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Respiratory infections in Eñepa Amerindians are related to malnutrition and *Streptococcus pneumoniae* carriage

Lilly M. Verhagen [a,b], Keyla Gómez-Castellano [b], Eveline Snelders [c], Ismar Rivera-Olivero [b], Leonor Pocaterra [d], Willem J.G. Melchers [c], Jacobus H. de Waard [b], Peter W.M. Hermans [a,*]

[a] *Laboratory of Pediatric Infectious Diseases, Radboud University Medical Centre, PO Box 9101 (Internal Post 224), 6500 HB Nijmegen, The Netherlands*
[b] *Laboratorio de Tuberculosis, Instituto de Biomedicina, Universidad Central de Venezuela, 1010 Caracas, Venezuela*
[c] *Department of Medical Microbiology, Radboud University Medical Centre, 6500 HB Nijmegen, The Netherlands*
[d] *Catédra de Parasitología, Escuela de Medicina José María Vargas, Universidad Central de Venezuela, 1010 Caracas, Venezuela*

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Summary *Objectives:* High acute respiratory tract infection (ARTI) rates are observed in indigenous populations. We assessed the role of viral infections and nasopharyngeal bacterial carriage in ARTIs in Eñepa Amerindians from Venezuela.
Methods: In 40 children aged 0–10 years with ARTIs, healthy nearest-age sibling controls and their mothers the presence of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*/*psittachi* and 15 respiratory viruses was investigated.
Results: *S. pneumoniae* was the most frequently detected pathogen, with carriage rates of 75% and 38% in children and mothers respectively. In children, *S. pneumoniae* carriage was associated with ARTI risk in multivariate analysis (OR 14.1, 95% CI 1.4–137.7). Viral infections were not associated with ARTI risk. *S. pneumoniae* carriage was common in children of all ages while viral co-infections were more frequently present in children under 4 years compared to older children (46% vs. 17%, $p < 0.01$). An increase of one unit height-for-age Z score (i.e. improved chronic nutritional status) was associated with decreased odds of *S. pneumoniae* colonization in multivariate analysis (OR 0.66, 95% CI 0.44–0.99).

* Corresponding author. Tel.: +31 24 3666406; fax: +31 24 3666352.
E-mail address: P.Hermans@cukz.umcn.nl (P.W.M. Hermans).

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Conclusions: In Eñepa children high *S. pneumoniae* carriage rates associated with a poor nutritional status contribute to the development of ARTIs.

Introduction

Acute respiratory tract infections (ARTIs) are among the leading causes of childhood mortality worldwide, responsible for about two million child deaths annually.[1] Pneumonia rates are especially high in low-income countries and underlying malnutrition is a major risk factor for pneumonia in children from developing countries.[2] Recent estimates suggest that *Streptococcus pneumoniae* and *Haemophilus influenzae* together account for more than 50% of childhood pneumonia deaths each year.[3,4] Acquisition of nasopharyngeal carriage of these bacteria is an initial step in the process leading to invasive bacterial diseases.[5] The increased ability to simultaneously test for multiple pathogens has highlighted the potential role of co-colonization with multiple respiratory tract bacteria and co-infection with viral infections in progression to disease after colonization.[6–9]

In indigenous children ARTIs, in particular acute lower respiratory tract infections (ALRTIs), are more common and associated with higher morbidity than among non-indigenous age-matched counterparts in the same region. In a population-based birth cohort study performed in Australia, pneumonia rates in Aboriginal children were 13.5 times higher than in non-Aboriginal children (95% CI 12.8–14.4).[10] In the United States, The ALRTI-associated hospitalization rate was 3-fold higher for American Indian/Alaska Native children than for the general U.S. population in retrospective analyses.[11,12] The high prevalence rates of ARTIs, including ALRTIs and acute otitis media (AOM), in Australian and North American native children have been associated with increased carriage of viral and bacterial respiratory tract pathogens compared to non-natives.[13–18] While in Australia, Canada and the U.S. native populations make up only two to four percent of the population, ten percent of the South American population consists of indigenous people.[19–21] There is a lack of health research reports concerning the principal clinical presentations and infectious etiologies of ARTIs in indigenous people from the South American region.[19]

The Eñepa (or Panare) Amerindians inhabit the Cedeño Municipality of the Venezuelan state of Bolívar, characterized by a forest-savanna landscape. They live in around 40 isolated communities where they have very little interaction with other indigenous or non-indigenous Venezuelan populations. Pneumococcal vaccinations have not been introduced in this population. We investigated bacterial nasopharyngeal carriage, viral infections and nutritional status in Eñepa Amerindian children aged 0–10 years with and without ARTIs and their mothers.

Patients and methods

Study population and sampling

In August 2011, during the rainy season, five geographically isolated Eñepa communities (Biscochuelo, Colorado, El Guamal, Macanilla, Quebrada seca) were visited for primary health care services. During these visits, all inhabitants were registered. Of the 145 children aged 0–10 years present in the 5 communities at the time of survey, 40 (28%) were diagnosed with an ARTI, including AOM. Nasopharyngeal samples were taken of these children and of 40 nearest-age full sibling controls aged 0–10 years and within 5 years of patient age. When several siblings fulfilled these eligibility criteria, the sibling whose age was nearest to that of the case was included. When no siblings fulfilled these criteria ($n = 3$), the nearest-age cousin living in the same household was included as a matched control. Nasopharyngeal samples were also taken of mothers of the included children ($n = 43$). As nasopharyngeal samples alone may be insufficient to detect colonization by *S. pneumoniae* and *H. influenzae*,[22] oropharyngeal swabs for bacterial isolation were taken as well. Nasopharyngeal samples for bacterial and viral isolation were obtained with a flexible swab (Copan Italia). Oropharyngeal samples were obtained by use of rigid cotton-tipped applicators. Swabs were transported at 4°C–7°C within 4 h after sampling, in STGG medium[23] for bacterial isolation and in TE (10 mM Tris–HCl, 1 mM EDTA, pH 8) for virus isolation, to a −20 °C freezer. Within 7 days, swabs were transferred to −70 °C where they were stored until microbiological and virological analyses. Physical examinations, including ear examination by pneumatic otoscopy and anthropometric measurements of children and mothers, were performed and documented on a standardized data collection sheet.

Study definitions

ARTIs were classified as upper respiratory tract infection (URTI) or ALRTI. A diagnosis of URTI was made when at least 2 common cold symptoms (fever, rhinorrhea, sore throat, headache, cough, muscle aches) with at least 1 symptom involving the respiratory tract (rhinorrhea, sore throat, cough) in the absence of an increased respiratory rate, chest indrawing, or auscultatory findings such as crepitations or rhonchi, were present.

ALRTIs were classified as follows:

- Pneumonia: the presence of (1) a history of cough or difficulty in breathing and (2) chest indrawing or increased respiratory rate ($\geq$60 breaths/min for children $<$2 months of age, $\geq$50 breaths/min for children 2–11 months of age,

≥40 breaths/min for children 1–5 years of age and ≥30 breaths/min for children 6–10 years of age).[24,25]
- Acute bronchitis: productive cough of <3 weeks' duration following a URTI, without wheezing, and no parental history of recurrent wheezing.[25]
- Acute bronchiolitis: an upper respiratory prodrome followed by wheezing and increased respiratory effort, manifested as tachypnoea or chest indrawing, in children <24 months of age.[25,26]
- Acute wheezing: an attack of wheezing preceded by upper respiratory tract symptoms in children ≥24 months of age, with or without a parental history of recurrent wheezing.

Diagnosis of AOM was based on the combined presence of acute symptoms (fever, irritability, pulling at the ears), at least two signs of middle-ear effusion detected by means of pneumatic otoscopic examination (bulging, decreased or absent mobility, abnormal color or opacity not due to scarring, air-fluid interfaces) and at least one sign of middle-ear inflammation (erythema, increased vascularity over full, bulging or yellow tympanic membrane).[27]

A history of breastfeeding was determined by parent's response and defined as breastfeeding at the time of survey in children aged ≤12 months and breastfeeding during the first year of life in children aged >12 months.

Microbiological and virological analysis

Swabs in STGG were plated on blood and chocolate agar plates and cultured for *S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* in the laboratory of the 'Instituto de Biomedicina' in Caracas, Venezuela, using standard methods.[28] Virological analysis was performed in the Radboud University Medical Centre in Nijmegen, The Netherlands, using multiplex PCR as previously described.[29] Briefly, respiratory swabs were dissolved in TE and spiked with equine arthritis virus (EAV) as an internal control.[30] DNA/RNA was isolated using the *MagNA Pure 96 DNA and Viral NA Small Volume Kit* (Roche Diagnostics) and the *Viral NA Plasma SV protocol*. Real-time multiplex PCR for detection of the atypical bacteria *Mycoplasma pneumoniae* (*MP*) and *Chlamydophila pneumoniae/psittachi* (*CP*) and for respiratory syncytial virus (RSV), influenza A (FluA), influenza B (FluB), human rhinovirus (HRV), human metapneumovirus (HMPV), parainfluenza 1, 2, 3 and 4 (PIV1-4), human coronaviruses OC43 and 229E (HCoV), enteroviruses (EV), human parechoviruses (HPeV), human bocavirus (HBoV) and human adenoviruses (AdV) was performed on the Roche LightCycler® 480 system.

Anthropometric measurements

Anthropometric measurements were transformed into weight-for-height, height-for-age, and body mass index (BMI)-for-age Z scores based on WHO standard reference populations[31,32] using WHO anthro software.[33] Children under 5 years of age with weight-for-height or height-for-age Z scores < -2 standard deviations (SD) were defined as malnourished. Children aged 5–10 years with BMI-for-age or height-for-age Z scores < -2 SD were defined as malnourished. Weight-for-height and BMI-for-age Z scores are indicators of wasting (acute malnutrition) in respectively children under 5 years and those aged 5 years and above. The height-for-age Z score is an indicator for grading stunting (chronic malnutrition) in children of all ages.[34–36] Mothers with a BMI <18.5 were classified as malnourished.[37]

Ethical aspects

Before the start of the survey, meetings were held with village elders and members of the communities to explain to them in Spanish and/or in their native language the nature and objectives of the study. Children were included on the basis of written informed consent from parents. Mothers also provided written informed consent. The ethical committee of the Instituto de Biomedicina (Comité de Bioética, Estado portador nasofaríngeo de *S. pneumoniae* en adultos y niños de la población indígena Panare y relación con neumonía-CB-2011-07) and the Regional Health Services approved the study protocol.

Statistical analyses

The Mc Nemar test was used to assess the association of viral and bacterial pathogens with ARTI univariately. For univariate analysis of the association of continuous variables with ARTI the paired Student's *t* test or nonparametric Wilcoxon signed rank test was used, depending on whether or not the variables were normally distributed (Kolmogorov–Smirnov's test, $p > 0.05$). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by means of multivariate conditional logistic regression analysis. Those pathogens and variables associated with ARTI with a *p*-value ≤0.20 in univariate analyses were entered into the multivariate model. Sex and indicators of nutritional status (weight-for-height, BMI-for-age and height-for-age Z scores) were retained in the multivariate model, irrespective of their *p*-values in univariate analysis.

Determinants of viral infection and bacterial colonization were identified univariately using Chi-square test or Fisher's exact test, as appropriate, for categorical variables. For continuous variables, the unpaired Student's *t* test or nonparametric Mann–Whitney's test was used depending on whether or not the variables were normally distributed (Kolmogorov–Smirnov's test, $p > 0.05$). Variables associated with *S. pneumoniae* colonization with a *p*-value ≤0.20 in univariate analysis were entered into a multivariate logistic regression model. Age, sex and indicators of nutritional status were retained in the multivariate model, irrespective of their *p*-values in univariate analysis.

Statistical significance was set at $p < 0.05$. The SPSS program for Windows version 20.0 (SPSS Inc, Chicago, IL) was used for statistical analyses.

Results

Samples for viral and bacterial analyses were taken in 79/80 children (99%). Of the 43 included mothers, samples for bacterial analyses were taken in 42 mothers (98%), while swabs for viral analyses were taken in all mothers.

Table 1 Characteristics of cases and controls.

	Cases	Controls	*p*-value
Demographic figures			
Sex, *n* (%)			0.82
Female	22 (55)	20 (50)	
Male	18 (45)	20 (50)	
Age (years), mean (SD)	4.1 (2.7)	4.3 (3.2)	0.63
Nutritional status			
Weight-for-height Z score in children <5 years, mean (SD) BMI-for-age Z score in children ≥5 years, mean (SD)	−1.46 (2.18)	−0.55 (2.23)	0.052
Height-for-age Z score in children of all ages, mean (SD)	−1.84 (2.36)	−1.74 (1.88)	0.85
Malnourished, *n* (%)	33 (83)	23 (58)	0.013
History of breastfeeding, *n* (%)	35 (88)	36 (90)	0.67

Determinants of acute respiratory tract infections

Most of the 40 cases were classified as having ALRTI (58%, $n = 23$), further differentiated as pneumonia (15%, $n = 6$), acute bronchitis (33%, $n = 13$), acute bronchiolitis (3%, $n = 1$) and acute wheezing (8%, $n = 3$). Eleven children (28%) were classified as URTI. Twenty-one children (53%) were diagnosed with AOM, of which 18 were unilateral and three were bilateral. Fifteen (71%) of the children with an AOM were also diagnosed with an ARTI at the time of the survey. Characteristics of cases and controls are shown in Table 1. Cases were significantly more often malnourished compared to controls (83% vs. 58%, $p = 0.013$). In particular indicators of acute malnutrition, i.e. weight-for-height and BMI-for-age Z scores, were lower in cases compared to controls (Table 1).

Occurrence of respiratory tract pathogens

Viral or atypical bacterial pathogens were detected in 24/79 (30%) of the children. The most commonly identified viruses were HRV (10%), EV (6%), FluA H1N1 (6%), and HBoV (3%). AdV, HMPV and RSV were all detected in 1% of the children. Of the atypical bacterial pathogens, *CP* was detected in 1 child (1%); *MP* was not detected. In three mothers a viral infection was detected, either FluA H1N1, HRV or HBOV. No children or mothers were infected with more than one virus. Fifty-nine children (75%) were colonized with *S. pneumoniae*, 17 (22%) were colonized with *H. influenzae*, 17 (22%) were colonized with *S. aureus* and 22 (28%) were colonized with *M. catarrhalis*. Nasopharyngeal colonization rates of all four bacteria in children of different ages were approximately equal (Table 2). For mothers, colonization rates were lower but still considerable; respectively 38%, 2%, 12% and 2% for *S. pneumoniae*, *H. influenzae*, *S. aureus* and *M. catarrhalis*. Thirty-two children (41%) and one mother (2%) were colonized with multiple bacterial pathogens. In 12% of the children and in 81% of the mothers that were colonized with *S. pneumoniae*, pneumococci were detected only in the oropharyngeal sample and not in the nasopharyngeal sample. In contrast, no *H. influenzae* was isolated from the oropharyngeal sample alone.

Association of respiratory tract pathogens with ARTIs

S. pneumoniae carriage rates were significantly higher in cases compared to controls (90% vs. 59%, $p < 0.01$). In contrast, *M. catarrhalis* was less often detected in cases compared to controls, but this was not statistically significant (18% vs. 36%, $p = 0.14$). Colonization with *H. influenzae* or *S. aureus* was not significantly associated with ARTI ($p = 1.0$ and $p = 0.58$ respectively). None of the detected viral infections showed a significant association with ARTI with *p*-values varying from $p = 0.38$ to $p = 0.73$. In multivariate conditional logistic regression analyses, *S. pneumoniae* colonization was a significant determinant of ARTI risk (OR 14.1, 95% CI 1.4—137.7). Furthermore, the indicator of acute malnutrition (weight-for-height and BMI-for-age Z score in respectively children under 5 years of age and those aged 5 years and above) was associated with ARTI in multivariate analysis: an increase of one unit Z score (i.e. improved acute nutritional status) was significantly

Table 2 Colonization with bacterial pathogens in children of different ages.

Age in years	No. of children	>1 bacteria, *n* (%)	*S. pneumoniae*, *n* (%)	*H. influenzae*, *n* (%)	*S. aureus*, *n* (%)	*M. catarrhalis*, *n* (%)
0—1	17	8 (47)	13 (76)	3 (18)	4 (24)	7 (41)
2—3	17	7 (41)	13 (76)	2 (12)	3 (18)	6 (35)
4—5	19	7 (37)	17 (89)	4 (21)	4 (21)	2 (11)
6—7	13	4 (31)	8 (62)	5 (38)	1 (8)	3 (23)
8—10	13	6 (46)	8 (62)	3 (23)	5 (38)	4 (31)
All children	79	32 (41)	59 (75)	17 (22)	17 (22)	22 (28)

associated with decreased odds of ARTI (OR 0.61, 95% CI 0.40—0.94). *M. catarrhalis* carriage and height-for-age Z score were not significantly associated with ARTI in multivariate analysis.

Determinants of bacterial colonization and viral infection

As the prevalence of *S. pneumoniae* colonization was high in both children and mothers, we determined factors associated with carriage in both groups (Table 3). No significant differences in sex or number of people living in the household between *S. pneumoniae* colonized and non-colonized children were seen. Also, age was not significantly correlated with *S. pneumoniae* colonization. Children colonized by *S. pneumoniae* were significantly more often malnourished than non-colonized children (76% vs. 50%, $p = 0.027$). In particular the indicator of chronic malnutrition, height-for-age Z score, was lower in children colonized with *S. pneumoniae* compared to non-colonized children (−2.08 vs. −1.03, $p = 0.056$). In multivariate analysis including all variables with a $p \leq 0.20$ an increase of one unit Z score of height-for-age (i.e. improved chronic nutritional status) was significantly associated with decreased odds of *S. pneumoniae* colonization (OR 0.66, 95% CI 0.44—0.99).

The absence of a statistically significant relationship between age and *S. pneumoniae* colonization was observed in both cases and controls. Of the cases aged 0—5 years 92% was colonized by *S. pneumoniae* while 78% of cases aged 6—10 years were *S. pneumoniae* positive ($p = 0.30$). In controls, *S. pneumoniae* was observed in 70% of children aged 0—5 years vs. 50% of children aged 6—10 years ($p = 0.23$).

Children with a viral infection were younger than children without a viral infection, but this was not statistically significant (3.3 vs. 4.5 years, $p = 0.090$). Sex and indicators of nutritional status were not significantly associated with detection of a respiratory virus.

In mothers, *S. pneumoniae* colonization was significantly associated with a lower mean BMI (20.9 vs. 23.4, $p = 0.049$ in colonized vs. non-colonized mothers, Table 3). Furthermore, the median number of people living in the household was significantly lower in mothers colonized with *S. pneumoniae* compared to mothers not colonized with *S. pneumoniae* (4.5 vs. 5, $p = 0.041$). *S. pneumoniae* colonization rates in mothers of control children were not significantly different from rates observed in mothers of cases (36% vs. 46%, $p = 0.72$).

There were no significant associations between bacterial colonization in mothers and children. *S. pneumoniae* was more often detected in mothers of children colonized by *S. pneumoniae* than in mothers of children not colonized by *S. pneumoniae*, but this was not statistically significant (39% vs. 20%, $p = 0.098$, Table 3).

Co-occurrence of bacteria and respiratory viruses

The effect of the co-occurrence of bacteria and viruses on the risk of ARTI was investigated by dividing the data according to whether at least one vs. no bacterial pathogen was detected. This showed no significant difference in the

Table 3 Determinants of *S. pneumoniae* colonization in Eñepa Amerindian children and mothers.

		S. pneumoniae present	*S. pneumoniae* not present	p-value
Children	Demographic figures			
	Sex, *n* (%)			0.84
	Female	31 (53)	10 (50)	
	Male	28 (47)	10 (50)	
	Age (years), mean (SD)	3.9 (2.8)	4.7 (3.2)	0.31
	Number of people in household, median (IQR)	5 (4—6)	5 (4—6)	0.73
	Nutritional status			
	Weight-for-height Z score in children < 5 years, mean (SD) BMI-for-age Z score in children ≥5 years, mean (SD)	−0.94 (2.27)	−1.26 (2.23)	0.59
	Height-for-age Z score in children of all ages, mean (SD)	−2.08 (1.97)	−1.03 (2.42)	0.056
	Malnourished, *n* (%)	45 (76)	10 (50)	0.027
	History of breastfeeding, *n* (%)	50 (91)	20 (100)	0.32
	Mother colonized with *S. pneumoniae*	23 (39)	4 (20)	0.098
	Respiratory tract infection			
	Any respiratory tract infection (ARTI + AOM), *n* (%)	35 (59)	4 (20)	<0.01
	ARTI, *n* (%)	31 (53)	2 (10)	<0.01
	ALRTI, *n* (%)	22 (37)	1 (5)	<0.01
	URTI, *n* (%)	9 (15)	1 (5)	0.44
	AOM, *n* %)	19 (32)	2 (10)	0.052
Mothers	Demographic figures			
	Age (years), mean (SD)	29.8 (8.9)	30.3 (9.7)	0.86
	Number of people in household, median (IQR)	4.5 (4—6)	5 (4—7)	0.041
	Nutritional status			
	BMI, mean (SD)	20.9 (3.44)	23.4 (4.06)	0.049
	Malnourished, *n* (%)	2 (13)	2 (8)	0.63

prevalence of a viral infection in addition to bacterial colonization between cases and controls (31% vs. 26%, $p = 0.80$). Children that suffered from a viral infection in addition to colonization with a bacterial pathogen were significantly younger than children in which a bacterial pathogen alone, without viral co-infection, was detected (3.4 years vs. 4.8 years, $p = 0.043$). Of the children younger than 4 years of age with bacterial colonization 46% suffered from a viral co-infection compared to 17% of the children aged 4–10 years ($p < 0.01$).

Discussion

In Eñepa Amerindian children, acute malnutrition and *S. pneumoniae* colonization were significantly associated with the presence of an ARTI in multivariate analysis. *S. pneumoniae* carriage rates were high in children up to 10 years of age and chronic malnutrition was significantly associated with an increased risk for *S. pneumoniae* colonization. A viral infection in addition to colonization by respiratory tract bacteria occurred significantly more often in children under 4 years of age. To our knowledge, this is the first report describing the detection of both respiratory viruses and bacteria in South American indigenous children.

In case–control studies performed in Vietnam, Turkey and Greenland a significant association of *S. pneumoniae* colonization with ARTIs was also observed.[7,38,39] In healthy children, *S. pneumoniae* carriage rates observed in studies performed in industrialized areas before the introduction of pneumococcal vaccines decrease from 30% to 40% in the first 5 years of life to below 20% in children aged 6–10 years.[40–42] In a study performed in another Venezuelan Amerindian population, the Warao people, *S. pneumoniae* carriage rates in children under 2 years of age were higher compared to carriage rates in children older than 2 years of age (50% vs. 25%).[43] However, in other tropical rural areas *S. pneumoniae* carriage rates up to 40% have been observed in healthy children between 5 and 10 years of age.[44–46] We observed a *S. pneumoniae* carriage rate of 50% in healthy controls older than 5 years of age. The high *S. pneumoniae* colonization rate in Eñepa Amerindians increases the size of the pneumococcal reservoir in these communities. As in these populations approximately half of the population is less than 15 years of age, those children aged 5–10 years represent a sizeable fraction of the total population and one that is likely to interact frequently with young children who are most susceptible to the development of disease after nasopharyngeal colonization.[5]

Carriage rates of *S. pneumoniae* in Eñepa mothers were extremely high (38%) compared to the carriage rates of 7%–9% that are observed in mothers and other close adult contacts in cross-sectional studies from industrialized as well as rural areas.[45–47] A previously performed study in the Eñepa Amerindian population during the dry season showed a prevalence of *S. pneumoniae* colonization in mothers of children under 5 years of age of only 11%.[48] This suggests that the high prevalence observed in our study was due to seasonal influences. Longitudinal studies have demonstrated seasonal variations in the rate of pneumococcal colonization and rainy season has been identified as a significant risk factor for *S. pneumoniae* carriage in children and adults.[16,44,49,50] However, the prevalence of *S. pneumoniae* colonization observed in healthy Eñepa children under 5 years of age in the previous study (69%) was similar to the rate we observed in healthy controls up to 5 years of age (70%). Another possible explanation for the high carriage rate in mothers in our study is the observation that in 81% of the *S. pneumoniae* positive mothers, pneumococci were only isolated from the oropharyngeal sample. In the previously performed study, oropharyngeal samples were not taken.[48] The percentage of *S. pneumoniae* positive children in which *S. pneumoniae* was isolated from the oropharyngeal sample alone was lower (12%). The importance of oropharyngeal sampling for detection of *S. pneumoniae* and *H. influenzae* in children and mothers was previously demonstrated by Greenberg et al.[22] However, in contrast to their findings, in our study no *H. influenzae* was isolated from the oropharyngeal sample alone. This might be due to the high number of children with ARTI in our study, as *H. influenzae* detection in the nasopharyngeal swab increased during illness in the study of Greenberg et al.[22]

We did not study the molecular characteristics of identified pathogens. As HRV was the viral infection with the highest prevalence in our study population, it would be of interest to perform phylogenetic analysis of the HRV strains circulating in this population in order to compare the strains detected in this area with published HRV sequences from other parts of the world.[51] Respiratory viruses were, however, not significantly associated with ARTIs in our study. *S. pneumoniae* carriage was significantly associated with ARTIs in our study and further characterization of *S. pneumoniae* isolates by serotyping, molecular typing and susceptibility testing is recommendable to obtain insight into resistance patterns and pneumococcal vaccination coverage in this population.

Carriage rates of *S. pneumoniae* in children included in our study were significantly increased in chronically malnourished children. Due to the case–control study design, it was unknown whether chronic malnutrition is a risk factor for *S. pneumoniae* colonization or whether *S. pneumoniae* carriage affects growth leading to growth deficits and chronic malnutrition. In a longitudinal study including South Indian infants, *S. pneumoniae* carriage was significantly associated with increased odds of chronic malnutrition (OR 3.1, 95% CI 1.3–7.4), suggesting that *S. pneumoniae* colonization affects growth.[52] In Bangladesh, immunization of infants with a pneumococcal conjugate vaccine (PCV7) was independently associated with an increase in height-for-age.[53] Acute malnutrition was significantly associated with ARTI risk in our study. A significant relationship between acute malnutrition and respiratory tract infections has also been observed in other studies and undernutrition, either acute or chronic, negatively affects disease outcome.[1,54–56]

While bacterial colonization was not associated with the age of the child in our study, children with viral-bacterial co-occurrence were significantly younger than children with bacterial colonization alone. A mixed nasopharyngeal flora containing respiratory viruses and bacteria was also significantly more often observed in children under 4 years of age compared to children aged 5–8 years in Greenland.[39] Interestingly, we did not observe a significant association of respiratory viral infections with ARTI risk. In studies

performed in developed countries, viruses have been identified as the potential etiological cause in more than half of the ARTI cases.[57,58] It is important to know the relative contributions of viruses and bacteria to the burden of ARTI and AOM in different populations to ensure appropriate population-specific case management and preventive strategies, including vaccination.

Conclusions from the present study must be tempered by an appreciation of limitations. As we matched cases with sibling controls, we may underestimate the effect of factors related to residence and household exposure. An example of a factor related to residence that has been associated with ARTIs is exposure to biomass smoke.[55,59] Larger cross-sectional studies are needed to examine the associations between household-related factors and ARTI risk. Additionally, the magnitude of the associations between demographic and nutritional variables and *S. pneumoniae* colonization in our study may differ from the effect magnitude in the general population as our study population was not a representative sample of the general population of Eñepa Amerindian children due to the case—control study design. Finally, confidence intervals of the OR determined in multivariate analyses of factors associated with ARTI and *S. pneumoniae* colonization were wide due to the small sample size of our study. Rural areas are generally difficult to reach during the winter season due to heavy rain falls, limiting possibilities for field workers and researchers. The communities included in our study were very isolated communities that could be reached only after several hours of walking through an undulating forest-savanna area as heavy rains had flooded area roads. As most respiratory viruses circulate in the rainy season,[60,61] this is the preferred period for research focusing on the correlation of respiratory tract bacteria with viral infections.

In conclusion, our study shows that high *S. pneumoniae* carriage rates in Eñepa Amerindian children up to 10 years of age are a risk factor for ARTI while viral infections were not significantly associated with ARTI risk. These data highlight the need for the further investigation of relative contributions of viruses and bacteria to the burden of ARTI in South American indigenous children, taking into consideration population-specific epidemiological and anthropometric characteristics.

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References

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. *Lancet* 2005;**365**(9465):1147—52.
2. Schlaudecker EP, Steinhoff MC, Moore SR. Interactions of diarrhea, pneumonia, and malnutrition in childhood: recent evidence from developing countries. *Curr Opin Infect Dis* 2011; **24**(5):496—502.
3. Watt JP, Wolfson LJ, O'Brien KL, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *Lancet* 2009;**374**:903—11.
4. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* 2009;**374**:893—902.
5. Bogaert D, de Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *Lancet Infect Dis* 2004;**4**(3):144—54.
6. Diavatopoulos DA, Short KR, Price JT, Wilksch JJ, Brown LE, Briles DE, et al. Influenza A virus facilitates *Streptococcus pneumoniae* transmission and disease. *FASEB J* 2010;**24**(6): 1789—98.
7. Vu HT, Yoshida LM, Suzuki M, Nguyen HA, Nguyen CD, Nguyen AT, et al. Association between nasopharyngeal load of *Streptococcus pneumoniae*, viral coinfection, and radiologically confirmed pneumonia in Vietnamese children. *Pediatr Infect Dis J* 2011;**30**(1):11—8.
8. Pettigrew MM, Gent JF, Revai K, Patel JA, Chonmaitree T. Microbial interactions during upper respiratory tract infections. *Emerg Infect Dis* 2008;**14**(10):1584—91.
9. Chorazy ML, Lebeck MG, McCarthy TA, Richter SS, Torner JC, Gray GC. Polymicrobial acute respiratory infections in a hospital-based pediatric population. *Pediatr Infect Dis J* 2013. [Published online ahead of print].
10. Moore H, Burgner D, Carville K, Jacoby P, Richmond P, Lehmann D. Diverging trends for lower respiratory infections in non-Aboriginal and Aboriginal children. *J Paediatrics Child Health* 2007;**43**(6):451—7.
11. Peck AJ, Holman RC, Curns AT, Lingappa JR, Cheek JE, Singleton RJ, et al. Lower respiratory tract infections among american Indian and Alaska Native children and the general population of U.S. Children. *Pediatr Infect Dis J* 2005;**24**(4): 342—51.
12. Singleton RJ, Holman RC, Folkema AM, Wenger JD, Steiner CA, Redd JT. Trends in lower respiratory tract infection hospitalizations among American Indian/Alaska Native children and the general US child population. *J Pediatr* 2012;**161**(2):296—302.
13. Binks MJ, Cheng AC, Smith-Vaughan H, Sloots T, Nissen M, Whiley D, et al. Viral-bacterial co-infection in Australian Indigenous children with acute otitis media. *BMC Infect Dis* 2011;**11**:161.
14. Morris PS, Leach AJ, Silberberg P, Mellon G, Wilson C, Hamilton E, et al. Otitis media in young aboriginal children from remote communities in Northern and Central Australia: a cross-sectional survey. *BMC Pediatr* 2005;**5**:27.
15. Bowd AD. Otitis media: health and social consequences for aboriginal youth in Canada's north. *Int J Circumpolar Health* 2005;**64**(1):5—15.
16. Watson K, Carville K, Bowman J, Jacoby P, Riley TV, Leach AJ, et al. Upper respiratory tract bacterial carriage in aboriginal and non-Aboriginal children in a semi-arid area of Western Australia. *Pediatr Infect Dis J* 2006;**25**(9):782—90.
17. Alaghehbandan R, Gates KD, MacDonald D. Hospitalization due to pneumonia among Innu, Inuit and non-Aboriginal communities, Newfoundland and Labrador, Canada. *Int J Infect Dis* 2007;**11**(1):23—8.
18. Bhat N, Tokarz R, Jain K, Haq S, Weatherholtz R, Chandran A, et al. A prospective study of agents associated with acute respiratory infection among young American Indian children. *Pediatr Infect Dis J* 2013. [Published online ahead of print].

19. San SM, Hurtig AK. Review of health research on indigenous populations in Latin America, 1995–2004. *Salud Publica Mex*. 2007;**49**(4):316–20.
20. Aboriginal Peoples, 2006 Census. *Aboriginal peoples in Canada in 2006: Inuit, Métis and First Nations*. Statistics Canada Catalogue no. 97-558-XIE. Available from: www.statcan.ca; 2006 Census.
21. Humes KR, Jones NA, Ramirez RR. *Overview of race and hispanic origin: 2010*. Census Briefs 2010. United States Census Bureau. Available from:, www.census.gov/population/www/cen2010/glance/index.html; March 2011.
22. Greenberg D, Broides A, Blancovich I, Peled N, Givon-Lavi N, Dagan R. Relative importance of nasopharyngeal versus oropharyngeal sampling for isolation of *Streptococcus pneumoniae* and *Haemophilus influenzae* from healthy and sick individuals varies with age. *J Clin Microbiol* 2004;**42**(10):4604–9.
23. O'Brien KL, Bronsdon MA, Dagan R, Yagupsky P, Janco J, Elliott J, et al. Evaluation of a medium (STGG) for transport and optimal recovery of *Streptococcus pneumoniae* from nasopharyngeal secretions collected during field studies. *J Clin Microbiol* 2001;**39**(3):1021–4.
24. Fleming S, Thompson M, Stevens R, Heneghan C, Pluddemann A, Maconochie I, et al. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. *Lancet* 2011;**377**(9770):1011–8.
25. World Health Organization (WHO). *Acute respiratory infections in children: case management in small hospitals in developing countries*. Available from: https://apps.who.int/chd/publications/ari/mgt_ari/ws28095m.htm; 1990.
26. Subcommittee of the American Academy of Family Physicians, the American College of Chest Physicians, and the American Thoracic Society. Diagnosis and management of bronchiolitis. *Pediatrics* 2006;**118**(4):1774–93.
27. Subcommittee of the American Academy of Pediatrics and American Academy of Family Physicians. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**(5):1451–65.
28. Chapin KC, Lauderdale T, Funke G. Algorithms for identification of aerobic gram-positive cocci and aerobic gram-negative bacteria. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. *Manual of clinical microbiology*. 9th ed. Washington DC: ASM Press; 2007.
29. Stol K, Verhaegh SJ, Graamans K, Engel JA, Sturm PD, Melchers WJ, et al. Microbial profiling does not differentiate between childhood recurrent acute otitis media and chronic otitis media with effusion. *Int J Pediatr Otorhinolaryngol* 2013;**77**(4):488–93.
30. Balasuriya UB, Leutenegger CM, Topol JB, McCollum WH, Timoney PJ, MacLachlan NJ. Detection of equine arteritis virus by real-time TaqMan reverse transcription-PCR assay. *J Virol Methods* 2002;**101**(1–2):21–8.
31. De Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* 2007;**85**(9):660–7.
32. World Health Organization (WHO)Multicentre Growth Reference Study Group. *WHO child growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development*. Available from: http://www.who.int/childgrowth/standards/technical_report/en/; 2006.
33. World Health Organization (WHO). *Anthro and AnthroPlus for personal computers manual: software for assessing growth of the world's children and adolescents*. Available from: http://www.who.int/childgrowth/software/en/; 2009.
34. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;**371**(9608):243–60.
35. Duggan MB. Anthropometry as a tool for measuring malnutrition: impact of the new WHO growth standards and reference. *Ann Trop Paediatr* 2010;**30**(1):1–17.
36. The Harmonised Training Package (HTP). *Resource material for training on nutrition in emergencies, version 2. Nutrition works, emergency nutrition network, global nutrition cluster*. Available from: http://www.unscn.org/en/gnc_htp/; 2011.
37. World Health Organization (WHO). Global database on Body Mass Index. Available from: http://apps.who.int/bmi/.
38. Ciftci E, Dogru U, Aysev D, Ince E, Guriz H. Nasopharyngeal colonization with penicillin-resistant *Streptococcus pneumoniae* in Turkish children. *Pediatr Int* 2000;**42**(5):552–6.
39. Homoe P, Prag J, Farholt S, Henrichsen J, Hornsleth A, Kilian M, et al. High rate of nasopharyngeal carriage of potential pathogens among children in Greenland: results of a clinical survey of middle-ear disease. *Clin Infect Dis* 1996;**23**(5):1081–90.
40. Christenson B, Sylvan SP, Noreen B. Carriage of multiresistant *Streptococcus pneumoniae* among children attending day-care centres in the Stockholm area. *Scand J Infect Dis* 1997;**29**(6):555–8.
41. Bogaert D, van Belkum A, Sluijter M, Luijendijk A, de Groot R, Rumke HC, et al. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet* 2004;**363**(9424):1871–2.
42. Bae S, Yu JY, Lee K, Lee S, Park B, Kang Y. Nasal colonization by four potential respiratory bacteria in healthy children attending kindergarten or elementary school in Seoul, Korea. *J Med Microbiol* 2012;**61**(Pt 5):678–85.
43. Rivera-Olivero IA, Bogaert D, Bello T, del Nogal B, Sluijter M, Hermans PW, et al. Pneumococcal carriage among indigenous Warao children in Venezuela: serotypes, susceptibility patterns, and molecular epidemiology. *Clin Infect Dis* 2007;**45**(11):1427–34.
44. Abdullahi O, Nyiro J, Lewa P, Slack M, Scott AG. The descriptive epidemiology of *Streptococcus pneumoniae* and *Haemophilus influenzae* nasopharyngeal carriage in children and adults in kilifi district, Kenya. *Pediatr Infect Dis J* 2008;**27**:59–64.
45. Granat SM, Mia Z, Ollgren J, Herva E, Das M, Piirainen L, et al. Longitudinal study on pneumococcal carriage during the first year of life in Bangladesh. *Pediatr Infect Dis J* 2007;**26**(4):319–24.
46. Borer A, Meirson H, Peled N, Porat N, Dagan R, Fraser D, et al. Antibiotic-resistant pneumococci carried by young children do not appear to disseminate to adult members of a closed community. *Clin Infect Dis* 2001;**33**(4):436–44.
47. Vives M, Garcia ME, Saenz P, Mora MA, Mata L, Sabharwal H, et al. Nasopharyngeal colonization in Costa Rican children during the first year of life. *Pediatr Infect Dis J* 1997;**16**(9):852–8.
48. Bello González T, Rivera-Olivero IA, Pocaterra L, Spadola E, Araque M, Hermans PWM, et al. Estado de portador nasofaríngeo de *Streptococcus pneumoniae* en madres e hijos de la población indígena Panare del estado Bolívar, Venezuela. *Revista Argentina de Microbiología* 2010;**42**:30–4.
49. Tigoi CC, Gatakaa H, Karani A, Mugo D, Kungu S, Wanjiru E, et al. Rates of acquisition of pneumococcal colonization and transmission probabilities, by serotype, among newborn infants in Kilifi District, Kenya. *Clin Infect Dis* 2012;**55**(2):180–8.
50. Korona-Glowniak I, Malm A. Characteristics of *Streptococcus pneumoniae* strains colonizing upper respiratory tract of healthy preschool children in Poland. *ScientificWorldJournal* 2012;**2012**:732901.

51. Arden KE, Mackay IM. Newly identified human rhinoviruses: molecular methods heat up the cold viruses. *Rev Med Virol* 2010;**20**(3):156—76.
52. Coles CL, Rahmathullah L, Kanungo R, Katz J, Sandiford D, Devi S, et al. Pneumococcal carriage at age 2 months is associated with growth deficits at age 6 months among infants in South India. *J Nutr* 2012;**142**(6):1088—94.
53. Steinhoff MC, Omer SB, Roy E, El Arifeen S, Raqib R, Dodd C, et al. *Association of infant pneumococcal immunization with infant growth in Asia*. Presented at: Pediatric Academic Society Meeting in Vancouver, British Colombia, Canada April 30—May 3, 2010.
54. Chisti MJ, Tebruegge M, La Vincente S, Graham SM, Duke T. Pneumonia in severely malnourished children in developing countries - mortality risk, aetiology and validity of WHO clinical signs: a systematic review. *Trop Med Int Health* 2009; **14**(10):1173—89.
55. Bhat RY, Manjunath N. Correlates of acute lower respiratory tract infections in children under 5 years of age in India. *Int J Tuberc Lung Dis* 2013;**17**(3):418—22.
56. Shell-Duncan B, Wood JW. The evaluation of delayed-type hypersensitivity responsiveness and nutritional status as predictors of gastro-intestinal and acute respiratory infection: a prospective field study among traditional nomadic Kenyan children. *J Trop Pediatr* 1997;**43**(1):25—32.
57. Renois F, Lévêque N, Deliège PG, Fichel C, Bouin A, Abely M, et al. Enteroviruses as major cause of microbiologically unexplained acute respiratory tract infections in hospitalized pediatric patients. *J Infect* 2013;**66**(6):494—502.
58. Antunes H, Rodrigues H, Silva N, Ferreira C, Carvalho F, Ramalho H, et al. Etiology of bronchiolitis in a hospitalized pediatric population: prospective multicenter study. *J Clin Virol* 2010;**48**(2):134—6.
59. Taylor ET, Nakai S. Prevalence of acute respiratory infections in women and children in Western Sierra Leone due to smoke from wood and charcoal stoves. *Int J Environ Res Public Health* 2012;**9**(6):2252—65.
60. Alonso WJ, Laranjeira BJ, Pereira SA, Florencio CM, Moreno EC, Miller MA, et al. Comparative dynamics, morbidity and mortality burden of pediatric viral respiratory infections in an equatorial city. *Pediatr Infect Dis J* 2012;**31**(1):e9—14.
61. Dowell SF, Whitney CG, Wright C, Rose Jr CE, Schuchat A. Seasonal patterns of invasive pneumococcal disease. *Emerg Infect Dis* 2003;**9**(5):573—9.