngeal Polymerase Chain Reaction Density Cutpoints for Determining Case Status, and Associated Positive Rate in Cases and Negative Rate in Controls by Virus Among Chest Radiograph–Positive Cases and Controls With Positive Densities—Pneumonia Etiology Research for Child Health (PERCH) Study, August 2011–January 2014

| Virus | AUC | Optimal Cutpoint, \( \log_{10} \) Copies/mL | Proportion of \( \text{CXR}^+ \) Cases Above Cutpoint | Proportion of Controls Below Cutpoint |
|-------|-----|---------------------------------|---------------------------------|---------------------------------|
| Adenovirus | 0.60 | 4.88 | 0.44 | 0.78 |
| Coronavirus 43 | 0.57 | 6.94 | 0.36 | 0.74 |
| Coronavirus 63 | 0.58 | 7.24 | 0.22 | 0.89 |
| Influenza A | 0.61 | 5.12 | 0.50 | 0.68 |
| Influenza B | 0.55 | 3.79 | 0.89 | 0.28 |
| HBOV | 0.54 | 5.81 | 0.20 | 0.89 |
| HMPV A/B | 0.54 | 3.9 | 0.91 | 0.20 |
| Parainfluenza 1 | 0.65 | 4.62 | 0.75 | 0.55 |
| Parainfluenza 2 | 0.54 | 5.64 | 0.26 | 0.91 |
| Parainfluenza 3 | 0.69 | 4.75 | 0.81 | 0.54 |
| PV/EV | 0.53 | 4.38 | 0.40 | 0.71 |
| Rhinovirus | 0.56 | 3.64 | 0.55 | 0.56 |
| RSV | 0.78 | 6.30 | 0.84 | 0.59 |

Abbreviations: AUC, area under the curve; \( \text{CXR}^+ \), chest radiograph positive; HBOV, human bocavirus; HMPV, human metapneumovirus; PV/EV, parechovirus/enterovirus; RSV, respiratory syncytial virus.

*Viruses with adjusted odds ratios <1 (see Table 2) were excluded from table (coronavirus 229, coronavirus HKU1, influenza C, and parainfluenza 4).

*Calculated using Youden index and, where possible, leave-one-out cross-validation. Leave-one-out cross-validation was not performed for influenza B, parainfluenza 2, or parechovirus/enterovirus.

*\( \text{CXR}^+ \) defined as having radiographic evidence of pneumonia.

### DISCUSSION

In the PERCH study, the evidence for the utility of NP/OP viral load in distinguishing radiographically confirmed cases of severe or very severe pneumonia from controls was mixed. On the one hand, we found a higher mean viral load in NP/OP samples from severe and very severe pneumonia cases than from community controls without pneumonia for several respiratory viruses. Moreover, for many viruses, using a higher viral load threshold to define positivity that maximized the combination of sensitivity and specificity increased the odds ratio for case status over a simple binary (presence/absence) definition of positivity based on viral detection, which has high sensitivity but low specificity. On the other hand, there was substantial overlap in the distribution of NP/OP viral load densities among cases and controls, even for RSV, the virus most strongly associated with case status. No cutoffs clearly distinguished cases from controls.

**Predictors of Viral Load**

We explored several potential predictors of viral density among cases. When NP/OP specimens were collected earlier in the course of illness, mean viral load was higher for RSV, PIV1, and PIV3 and was lower for adenovirus; no significant difference was observed for the other viruses (Supplementary Table 1). The viral load among cases did not vary by age for most viruses, including RSV, but a significant trend toward decreasing viral load with increasing age was observed for a few viruses, including adenovirus and RSV, which was also observed among controls (Supplementary Figure 2). A slight, but significant trend toward increasing viral load with increasing age was seen for rhinovirus among cases, but a significant trend in the opposite direction was observed for controls. In general, viral load did not vary by study site. One notable exception was higher PIV1 viral load in The Gambia site (the only site with a PIV1 outbreak, data not shown). HIV-infected cases had a higher mean viral load for coronavirus 43 and a lower viral load for HMPV, PIV3, and RSV (Supplementary Table 2). There were no significant differences in the viral load between cases who were normally nourished vs malnourished (except PIV1 viral load was higher in normally nourished, data not shown).

We assessed whether viral load was associated with pneumonia severity. Rhinovirus was the only virus with higher mean viral load for very severe pneumonia compared to severe pneumonia (Supplementary Figure 3). Influenza A was the only virus with higher mean viral load in fatal compared with surviving cases (Supplementary Figure 4). Furthermore, we compared mean viral densities between cases with evidence of an other infiltrate (without alveolar consolidation) on chest radiograph to cases with evidence of alveolar consolidation (without evidence of an other infiltrate). No significant differences were found for any virus after adjusting for site and age.
Previous studies have also shown that the median or mean RSV concentration of NP/OP specimens among children is higher in cases of severe illness than among a healthy or mildly ill control population [7–10, 20, 21]. However, few of these studies compared the distribution of viral loads between severe cases and controls. Those that did compare these 2 groups showed an overlapping distribution similar to our study [8, 22]. Some studies of RSV viral load failed to show an association with severe lower respiratory tract infection [23, 24], but these studies included older children and adolescents in whom the
pathogenic significance of detecting RSV in the NP/OP is less clear. One study of RSV viral load in infants showed no association with severe bronchiolitis [25] while another suggested that viral load only influences clinical severity for first RSV infections in young infants [10].

In some studies, influenza viral load was associated with severe disease [4, 26–29], while in others there was no association [8, 23, 27, 30–32]. A few studies have shown a higher viral load in severe cases for HMPV [24, 33, 34]. Higher viral loads of HBoV in nasopharyngeal aspirates were associated with greater severity of illness among Chinese children [35]. Rhinovirus viral load has been associated with more severe illness [22], but not in some studies [8, 21]. Again, the majority of these studies looked only at the central tendency of the viral load and did not demonstrate a clear dichotomy in the distribution of viral loads based on case status or severity category.

We did not find a higher viral load associated with greater severity among pneumonia cases for most viruses. Cases who died had a similar viral load as those who survived, and those with very severe pneumonia had similar viral loads to those children with severe pneumonia. This is in contrast with some other studies of viruses in which higher viral load was observed among RSV-infected children requiring mechanical ventilation [9], and severe acute respiratory syndrome coronavirus– and Middle East respiratory syndrome coronavirus–infected adults who died [36, 37].

We undertook this analysis, in part, to determine if viral loads of NP/OP specimens could be incorporated into the PERCH analysis to identify etiologies of severe/very severe pneumonia. Using a higher density threshold also did not have an appreciable effect on the population attributable fraction for most viruses, suggesting that using higher thresholds to assign viral etiology to cases would likely have little impact on the analysis of the etiologic distribution among the population of PERCH cases [38]. In the final PERCH analyses, we will be able to run sensitivity analyses to assess the impact of incorporating viral density thresholds on the assessment of etiology.

In contrast to the accompanying analyses of bacterial pneumonia, our conclusions about the interpretation of viral load and whether to include it in the main PERCH etiology analysis were limited by the lack of a gold standard to diagnose viral pneumonia [5, 16]. There were few PERCH cases who underwent lung aspirate procedures and even fewer who had a lung aspirate in which a virus was detected in their lungs. Among a population of pneumonia cases in which a virus was detected in the NP/OP, there was likely a mixture of those in whom the virus had a causal role in pneumonia and those in whom it did not. The inability to identify which children had pneumonia due to which virus hampered the study's ability to determine if higher viral loads in the NP/OP were associated with pneumonia.

Besides the lack of a gold standard, other limitations might have affected our results. First, specimens were taken at one point in time on admission to the hospital. We observed that viral load varied with the time since illness onset, with higher viral load earlier in the course of symptomatic illness for some viruses. Taking sequential samples in which we could compare the peak viral load between cases of different clinical severity would have been optimal. Second, our design precluded us from assessing the role of viral load in the lung. Upper respiratory tract viral load might reflect the amount of replication in the local epithelial cells rather than the viral burden in the lung parenchyma. Evaluation of viral load of specimens from the lung, either through lung aspirates or bronchoalveolar lavage, would provide more direct evidence of the role of viral load in pneumonia severity.

The widespread use of sensitive PCR assays for testing NP/OP specimens has led to a higher reported prevalence of pneumonias attributed to respiratory viruses in both adults and children [2]. Due to the high prevalence of viral infections of the URT itself, however, it is difficult to conclude that detection of a virus in the URT of a pneumonia patient is equivalent to having pneumonia due to that virus. In the PERCH study, the viral loads in the NP/OP of pneumonia patients are unlikely to further clarify the role of that virus in causing pneumonia. However, the PERCH study design was not optimal to answer this question definitively. Further research, such as longitudinal studies and animal models, is needed to better elucidate the interpretation of viral load in the diagnosis and clinical management of viral pneumonia.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes
Author contributions. D. R. F. led the analysis, interpreted results, and drafted initial manuscript. W. F. and Q. S. performed the analysis. W. F., Q. S., D. E. P., M. M. H., and D. R. M. provided significant guidance on the development of the manuscript. O. S. L., K. L. O., D. R. F., L. L. H., R. A. K., D. R. M., M. D. K., H. C. B., W. A. B., S. R. C. H., K. L. K., S. A. M., J. A. G. S., D. M. T. conceived and designed the study and supervised study conduct. W. F., D. E. P., Q. S., M. M. H., P. V. A., M. A., J. O. A., V. L. B., A. N. D., A. J. D., B. E. E., D. G., M. L., S. C. M., J. M., J. M., C. P., P. S., S. O. S., M. D. T., T. W., and K. Z. were involved in study conduct. S. L. Z. provided statistical expertise and led the integrated etiology analysis. All authors reviewed and approved the manuscript. D. R. F. had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Acknowledgments. We acknowledge the significant contributions of the PERCH Study Group and all PERCH investigators. We offer our gratitude to the members of the Pneumonia Methods Working Group, PERCH Expert Group, and PERCH Chest Radiograph Reading Panel for their time and lending expertise to assist the PERCH Study Group. See Supplementary Materials for a full list of names. We offer sincere thanks to the patients and families who participated in this study.

S344 • CID 2017:64 (Suppl 3) • Feikin et al
PERCH Study Group. Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland. Katherine L. O’Brien (principal investigator [PI]), Orin S. Levine (former PI, current affiliation Bill & Melinda Gates Foundation, Seattle, Washington), Maria Deloria Knoll (co-PI), Daniel R. Feikin (joint affiliation with Centers for Disease Control and Prevention [CDC], Atlanta, Georgia), Andrea N. DeLuca, Amanda J. Driscoll, Nicholas Fancourt, Wei Fu, Laura L. Hammitt, Melissa M. Higdon, E. Wangeci Kagucia, Ruth A. Karron, Mengying Li, Daniel E. Park, Christine Prosperi, Zhenke Wu, Scott L. Zeger; Emmes Corporation, Rockville, Maryland: Nora L. Watson; Nuffield Department of Clinical Medicine, University of Oxford, United Kingdom: Jane Crawley; University of Otago, Christchurch, New Zealand: David R. Murdoch; icdrib, Dhaka and Matlab, Bangladesh: W. Abdullah Brooks (site PI), Hubert P Endtz, Khaled Zaman, Doli Goswami, Lokman Hossain, Yasin Hamid, Hasan Ashraf; Medical Research Council, Basse, The Gambia: Stephen R. C. Howie (site PI), Bernard E. Ebruke, Martin Antonio, Jessica McClean, Eunice Machuka, Ariffin Shams; Syed M. A. Zaman, Grant Mackenzie; KEMRI–Wellcome Trust Research Programme, Kihii, Kenya: J. Anthony G. Scott (site PI and PERCH co-PI), Juliet O. Awori, Susan C. Morpeth, Alice Kamau, Sidi Kazungu, Micah Silaba Omide; Division of Infectious Disease and Tropical Pediatrics, Department of Pediatrics, Center for Vaccine Development, Institute of Global Health, University of Minnesota School of Medicine, Baltimore, Maryland and Centre pour le Développement des Vaccins (CVD-Mali), Bamako, Mali: Karen L. Koff (site PI), Milagritos D. Tapia, Samba O. Sow, Mamadou Sylla, Boubou Tamboura, Uma Owuchekwu, Nana Kourouma, Alou Toure; Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa: Shabir A. Madhi (site PI), David P. Moore, Peter V. Adrian, Vicky L. Baillie, Locadiah Kuvanda, Azwifwari Mudau, Michelle J. Groome, Nasreen Hamed; Thailand Ministry of Public Health–US CDC Collaboration, Nonthaburi, Thailand: Henry C. Baggett (site PI), Somsak Thamthitiwat, Susan A. Maloney (former site PI), Charadathu Bunthi, Julia Rhodes, Pongpunt Sawatwong, Pasakorn Akarasewi (site co-PI, Ministry of Public Health); Boston University School of Public Health, Boston, Massachusetts and University Teaching Hospital, Lusaka, Zambia: Donald M. Thea (site PI), Lawrence Mwananyanda, James Chipeta, Phil Seidenberg, James Mwansa, Somwe wa Somwe, Geoffrey Kwenda. Canterbury Health Laboratory, Christchurch, New Zealand: Trevor P. Anderson, Joanne Mitchell.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, Department of Health and Human Services, or the US government. This article is published with the permission of the Director of the Kenya Medical Research Institute.

Financial support. PERCH was supported by the Bill & Melinda Gates Foundation (grant number 58968 to the International Vaccine Access Center, Department of International Health, Johns Hopkins Bloomberg School of Public Health). J. A. G. S. was supported by a clinical fellowship from the Wellcome Trust of Great Britain (award number 098532).

Supplement sponsorship. This article appears as part of the supplement “Pneumonia Etiology Research for Child Health (PERCH): Foundational Basis for the Primary Etiology Results,” sponsored by a grant from the Bill & Melinda Gates Foundation to the PERCH study of Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

Potential conflicts of interest. M. D. K. has received funding for consultancies from Merck, Pfizer, and Novartis, and grant funding from Merck. L. L. H. has received grant funding from Pfizer and GlaxoSmithKline. K. L. K. has received funding from Merck Sharp & Dohme. S. A. M. has received honoraria for advisory board membership from the Bill & Melinda Gates Foundation, Pfizer, Medimmune, and Novartis; has received institutional grants from GSK, Novartis, Pfizer, Minervax, and the Bill & Melinda Gates Foundation; and has served on speaker’s bureaus for Sanofi Pasteur and GSK. K. L. O. has received grant funding from Pfizer and GlaxoSmithKline and has served on technical advisory boards for Merck, Sanofi Pasteur, PATH, Affinivax, and ClearPath. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Hammitt LL, Murdoch DR, Scott JA, et al. Pneumonia Methods Working Group. Specimen collection for the diagnosis of pediatric pneumonia. Clin Infect Dis 2012; 54(suppl 2):S132–9.

2. Rusakanok O, Labht E, Jennings LC, Murdoch DR. Viral pneumonia. Lancet 2011; 377:1264–75.

3. Murdoch DR, O’Brien KL, Scott JA, et al. Breathing new life into pneumonia diagnostics. J Clin Microbiol 2009; 47:3405–8.

4. Jansen RR, Wierenga J, Roekkock SM, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. J Clin Microbiol 2011; 49:2631–6.

5. Baggett HC, Watson NL, Deloria Knoll M, et al. Density of upper respiratory colonization with Streptococcus pneumoniae and its role in the diagnosis of pneumococcal pneumonia among children aged <5 years in the PERCH study. Clin Infect Dis 2017; 64(suppl 3):S317–27.

6. Albrich WC, Madhi SA, Adrian PV et al. Use of a rapid test of pneumococcal colonization density to diagnose pneumococcal pneumonia. Clin Infect Dis 2012; 54:601–9.

7. Fuller JA, Njenga MK, Biggo G, et al. Association of the CT values of real-time PCR of viral upper respiratory tract infection with clinical severity. Kenya J Med Virol 2013; 85:924–32.

8. Uotikarp X, Marchant D, Gosselin J, et al. The relationship between respiratory viral loads and diagnosis in children presenting to a pediatric hospital emergency department. Pediatr Infect Dis J 2011; 30:218–23.

9. Buckingham SC, Bush AJ, Devincenzo JP. Nasal quantity of respiratory syncytial virus correlates with disease severity in hospitalized infants. Pediatr Infect Dis J 2000; 19:113–7.

10. Devincenzo JP, El Saleby CM, Bush AJ. Respiratory syncytial virus load predicts disease severity in previously healthy infants. J Infect Dis 2005; 191:1861–8.

11. Devincenzo JP, Wilkinson T, Vasishaw A, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. Am J Respir Crit Care Med 2010; 182:1305–14.

12. Deloria-Knoll M, Feikin DR, Scott JA, et al; Pneumonia Methods Working Group. Identification and selection of cases and controls in the pneumonia etiology research for child health project. Clin Infect Dis 2012; 54(suppl 2):S117–23.

13. Scott JA, Wonodi C, Moisi JC, et al; Pneumonia Methods Working Group. The definition of pneumonia, the assessment of severity, and clinical standardization in the Pneumonia Etiology Research for Child Health study. Clin Infect Dis 2012; 54(suppl 2):S109–16.

14. Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. Bull World Health Organ 2005; 83:353–9.

15. Driscoll AJ, Karron RA, Morpeth SC, et al. Standardization of laboratory methods for the pneumonia etiology research for child health study. Clin Infect Dis 2017; 54(suppl 3):S245–52.

16. Park DE, Baggett HC, Howie SRC, et al. Colonization density of the upper respiratory tract as a predictor of pneumonia—Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, and Pneumocystis jirovecii. Clin Infect Dis 2017; 64(suppl 3):S328–36.

17. Falade AG, Mulholland EK, Adegbola RA, Greenwood BM. Bacterial isolates from blood and lung aspirate cultures in Gambian children with lobar pneumonia. Ann Trop Paediatr 1997; 17:315–39.

18. Youden WI. Index for rating diagnostic tests. Cancer 1950; 3:322–5.

19. Leelathom MG, Moons KGM, Reitsma JB, Zwinderman AH. Bias in sensitivity and specificity caused by data-driven selection of optimal cutoff values: mechanisms, magnitude, and solutions. Clin Chem 2008; 54:729–37.

20. Fodha I, Vahid A, Gheheda L, et al. Respiratory syncytial virus infections in hospitalized infants: association between viral load, virus subgroup, and disease severity. J Med Virol 2007; 79:1951–8.

21. Houben ML, Coenjaerts FE, Rossen JW, et al. Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. J Med Virol 2010; 82:1266–71.

22. Jansen RR, Schinkel J, Dek I, et al. Quantitation of respiratory viruses in relation to clinical course in children with acute respiratory tract infections. Pediatr Infect Dis J 2010; 29:82–4.

23. Franz A, Adams O, Willems E et al. Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection. J Clin Virol 2010; 48:239–45.

24. Martin ET, Kaypers J, Heugel J, Englund JA. Clinical disease and viral load in children infected with respiratory syncytial virus or human metapneumovirus. Diagn Microbiol Infect Dis 2008; 62:382–8.

25. Wright PE, Gruber WC, Peters M, et al. Illness severity, viral shedding, and antibody responses in infants hospitalized with bronchiolitis caused by respiratory syncytial virus. J Infect Dis 2002; 185:1011–8.
26. Hall CB, Douglas RG Jr, Geiman JM, Meagher MP. Viral shedding patterns of children with influenza B infection. J Infect Dis 1979; 140:610–3.
27. Lee CK, Lee HK, Loh TP, et al. Comparison of seasonal influenza A (H1N1) 2009 and seasonal influenza viral loads, Singapore. Emerg Infect Dis 2011; 17:287–91.
28. Li CG, Wang L, Eng HL, et al. Correlation of pediatric H1N1 2009 viral load with disease severity and prolonged viral shedding in children. Emerg Infect Dis 2010; 16:1265–72.
29. Lu PX, Deng YY, Yang GL, et al. The relationship between chest CT findings and viral load in patients with novel influenza A (H1N1). Natl Cent Biotechnol Inf 2010; 33:746–9.
30. Duchamp MB, Casalegno JS, Gillet Y, et al. Pandemic A(H1N1)2009 influenza virus detection by real-time RT-PCR: is viral quantification useful? Clin Microbiol Infect 2010; 16:317–21.
31. Meschi S, Selleri M, Lalle E, et al. Duration of viral shedding in hospitalized patients infected with pandemic H1N1. BMC Infect Dis 2011; 11:140.
32. To KK, Hung IF, Li IWS, et al. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin Infect Dis 2010; 50:850–9.
33. Bosis S, Esposito S, Osterhaus AD, et al. Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. J Clin Virol 2008; 42:286–90.
34. Gerna G, Sarasini A, Percivalle E, et al. Prospective study of human metapneumovirus infection: diagnosis, typing and virus quantification in nasopharyngeal secretions from pediatric patients. J Clin Virol 2007; 40:236–40.
35. Zhao B, Yu X, Wang C, et al. High human bocavirus viral load is associated with disease severity in children under five years of age. PLoS One 2013; 8:e62318.
36. Peiris JS, Guan Y, Yuen KY. Severe acute respiratory syndrome. Nat Med 2004; 10(12 suppl):S88–97.
37. Feikin DR, Al-Raddadi R, Qutub M, et al. Association of higher MERS-CoV virus load with severe disease and death, Saudi Arabia, 2014. Emerg Infect Dis 2015; 21:2029–35.
38. Deloria Knoll M, Fu W, Shi Q, et al. Bayesian estimation of pneumonia etiology: epidemiologic considerations and applications to pneumonia etiology research for child health study. Clin Infect Dis 2017; 64(suppl 3):S213–27.