Upper Respiratory Tract Co-detection of Human Endemic Coronaviruses and High-density Pneumococcus Associated With Increased Severity Among HIV-Uninfected Children Under 5 Years Old in the PERCH Study

Daniel E. Park, MSPH,* † Melissa M. Higdon, MPH,* Christine Prosperi, MS,* † Henry C. Baggett, MD,‡ W. Abdullah Brooks, MD,§ †† Daniel R. Feikin, MD, † Laura L. Hammitt, MD,* † Steve R. C. Howie, MD,¶ ¶ Karen L. Kotloff, MD, ‡‡ Orin S. Levine, PhD,† ††† Shabir A. Madhi, MD,§§§ David R. Murdoch, MD,¶¶¶¶ ¶ Katherine L. O’Brien, MD MPH,* †‡ J. Anthony G. Scott, MD, ‡‡‡‡‡‡‡‡ Donald M. Thea, MD MSc,§§§§

Background: Severity of viral respiratory illnesses can be increased with bacterial coinfection and can vary by sex, but influence of coinfection and sex on human endemic coronavirus (CoV) species, which generally cause mild to moderate respiratory illness, is unknown. We evaluated CoV and pneumococcal co-detection by sex in childhood pneumonia.

Methods: In the 2011–2014 Pneumonia Etiology Research for Child Health study, nasopharyngeal and oropharyngeal (NP/OP) swabs and other samples were collected from 3981 children <5 years hospitalized with severe or very severe pneumonia in 7 countries. Severity by NP/OP detection status of CoV (NL63, 229E, OC43 or HKU1) and high-density (≥6.9 log10 copies/mL) nath Academic Hospital and University of the Witwatersrand, South Africa; †††††Microbiology Laboratory, Middlemore Hospital, Counties Manukau District Health Board, Auckland, New Zealand; ‡‡‡‡‡‡‡‡Virology Laboratory, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Bangladesh; ¶¶¶¶¶Centre pour le Développement des Vaccins (CVD-Mali), Bamako, Mali; and ‡‡‡‡‡‡‡‡Department of Pediatrics, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, Maryland.

July Rhodes, PhD,***** Samba O. Sow, MD,Глав Amino, MSc,***** and Maria Deloria Knoll, PhD*

Accepted for publication February 25, 2021
From the *Department of International Health, International Vaccine Access Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; †Department of Environmental and Occupational Health, Milken Institute School of Public Health, George Washington University, Washington, District of Columbia; ‡Division of Global Health Protection, Centers for Disease Control and Prevention, Atlanta, Georgia; ‡‡International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Bangladesh; §§Medical Research Council Unit, Basse, The Gambia; **Department of Paediatrics, University of Auckland, New Zealand; §§§Department of Pediatrics and Department of Medicine, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, Maryland; ¶¶Bill & Melinda Gates Foundation, Seattle, Washington; §§§Medical Research Council: Respiratory and Meningeal Pathogens Research Unit; ¶¶¶Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases Unit, University of the Witwatersrand, Johannesburg, South Africa; §§§Department of Pathology and Biomedical Sciences, University of Otago; ***Microbiology Unit, Canterbury Health Laboratories, Christchurch, New Zealand; †††KEMRI Wellcome Trust Research Programme, Centre for Geographic Medicine Research, Coast, Kilifi, Kenya; §§§Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom; §§§Department of Global Health and Development, Boston University School of Public Health, Boston, Massachusetts; §§§Division of Global Health Protection, Thailand Ministry of Public Health–US Centers for Disease Control and Prevention Collaboration, Nonthaburi, Thailand; †††††Microbiology and Infectious Disease Unit, Warwick Medical School, University of Warwick, Coventry, United Kingdom; †††††Division of Global Health Protection, Thailand Ministry of Public Health–US Centers for Disease Control and Prevention Collaboration, Nonthaburi, Thailand; †††††Right to Care-Zambia, Lusaka, Zambia; §§§§§National Institute of Health, Ministry of Public Health, Nonthaburi, Thailand; †††††Virology Laboratory, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Bangladesh; ¶¶¶¶¶Centre pour le Développement des Vaccins (CVD-Mali), Bamako, Mali; and ‡‡‡‡‡‡‡‡Department of Pediatrics, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, Maryland.

J.A.G.S was supported by a clinical fellowship from the Wellcome Trust of Great Britain (UK, 098532). W.A.B. reported funding from Sanofi, PATH, Bill & Melinda Gates Foundation, and contributions to contemporaneous studies from Serum Institute of India, LTD Roche and Sanofi. M.D.K. has received funding for consultancies from Merck, Pfizer, Novartis, and grant funding from Merck and Pfizer. M.M.H. has received grant funding from Pfizer. L.L.H. has received grant funding from GlaxoSmithKline, Pfizer and Merck. K.L.O. has received grant funding from GlaxoSmithKline and Pfizer and participates on technical advisory boards for Merck, Sanofi Pasteur, PATH, Afinvax and ClearPath. C.P. has received grant funding from Merck. S.R.C.H. has a patent Lipocalin-2 as a Biomarker for Pneumococcal Infection, Status pending. K.L.K. has received grant funding from Merck Sharp & Dohme. S.A.M. has received honorarium for advisory board for the Bill & Melinda Gates Foundation, Pfizer, Medimmune and Novartis; institutional grants from GlaxoSmithKline, Novartis, Pfizer, Minervax and Bill & Melinda Gates Foundation; and speakers bureau for Sanofi Pasteur and GlaxoSmithKline. This paper is published with the permission of the Director of the Kenya Medical Research Institute. 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. The other authors have no conflicts of interest to disclose.

Address for correspondence: Daniel E. Park, MSPH, George Washington Milken Institute School of Public Health, 800 22nd St. NW, 7571 Science and Engineering Hall, Washington, DC 20052. E-mail: danpark@gwu.edu.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal’s website (www.pidj.com)
pneumococcus (HDSpn) by real-time polymerase chain reaction was assessed by sex using logistic regression adjusted for age and site.

**Results:** There were 43 (1.1%) CoV+/HDSpn+, 247 CoV+/HDSpn−, 449 CoV−/HDSpn+ and 3149 CoV−/HDSpn− cases with no significant difference in co-detection frequency by sex (range 51.2%–64.0% male, P = 0.06). More CoV+/HDSpn+ pneumonia was very severe compared with other groups for both males (13/22, 59.1% versus range 29.1%–34.7%, P = 0.04) and females (10/21, 47.6% versus 32.5%–43.5%, P = 0.009), but only male CoV+/HDSpn+ required supplemental oxygen more frequently (45.0% versus 20.6%–28.6%, P < 0.001) and had higher mortality (35.0% versus 5.3%–7.1%, P = 0.004) than other groups. For females with CoV+/HDSpn+, supplemental oxygen was 25.0% versus 24.8%–33.3% (P = 0.58) and mortality was 10.0% versus 9.2%–12.9% (P = 0.69).

**Conclusions:** Co-detection of endemic CoV and HDSpn was rare in children hospitalized with pneumonia, but associated with higher severity and mortality in males. Findings may warrant investigation of differences in severity by sex with co-detection of HDSpn and SARS-CoV-2.

**Key Words:** coronavirus, pneumococcus, coinfection, pneumonia, COVID-19

(Pediatr Infect Dis J 2021;XX:00–00)

**B**acterial coinfection increased morbidity and mortality in both the 1918 and 2009 influenza A pandemics.\(^1\) By some estimates, >95% of the deaths during the 1918 influenza pandemic involved complication with bacterial pneumonia, most commonly with *Streptococcus pneumoniae*.\(^1\) In a large-scale clinical study across the United States during the 2009 H1N1 pandemic, 28% of H1N1 2009 virus-positive samples had at least 1 other pathogen detected.\(^4\) In nonpandemic contexts, pneumonia etiology studies have attributed around 10%–30% of hospitalized pneumonia diagnosis relies on sputum and bronchoscopy, which is challenging because similar clinical presentation and poor sensitivity with immunologic priming, upper respiratory tract microbiome dysbiosis and increased susceptibility to viral coinfection.\(^22,23\)

Differences in severity of disease by sex have been observed for influenza, SARS-CoV-2 and pneumococcus. Severity of influenza disease and COVID-19 is generally greater in males, including in adult men.\(^24,25\) In an era of personal protective equipment and social distancing, adult men have had higher rates of hospitalization, intensive care unit (ICU) admission, and mortality.\(^26,27\) However, few studies have examined evidence for co-pathogenesis of endemic CoV with pneumococcus,\(^25\) and in vivo data from a pneumococcal conjugate vaccine trial suggesting that pneumococcal carriage itself may also contribute to severity of disease.\(^28\)

**Materials and Methods**

**PERCH enrollment** occurred between August 2011 and November 2014 for 24 months at each of 9 study sites 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. Identification and selection of cases and controls have been described previously.\(^36\) Cases included community controls to evaluate the background prevalence of infection in children without pneumonia.\(^39\) To explore severity of pneumonia associated with *S. pneumoniae* and endemic CoV coinfection and evaluate differences by sex, we evaluated the clinical and epidemiologic characteristics of NP/OP co-detection in the PERCH study.

**Key Words:** coronavirus, pneumococcus, coinfection, pneumonia, COVID-19

© 2021 The Author(s). Published by Wolters Kluwer Health, Inc.
Clinical characterization of the illness in cases was assessed at admission. Digital chest radiograph images were assessed by members of a panel of 14 radiologists and pediatrians who were trained in the standardized interpretation of pediatric chest radiographs.42

Co-infection status for primary analyses were defined using NP/OP detection as follows: coronavirus with high-density S. pneumoniae (CoV+/HDSpn+), coronavirus without high-density S. pneumoniae (CoV+/HDSpn−), HDSpn without coronavirus (CoV−/HDSpn−) and neither HDSpn nor coronavirus (CoV−/HDSpn−). A secondary analysis evaluated NP/OP CoV co-detection with 3 categories of S. pneumoniae density: (1) no S. pneumoniae; (2) low-density pneumococcus (<6.9 log_{10} copies/mL); and (3) high-density pneumococcal. Prevalence of co-infection in cases was compared with controls. We evaluated associations between density of CoV and pneumococcal density category, sex and mortality. To assess whether findings were unique to CoV and S. pneumoniae co-detections, supplemental analyses evaluated co-detection of CoV with Haemophilus influenzae and Staphylococcus aureus, and co-detection of S. pneumoniae with influenza A, B or C, human metapneumovirus, parainfluenzavirus 1 or 3 (Para 1/3) and respiratory syncytial virus A and B (RSV A/B). A sensitivity analysis was conducted to expand the definition of CoV+ to include CoV detected in induced sputum by PCR and the definition of HDSpn+ to include IPD cases that fell below the threshold, that is, had S. pneumoniae recovered from blood by culture or from lung aspirate or pleural fluid by culture or PCR. A second sensitivity analysis lowered the pneumococcal density threshold to 6.6 log_{10} copies/mL, which better aligned with detection of pneumococcal pneumonia from children with prior antibiotic use.59

Statistical Analysis

Demographic, clinical and laboratory characteristics were compared by co-detection category using logistic regression adjusted for age and site for categorical variables or the Wilcoxon signed-rank test for continuous variables, with and without stratifying by sex. Wilson score intervals were used to generate binomial proportional confidence intervals (CIs). Certain models were only adjusted for age and subregion (Asia, Western Africa, Southern Africa and Eastern Africa) due to sample size limitations. Interaction terms for sex were included in regression models to test for differences in association between co-detection and covariates by sex. Other pathogen combinations were selected based on prior evidence in the literature and through Random Forest models to evaluate all potential pathogens as predictors of CoV detection. Statistical analyses were conducted in SAS, version 9.4, and R, version 3.3.1.

RESULTS

Of 3888 HIV-negative cases enrolled between 2011 and 2014 with WHO-defined severe or very severe pneumonia with available NP/OP results, 7.5% (n = 290) had endemic coronavirus detected by NP/OP PCR (2.2% NL63, 1.1% 229E, 2.8% OC43 and 1.6% HKU1) and 492 (12.6%) had HDSpn detected. CoV+/HDSpn+ was observed in 43 (1.1%) cases, CoV+/HDSpn− in 247 (6.4%), CoV−/HDSpn+ in 448 (11.5%) and 3149 (81.0%) had neither. S. pneumoniae was detected by blood culture more frequently in HDSpn+ cases and most frequently in those also CoV+: CoV+/HDSpn+ 7.1%, CoV−/HDSpn+ 4.3%, CoV+/HDSpn− 1.2% and CoV−/HDSpn− 0.3% (Table S12, Supplemental Digital Content 1, http://links.lww.com/INF/E363). Controls (n = 4977) had higher CoV prevalence than cases (10.0% versus 7.5%), but CoV+/HDSpn+ prevalence was similar between cases and controls (1.1% versus 0.9%) (Table 1).

Co-detection of Endemic CoV and HDSpn

Co-detection was not associated with age, sex or pneumococcal conjugate vaccination status, but CoV+/HDSpn+ cases were disproportionately from Mali (Table 2) (Supplemental Digital Content 1 and 2, http://links.lww.com/INF/E363). Cases with high pneumococcal density, with or without CoV, were half as likely to have received antibiotics before NP/OP swab collection compared with cases without high-density pneumococcus (24.4% versus 47.4%, P < 0.001).

The association between the case co-detection group and clinical signs and symptoms at admission differed by gender (Table 3). For males only, CoV+/HDSpn+ cases were significantly more likely than the other co-detection groups to have WHO-defined very severe pneumonia (59.1% versus range 29.1%–34.7%, P = 0.04), require supplemental oxygen (45.0% versus 20.6%–28.6%, P < 0.001), have an mid-upper arm circumference-for-age Z-score < -3 SD (21.4% versus 2.6%–14.6%, P = 0.005). Among females only, leukocytosis was highest in CoV+/HDSpn+ cases (P = 0.01; interaction by sex P = 0.06) and an abnormal chest radiograph was least common among CoV+/HDSpn+ cases (28.6%) compared with the other co-detection groups (53.4%–65.8%, P = 0.03); for males, the abnormal chest radiograph was more common in HDSpn+ cases regardless of CoV status (64.7% and 58.1% for CoV+ and CoV−, respectively) compared with HDSpn− cases (43.8% and 49.4%, respectively; P = 0.03; interaction by sex P = 0.04). Other sex differences included less tachypnea among CoV+ in males (P = 0.009) but no difference among co-detection groups in females (interaction by sex P = 0.04). Fever at admission was common in all groups, but in males was more common among HDSpn+ cases (92.7%) compared with HDSpn− cases (79.8%, P = 0.03), whereas fever in females was most common in CoV+/HDSpn+ cases (90.8%) compared with other groups (range 80.9%–81.0%, P = 0.02; interaction by sex P = 0.09). Median C-reactive protein was highest among CoV+/HDSpn+ cases (P < 0.001) and was highest in HDSpn+ cases regardless of CoV status for females (Supplemental Digital Content 3 and 4, http://links.lww.com/INF/E363).

The overall case fatality ratio (CFR) among all PERCH HIV-negative cases was higher in females (8.9%) than males (7.4%, P < 0.001) (Supplemental Digital Content 5, http://links.lww.com/INF/E363). The CFR was highest in CoV+/HDSpn+ cases (22.5%, n = 9/40) compared with the other coinfection groups (7.2%–9.7%, P = 0.053) (Supplemental Digital Content 6, http://links.lww.com/INF/E363). When stratified by sex, this association was seen only among males (interaction by sex P = 0.02) among whom CoV+/HDSpn+ CFR was 35.0% (n = 7/20, 95% CI 18.1%–56.7%) compared with 5.3%–7.1% in the other groups (P = 0.004). In females, the CFR among CoV+/HDSpn+ cases was 10.0% (n = 2/20) and 9.2%–12.9% in other groups (P = 0.69, Fig. 1). After adjusting for age, site and malnutrition, the odds ratio of mortality in CoV+/HDSpn+ male cases compared with CoV+/HDSpn−, CoV−/HDSpn+ and CoV−/HDSpn− male cases was 11.6 (95% CI 3.1–44.4), 5.9 (1.7–20.3) and 8.6 (3.1–24.2), respectively (Supplemental Digital Content 7, http://links.lww.com/INF/E363). Among male children with CoV who died, 46.7% (n = 7/15, 95% CI 21.4%–71.9%) were HDSpn+ in NP/OP. Males without pneumococcus detected on NP/OP swabs had significantly higher CFR than males with low-density (<6.9 log_{10} copies/mL) pneumococcal upper respiratory tract carriage (CoV+: 10.2% versus 3.0%, P = 0.05; CoV−: 7.4% versus 4.9%, P = 0.02) Supplemental Digital Content 8, http://links.lww.com/INF/E363). CFR was similar across pneumococcal density categories for female children (range 8.2%–13.3%).

The CoV NP/OP viral load was similar among males and females (median 5.2 and 5.3 log_{10} copies/mL, respectively) and did not differ significantly by pneumococcal load (range 4.9–5.8 log_{10}
TABLE 1. Distribution of Human Endemic Coronavirus (CoV-NL63, CoV-229E, CoV-OC43 or CoV-HKU1) and HDSpn Co-detection Status in NP/OP by PERCH Case-control Status

| Characteristic          | Case (n = 3888) | Control (n = 4977) | aOR*; Case vs. Control (Ref) | P     |
|-------------------------|-----------------|--------------------|------------------------------|-------|
| CoV+/HDSpn+            | 290 (7.5)       | 501 (10.1)         | 0.67 (0.58–0.78)             | <0.001|
| CoV+/HDSpn−            | 492 (12.7)      | 379 (7.6)          | 1.62 (1.40–1.87)             | <0.001|
| CoV−/HDSpn+            | 227 (6.4)       | 454 (9.1)          | 0.63 (0.54–0.75)             | <0.001|
| CoV−/HDSpn−            | 3149 (11.5)     | 4143 (11.5)        | 0.96 (0.86–1.07)             | 0.433 |

*Odds ratio for case status compared with control, adjusted for age in months and site.

TABLE 2. Characteristics of Children Hospitalized with Severe or Very Severe Pneumonia by NP/OP Co-detection Status of Endemic Coronavirus (CoV-NL63, CoV-229E, CoV-OC43 or CoV-HKU1) and HDSpn

| Characteristic | A. CoV+/HDSpn+ | B. CoV+/HDSpn− | C. CoV−/HDSpn+ | D. CoV− and HDSpn− | P† |
|----------------|----------------|---------------|----------------|--------------------|-----|
| Total          | 43 (100)       | 247 (100)     | 449 (100)      | 3149 (100)         |     |
| Age <1 yr      | 27 (62.8)      | 174 (70.4)    | 284 (63.3)     | 1980 (62.9)        | 0.129|
| Male sex       | 22 (51.2)      | 158 (64.0)    | 242 (53.9)     | 1826 (58.0)        | 0.058|
| Site           |                |               |                |                    |     |
| Bangladesh     | 5 (11.6)       | 33 (13.4)     | 63 (14.0)      | 424 (13.5)         | <0.001|
| Thailand       | 0 (0)          | 10 (4.0)      | 3 (0.7)        | 208 (6.6)          |     |
| Mali           | 18 (41.9)      | 53 (21.5)     | 148 (33.0)     | 431 (13.7)         |     |
| The Gambia     | 9 (20.9)       | 48 (19.4)     | 95 (21.2)      | 457 (14.5)         |     |
| South Africa   | 6 (14.0)       | 54 (21.9)     | 81 (18.0)      | 654 (20.8)         |     |
| Zambia         | 3 (7.0)        | 25 (10.1)     | 31 (6.9)       | 401 (12.7)         |     |
| Kenya          | 2 (4.7)        | 24 (9.7)      | 28 (6.2)       | 574 (18.2)         |     |
| PCV vaccination status‡ |            |               |                |                    |     |
| No. doses      |                |               |                |                    | 0.226|
| 0              | 14 (35.0)      | 91 (38.2)     | 165 (37.8)     | 1359 (44.5)        |     |
| 1–2            | 10 (25.0)      | 80 (33.6)     | 128 (29.4)     | 787 (25.8)         |     |
| ≥3             | 16 (40.0)      | 67 (28.2)     | 143 (32.8)     | 910 (29.8)         |     |
| Fully vaccinated for age | 22 (55.0) | 129 (54.2) | 235 (53.9) | 1456 (47.6) | 0.307 |

*Odds ratio for case status compared with control, adjusted for age in months and site.

†P value obtained from multinomial logistic regression adjusted for age and site (where applicable).

§Defined as serum bioassy positive, antibiotics administered at the referral facility or antibiotic administration before the collection of NP/OP PCR specimens at the study facility.

© 2021 The Author(s). Published by Wolters Kluwer Health, Inc.
TABLE 3. Clinical Characteristics at Admission of Children Hospitalized with Severe or Very Severe Pneumonia by NP/OP Co-detection Status of Endemic Coronavirus (CoV-NL63, CoV-229E, CoV-OC43 or CoV-HKU1) and HDSpn*, by Sex

| Characteristics | No. (% with Available Information) | A. (CoV+/HDSpn+, n = 43) | B. (CoV+/HDSpn−, n = 247) | C. (CoV−/HDSpn+, n = 449) | D. (CoV−/HDSpn−, n = 3149) | Adjusted P* | P† for Interaction by Sex |
|----------------|-----------------------------------|---------------------------|---------------------------|---------------------------|----------------------------|-------------|---------------------------|
| Male           | 22 (51.2)                         | 158 (64.0)                | 242 (53.9)                | 1826 (58.0)               |                           |             |                           |
| Female         | 21 (48.8)                         | 89 (36.0)                 | 207 (46.1)                | 1323 (42.0)               |                           |             |                           |
| Very severe pneumonia (2005 WHO definition) | 23 | (53.5) | (32.8) | (38.8) | (30.8) | <0.001 | 0.335 |
| Male           | 13 | (59.1) | (29.1) | (34.7) | (29.5) | 0.0009 |             |
| Female         | 10 | (47.6) | (39.3) | (43.5) | (32.5) | 0.265 |             |
| Hypoxemia at admission | 17 | (39.5) | (36.8) | (40.9) | (35.0) | 0.061 | 0.873 |
| Male           | 8 | (36.4) | (31.0) | (37.6) | (32.5) | 0.182 |             |
| Female         | 9 | (42.9) | (46.8) | (44.7) | (38.3) | 0.002 | 0.697 |
| WHO defined very severe or hypoxemia | 30 | (69.8) | (52.9) | (56.6) | (50.9) | 0.002 | 0.240 |
| Male           | 16 | (72.7) | (56.8) | (51.7) | (48.5) | 0.015 |             |
| Female         | 14 | (66.7) | (63.6) | (62.3) | (54.2) | 0.004 |             |
| Supplemenal oxygen (ever)‡ | 13/36 | 48/192 | 99/367 | 687/2475 | 0.001 | 0.240 |             |
| Male           | 9/20 | 26/126 | 59/206 | 373/1460 | <0.001 |             |
| Female         | 4/16 | 22/86 | 40/161 | 314/1015 | 0.584 |             |
| Tachypnea      | 38 | (88.4) | (81.2) | (88.6) | (81.5) | 0.0009 |             |
| Male           | 17 | (77.3) | (77.2) | (90.9) | (81.5) | 0.044 | 0.036 |
| Female         | 21 | (100.0) | (88.5) | (86.0) | (81.5) | 0.017 |             |
| Fever          | 37 | (86.1) | (78.1) | (89.1) | (80.4) | 0.001 | 0.986 |
| Male           | 20 | (90.9) | (76.8) | (87.6) | (80.1) | 0.025 |             |
| Female         | 17 | (72) | (77) | (178) | (1071) | 0.175 | 0.597 |
| Observed cough | 22 | (81.0) | (80.9) | (90.8) | (81.0) | 0.204 |             |
| Male           | 10 | (45.5) | (46.9) | (69.0) | (71.9) | 0.382 | 0.109 |
| Female         | 12 | (57.1) | (65.2) | (65.5) | (68.7) | 0.720 |             |
| Vomiting       | 12 | (81.2) | (68.0) | (67.4) | (70.6) | 0.175 | 0.597 |
| Male           | 9 | (40.9) | (17.7) | (21.5) | (19.6) | 0.382 | 0.109 |
| Female         | 3 | (15.0) | (23.6) | (25.1) | (21.5) | 0.726 |             |
| Diarrhea       | 9 | (29.9) | (38) | (86) | 439 | 0.062 | 0.380 |
| Male           | 5 | (22.7) | (17.1) | (22.0) | (13.6) | 0.004 |             |
| Female         | 4 | (19.1) | (12.4) | (16.0) | (14.4) | 0.816 |             |
| Abnormal chest radiograph‡ | 15/31 | 110/213 | 220/377 | 1381/2706 | 0.077 | 0.043 |             |
| Male           | 11/17 | 60/137 | 119/205 | 783/1585 | 0.030 |             |
| Female         | 4/14 | 50/76 | 101/172 | 598/1121 | 0.030 |             |

(Continued)
which added three cases; (2) decreased the pneumococcal density threshold to 6.6 log10 copies/mL, which added 215 HDSpn+ cases (Supplemental Digital Content 14, http://links.lww.com/INF/E363). All findings were consistent with the primary analysis.

### Co-detection of Other Potential Pathogens

High-density *H. influenzae* colonization of the upper airway was the strongest bacterial predictor of CoV detection by random forest analysis (data not shown), but co-detection of CoV and

| Characteristics                                      | No. (% with Available Information) | CoV+/HDSpn+, n = 43 | CoV+/HDSpn−, n = 247 | CoV−/HDSpn+, n = 449 | CoV−/HDSpn−, n = 3149 | Adjusted P* | P† for Interaction by Sex |
|------------------------------------------------------|------------------------------------|----------------------|-----------------------|-----------------------|------------------------|-------------|--------------------------|
| Weight-for-height Z-score < −3 SDs                   | 5 (11.9)                           | 25 (10.6)            | 73 (16.6)             | 338 (11.1)            |                         | 0.118       | 0.077                    |
| Male                                                 | 4 (19.1)                           | 19 (12.3)            | 43 (18.1)             | 184 (10.4)            |                         | 0.052       |                          |
| Female                                               | 1 (4.8)                            | 6 (7.3)              | 30 (14.9)             | 154 (12.1)            |                         | 0.247       |                          |
| Weight-for-age Z-score < −3 SDs                      | 7 (16.7)                           | 31 (12.7)            | 84 (18.7)             | 491 (15.7)            |                         | 0.216       | 0.030                    |
| Male                                                 | 6 (18.6)                           | 18 (11.5)            | 54 (22.3)             | 280 (15.4)            |                         | 0.014       |                          |
| Female                                               | 1 (4.8)                            | 13 (11.5)            | 30 (14.5)             | 212 (16.1)            |                         | 0.604       |                          |
| Mid-upper arm circumference for age Z-score < −3 SDs | 4 (15.4)                           | 6 (4.6)              | 34 (12.8)             | 120 (6.5)             | 0.172                  | 0.339       |                          |
| Male                                                 | 3 (21.4)                           | 2 (2.6)              | 21 (14.6)             | 67 (6.4)              |                         | 0.005       |                          |
| Female                                               | 1 (8.3)                            | 4 (7.4)              | 13 (10.7)             | 53 (6.8)              |                         | 0.828       |                          |
| Severe acute malnutrition¶                           | 7 (16.3)                           | 43 (17.7)            | 97 (21.8)             | 556 (17.8)            | 0.635                  | 0.024       |                          |
| Male                                                 | 6 (27.3)                           | 26 (16.6)            | 55 (22.9)             | 295 (16.3)            |                         | 0.298       |                          |
| Female                                               | 1 (6.1)                            | 17 (8.3)             | 42 (13.7)             | 261 (8.0)             |                         | 0.308       |                          |
| Height-for-age Z-score < −3 SDs                      | 8 (21.1)                           | 29 (14.2)            | 67 (18.1)             | 522 (19.8)            | 0.192                  | 0.196       |                          |
| Male                                                 | 6 (30.0)                           | 21 (15.7)            | 37 (18.9)             | 329 (21.5)            |                         | 0.234       |                          |
| Female                                               | 1 (11.1)                           | 8 (11.4)             | 30 (17.1)             | 193 (17.4)            |                         | 0.427       |                          |
| Leukocytosis                                          | 22 (55.0)                          | 114 (49.1)           | 176 (42.6)            | 1273 (42.9)           |                         | 0.004       | 0.057                    |
| Male                                                 | 6 (27.3)                           | 69 (49.1)            | 96 (43.1)             | 723 (42.5)            |                         | 0.129       |                          |
| Female                                               | 13 (72.2)                          | 45 (51.1)            | 80 (42.1)             | 550 (43.4)            |                         | 0.010       |                          |
| Lymphopenia║                                         | 14 (32.6)                          | 62 (25.1)            | 138 (30.7)            | 796 (25.3)            | 0.086                  | 0.679       |                          |
| Male                                                 | 6 (27.3)                           | 42 (26.6)            | 71 (29.3)             | 453 (24.8)            |                         | 0.425       |                          |
| Female                                               | 8 (38.1)                           | 20 (22.5)            | 67 (32.4)             | 343 (25.9)            |                         | 0.146       |                          |
| C-reactive protein (mg/L), median, [IQR]             | 44.3 [8.1–176.4]                   | 14.8 [3.4–44.9]      | 27.8 [7.2–95.7]       | 12.9 [3.5–39.8]       | <0.001                 | 0.731       |                          |
| Male                                                 | 44.5 [7.8–176.4]                   | 14.6 [4.5–38.5]      | 22.6 [5.2–82.9]       | 12.5 [3.0–37.8]       | <0.001                 | <0.001      |                          |
| Female                                               | 43.5 [8.5–130.7]                   | 15.7 [2.4–53.6]      | 34.4 [11.9–107.6]     | 13.1 [3.6–41.3]       | <0.001                 | <0.001      |                          |
| Underlying condition**                               | 9 (20.9)                           | 61 (24.7)            | 129 (28.7)            | 803 (25.5)            | 0.637                  | 0.291       |                          |
| Male                                                 | 7 (21.4)                           | 35 (22.2)            | 69 (28.9)             | 429 (23.5)            |                         | 0.713       |                          |
| Female                                               | 2 (31.8)                           | 26 (26.2)            | 64 (28.9)             | 374 (23.5)            |                         | 0.988       |                          |

| *Overall P value obtained from multinomial logistic regression adjusted for age and site (where applicable). P values comparing co-detection groups are presented for males and females together, followed by sex-stratified P values listed below the grouped P values. Bold values denote statistical significance at the P < 0.05 level. |
| †Effect modification by sex, as indicated by interaction term P < 0.05 adjusted for site and age. |
| ‡Excludes South Africa due to near uniformity of receiving oxygen at South Africa. |
| §Restricted to children 6 months of age or older. |
| ¶Severe acute malnutrition: weight-for-height Z-score < −3 SD or middle arm circumference Z-score < −3 SDs or diagnosis of acute severe malnutrition. |
| ||Below 3000 cells per microliter of blood (3 × 10⁹/L). |
| **Underlying conditions: cerebral palsy, congenital heart disease/defect, congenital abnormalities, developmental delay, severe malnutrition, prematurity in an infant < 6 months old. The number of days with cough, fever, difficulty breathing, wheezing or runny nose, whichever symptom is longest. CoV indicates coronavirus; HDSpn, high-density *streptococcus pneumoniae*; IQR, interquartile range; NP/OP, nasopharyngeal/oropharyngeal; WHO, World Health Organization. |
high-density *H. influenzae* was not associated with mortality (CFR: 9.5% versus 6.7%–10.5% in other groups) (Supplemental Digital Content 15, http://links.lww.com/INF/E363). Similarly, mortality was not higher relative to other groups in cases where both CoV and *S. aureus* were detected in the NP/OP, or with co-detection of HDSpn and influenza A/B/C, human metapneumovirus, Para 1/3 or RSV A/B. Although influenza was rarely detected during PERCH, there were no deaths among the 22 HDSpn+/influenza+ co-detected cases. There were no differences by sex for any combination of pathogens except HDSpn and Para 1/3 where co-detection had a higher CFR among females (31.3% versus 7.6%–11.1%, *P* = 0.03) but not males (8.0% versus 2.6%–9.5%, *P* = 0.05).

**DISCUSSION**

Prevalence of endemic coronavirus species detected in the upper respiratory tract of children <5 years hospitalized with severe or very severe pneumonia in pre-COVID-19 years was 7.5%, which was lower than prevalence in age-matched community controls without pneumonia (10.0%). Co-detection of human endemic CoV species and HDSpn, a marker of pneumococcal pneumonia, was infrequent (1.1%), but in male children only was associated with higher case fatality and more severe disease compared with detection of CoV or *S. pneumoniae* alone. Case fatality was 35.0% in co-infected males compared with 5.3%–7.1% in the other infection combinations, whereas, in females, the case fatality was 10.0% versus 9.2%–12.9%, respectively. High-density pneumococcus was detected in 12.6% of cases overall, 14.8% among those with CoV detected and 18.2% of cases that died with no differences by sex, but high-density pneumococcus was detected in 47% (*n* = 7) of the 15 male children that died who had endemic CoV detected.

Endemic CoV species were not reported to be an important cause of severe pneumonia in the PERCH study because detection was low in cases and higher in controls. The more complex evaluation of pathogen and sex interaction presented here identified a subset of CoV-infected children with severe disease and fatal outcomes. Co-pathogenesis in pneumonia involves complex interactions between pathogens and host. Respiratory viruses may disrupt the lung physiology and generate immunopathologies that promote subsequent bacterial infection. Bacterial infections can increase morbidity of viral infections by increasing viral load and decreasing clearance. Among children with NP/OP co-detection of CoV and high-density pneumococcus, those that died had significantly higher CoV viral loads than those that survived (Supplemental Digital Content 9, http://links.lww.com/INF/E363). However, viral loads were similar between males and females among those with co-detection (Supplemental Digital Content 10, http://links.lww.com/INF/E363), so high viral load alone may not explain higher mortality in males. Certain pathogens inhibit the host immune response and increase susceptibility to secondary infections. There is evidence that CoV-NL63 strongly enhances streptococcal adherence to epithelial cells in human airway epithelium cultures and conversely does not affect adhesion of *S. aureus, H. influenzae* or *Pseudomonas aeruginosa*, which aligns with our findings of co-detection with these other pathogens. NP/OP pneumococcal carriage itself, and not solely super-infection in the lower respiratory tract, may play a role in severity. Virulence factors associated with nasopharyngeal colonization...
and biofilm formation are associated with lower respiratory tract adhesion, development of pneumonia, invasion, inflammation and cytotoxicity.\(^5\) High pneumococcal nasopharyngeal density also primes alveolar macrophages and leads to increased responsiveness to pneumococcus and other pathogens.\(^45\)\(^\text{55}\) High-density pneumococcal carriage in the upper respiratory tract may be a marker of microbiome dysbiosis, and pneumococcus may play a role in a wider relationship between the respiratory tract microbiome and severity. Studies have suggested that low-density pneumococcal carriage in adults is associated with lower microbiome perturbations, lower rates of viral coinfection and replication and decreased mucosal cytokine responses when compared with high-density carriage or noncarriage.\(^23\) This is consistent with our findings of highest mortality in children with noncarriage of pneumococcus and high-density pneumococcal carriage, particularly with CoV detection among male children (Supplemental Digital Content 8, http://links.lww.com/INF/E363).\(^22\) The microbiome has sex-dependent effects on immune function and priming, and males have higher absolute abundance of bacteria in the upper respiratory tract, which could contribute to observed differences by sex.\(^52\)\(^55\)

In most developing country settings, female children have lower mortality rates than males due to biological advantages, unless females have lower access to care or other disadvantages.\(^39\) In the context of COVID-19 in adults, males have generally constituted a higher proportion of hospitalized COVID-19 cases and had higher case fatality.\(^30\)\(^32\)\(^53\) IPD is also known to affect males disproportionately.\(^26\)\(^27\)\(^30\)\(^31\) Behavioral and immunologic factors are likely to contribute some of the differential severity by sex.\(^50\)\(^56\) However, immunologic differences may be less pronounced in children, and in the PERCH study, cases were more likely to be male and case fatality was higher in females (8.9% versus 7.4%) suggesting possible greater care-seeking for males in this study population. This suggests that external biologic factors may play a role in explaining the excess deaths observed in male children in the PERCH study and our results warrant consideration of the potential role of *S. pneumoniae* in differential severity by sex.

There are important limitations to this analysis. Although this was a large study with almost 4000 cases and 274 deaths with evaluable data, the analysis required multiple stratiﬁcations that resulted in a small sample size of the key subgroup of interest, that of males and females with co-detection of CoV and high-density pneumococcus. As a result, we were unable to evaluate outcomes by endemic CoV subtypes (Supplemental Digital Content 16, http://links.lww.com/INF/E363). Although the investigation was hypothesis-driven and the PERCH study was designed to evaluate causes and severity of pneumonia, this was not a prespecified analysis of the main study. Therefore, results shown here could be incidental and should be conﬁrmed in other studies. There was higher overall mortality among female children in PERCH, suggesting potential conservative bias in estimates of sex differences. We used high-density pneumococcal detection in the NP/OP as a marker of pneumococcal pneumonia, but it is not a conﬁrmatory measure as it has poor speciﬁcity,\(^29\) and sensitivity is reduced by prior exposure to antibiotics,\(^57\) which was common at PERCH sites. Furthermore, detection of organisms in the upper respiratory tract may not be a reliable surrogate for lower respiratory tract infection. Most cases and controls in PERCH had four or more pathogens detected on NP/OP, including the cases who died with CoV and high-density pneumococcal detected, making it difﬁcult to attribute causation for any specific pneumonia case.\(^35\) One had *S. aureus* detected in pleural ﬂuid and in PERCH was attributed at the cause of the pneumonia, but most of the additional organisms detected in the COV+\(^5\) high-density pneumococcal deaths were also commonly found in controls without pneumonia. A further limitation was our inability to fully explore the effect of malnutrition on participant outcomes in this analysis. Results adjusted for chronic malnutrition were consistent with overall ﬁndings, but because of the small sample size may not have adequately accounted for all factors that may have contributed to the higher mortality in males. Although effect of sex was not statistically signiﬁcant, males with co-detection were more likely to have height-for-age Z-score <−3 SDs (30.0%) compared to females (11.1%). Markers of severe acute malnutrition were statistically different between males and females, but this may indicate severity of illness as vomiting, diarrhea and systemic involvement were more prevalent in the co-detection group and in children that died. Nonetheless, malnutrition associated with pediatric pneumonia should be recognized as an important risk factor for mortality.\(^58\) None of the children with co-detection who died had underlying conditions other than severe malnutrition. The similar prevalence of co-detection in community controls suggests that human endemic CoV species may not be a sufﬁcient etiologic cause of pneumonia, alone or in combination with pneumococcus, but may interact with pneumococcus to exacerbate disease under speciﬁc conditions yet to be determined. Sensitivity analyses that increased sample size slightly were consistent with our primary analysis.

Any extensions from human endemic CoV species to COVID-19 may be inappropriate because epidemiologic and clinical manifestations of SARS-CoV-2 are different from endemic CoV species and ﬁndings from a pediatric population may not be relevant to adults as children have lower severity of COVID-19 compared with adults, possibly due to lower ACE2 receptor expres-\(^55\) Nonetheless, an analysis of adults in England’s national surveillance system reported coinfection of IPD and COVID-19 being rare, but associated with a signiﬁcant 7.8-fold increase in the case fatality rate.\(^36\) Carriage and IPD due to vaccine-type pneumococci can be reduced by pneumococcal vaccination,\(^46\) and recent reports have suggested a potential inverse association between pneumococcal vaccination and both endemic CoV and SARS-CoV-2 infection.\(^34\)\(^35\)\(^62\) However, pneumococcal conjugate vaccine vaccination status was not associated with co-detection in our study.

*S. pneumoniae* co-pathogenesis may contribute to increased morbidity and mortality from CoV infection among children with pneumonia. Coronavirus and HDSp co-detection was rare, but HDSp was present in almost a quarter of CoV-positive very severe cases, and in nearly half of CoV-positive males who died. Further studies are needed to conﬁrm these ﬁndings, and to elucidate the role of high-density pneumococcal carriage in the upper respiratory tract on immunologic priming, microbiome dysbiosis and other biological mechanisms of exacerbation. Further efforts to detect pneumococcal coinfection with endemic coronaviruses and SARS-CoV-2 may be warranted, along with potential evaluations of pneumococcal vaccination and colonization density as predictors of disease progression.

ACKNOWLEDGMENTS

We recognize the support provided by the Institutional Review Boards for study oversight. We appreciate the helpful discussions with Scott Zeger and our many colleagues. Finally, we gratefully recognize the parents and children who participated in this study and express our gratitude for their commitment to the advancement of knowledge toward better health for children in and beyond their community.

REFERENCES

1. McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. *Nat Rev Microbiol*. 2014;12:252–262.

2. Centers for Disease Control and Prevention (CDC). Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A
24. Klein SL, Pekosz A, Passaretti C, et al. Sex, gender and influenza. *Clin Microbiol Infect.* 2012;18:300–307.

25. CDC COVID-19 Response Team. Coronavirus Disease 2019 in Children—United States, February 12–April 2, 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69:22–26.

26. Wagenvoort GHI, Sanders EAM, Vlaminkx BJ, et al. Sex differences in invasive pneumococcal disease and the impact of pneumococcal conjugate vaccination in the Netherlands, 2004 to 2015. *Eurosurveillance*. 2017;22:30481.

27. Gutierrez F, Masii M, Miret C, et al. The influence of age and gender on the population-based incidence of community-acquired pneumonia caused by different microbial pathogens. *J Infectol*. 2006;193:166–74.

28. Jensen-Fangel S, Mohey R, Johnsen SP, et al. Gender differences in hospitalization rates for respiratory tract infections in Danish youth. *Scand J Infect Dis.* 2004;36:31–36.

29. Millett ER, Quint JK, Smeeth L, et al. Incidence of community-acquired lower respiratory tract infections and pneumonia among older adults in the United Kingdom: a population-based study. *PLoS One*. 2013;8:e75131.

30. Dong Y, Mo X, Hu Y, et al. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. *Pediatrics*. 2020;145:e20200702.

31. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708–1720.

32. Nasiri MJ, Haddadi S, Tahvildari A, et al. COVID-19 clinical characteristics, and sex-specific risk of mortality: systematic review and meta-analysis. *Front Med*. 2020;7:459.

33. Golda A, Malek N, Dudk B, et al. Infection with human coronavirus NL63 enhances streptococcal adherence to epithelial cells. *J Gen Virol*. 2011;92(Pt 6):1358–1368.

34. Nunes MC, Cutland CL, Klugman KP, et al. pneumococcal conjugate vaccine protection against coronavirus-associated pneumonia hospitalization in children living with and without HIV. *MBio*. 2021;12:e02547-e02520.

35. O’Brien KL, Baggett HC, Brooks WA, et al. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet*. 2019;394:757–779.

36. 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–S123.

37. Crawley J, Prosperi C, Baggett HC, et al; PERCH Study Group. Standardization of clinical assessment and sample collection across ALL PERCH Study Sites. *Clin Infect Dis*. 2017;64((suppl_3)):S228–S237.

38. Levine OS, O’Brien KL, Deloria-Knoll M, et al. The pneumonia etiology research for child health project: a 21st century childhood pneumonia etiology study. *Clin Infect Dis*. 2012;54((suppl 2)):S93–S101.

39. Driessil AJ, Karron RA, Morpeth SC, et al. Standardization of laboratory methods for the PERCH Study. *Clin Infect Dis*. 2017;64((suppl_3)):S245–S252.

40. Feikin DR, Fu W, Park DE, et al; PERCH Study Group. Is higher viral load in the upper respiratory tract associated with severe pneumonia? Findings from the PERCH Study. *Clin Infect Dis*. 2017;64((suppl_3)):S337–S346.

41. Park DE, Baggett HC, Howie SRC, et al; PERCH Study Group. Colonization density of the upper respiratory tract as a predictor of pneumonia-haemophilus influenzae, moraxella catarrhalis, *staphylococcus* and pneumocystis jirovecii. *Clin Infect Dis*. 2017;64((suppl_3)):S328–S336.

42. Fancourt N, Knoll MD, Baggett HC, et al. Chest radiograph findings in childhood pneumonia cases from the multisite PERCH study. *Clin Infect Dis*. 2017;64((suppl_3)):S262–S327.

43. Broggi A, Ghosh S, Sposito B, et al. Type III interferons disrupt the lung epithelial barrier upon viral recognition. *Science*. 2020;369:706–712.

44. Abood RN, McHugh KJ, Rich HE, et al. IL-22-binding protein exacerbates influenza, bacterial super-infection. *Mucosal Immunol*. 2012;5:1231–1243.

45. Bousiba S, Raoult D, La Scola B. Pneumonia pathogen detection and microbial interactions in polymicrobial episodes. *Future Microbiol*. 2013;8:633–660.

46. Moore DP, Dagan R, Madhi SA. Respiratory viral and pneumococcal coinfection of the respiratory tract: implications of pneumococcal vaccination. *Expert Rev Respir Med*. 2012;6:451–465.

47. Loughran AJ, Orluhela CJ, Tuomaneen EI. Streptococcus pneumoniae: invasion and inflammation. *Microbiol Spectr*. 2019;7:10.1128/microbiolspec.GPP3-0004-2018.

48. Mitsi E, Carniel B, Reiné J, et al. Nasal pneumococcal density is associated with microaspiration and heightened human alveolar macrophage production.
responsiveness to bacterial pathogens. *Am J Respir Crit Care Med.* 2020;201:335–347.

49. Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-mediated inflammatory responses: from mechanisms to potential therapeutic tools. *Virology.* 2020.

50. Weight CM, Venturini C, Pajar S, et al. Microinvasion by *Streptococcus pneumoniae* induces epithelial innate immunity during colonisation at the human mucosal surface. *Nat Commun.* 2019;10:3060.

51. de Steenhuijsen Piters WA, Heinonen S, Hasrat R, et al. Nasopharyngeal microbiota, host transcriptome, and disease severity in children with respiratory syncytial virus infection. *Am J Respir Crit Care Med.* 2016;194:1104–1115.

52. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol.* 2016;16:626–638.

53. Liu CM, Price LB, Hungate BA, et al. *Staphylococcus aureus* and the ecology of the nasal microbiome. *Sci Adv.* 2015;1:e1400216.

54. Sawyer CC. Child mortality estimation: estimating sex differences in childhood mortality since the 1970s. *PLoS Med.* 2012;9:e1001287.

55. Lu X, Zhang L, Du H, et al. SARS-CoV-2 infection in children. *N Engl J Med.* 2020;382:1663–1665.

56. Channappanavar R, Fett C, Mack M, et al. Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus infection. *J Immunol.* 2017;198:4046–4053.

57. Driscoll AJ, Knoll MD, Hammitt LL, et al. The effect of antibiotic exposure and specimen volume on the detection of bacterial pathogens in children with pneumonia. *Clin Infect Dis.* 2017;64(suppl_3):S368–S377.

58. Ahmed T, Begum B, Badzizzaman, et al. Management of severe malnutrition and diarrhea. *Indian J Pediatr.* 2001;68:45–51.

59. Bunyavanich S, Do A, Vicencio A. Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. *JAMA.* 2020;323:2427–2429.

60. Fleming-Dutra KE, Conklin L, Loo JD, et al. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on vaccine-type nasopharyngeal carriage. *Pediatr Infect Dis J.* 2014;33(suppl 2):S152–S160.

61. Noale M, Trevisan C, Maggi S, et al. The association between influenza and pneumococcal vaccinations and SARS-Cov-2 infection: data from the EPICOV19 web-based survey. *Vaccines (Basel).* 2020;8:E471.

62. Root-Bernstein R. Age and location in severity of COVID-19 pathology: do lactoferrin and pneumococcal vaccination explain low infant mortality and regional differences? *BioEssays.* 2020;42:e2000076.