



Serologic Analysis of Severe Respiratory Infections in HIV-Exposed Uninfected Infants

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ABSTRACT

Background: HIV-exposed uninfected (HEU) infants have increased rates of severe lower respiratory tract infection (LRTI), sepsis, hospitalization and death. We examined the incidence of LRTI in HEU according to maternal antibody transfer and infant antibody production.

Methods: We enrolled 247 HEU and 88 HIV-unexposed uninfected (HUU) Brazilian infant/mother pairs, including 107 HEU and 16 HUU with LRTI in the first 6 months of life. Antibodies to the following agents were measured by ELISA: respiratory syncytial virus (RSV) and pneumococcus (PNC) 1, 5, 6, 14 in mothers (delivery) and infants (0, 6 months); influenza A (Flu) and parainfluenza viruses (PIV) 1, 2, 3 (infants 0, 6 months); tetanus toxoid (infants 6 months).

Results: Compared to HUU, HEU infants had lower antibody levels at birth for all respiratory agents (p<0.0001), although maternal antibodies to PNC and RSV did not differ by HIV status. Transplacental transfer of maternal antibodies was lower for RSV in HEU vs. HUU (mean±SD ratios=1.3±3.5 vs. 1.8±0.8; p=0.05). Infant: mother PNC antibody ratios were <1 in both HEU and HUU, but the differences between HEU and HUU were not statistically significant. Compared to mothers of LRTI-, those of LRTI+ HEU had higher antibody levels to PNC 1 and 6 and those of LRTI+ HUU to PNC 5 and 14 (p≤0.04). Flu, PIV, RSV and PNC antibodies at birth were similar in LRTI+ vs. LRTI- HEU or HUU, except for higher PNC 5 and 14 levels in LRTI+ vs. LRTI- HUU (p≤0.05). At 6 months, HEU and HUU had similar antibody responses to tetanus vaccine regardless of LRTI status. After controlling for birth levels, HEU had lower RSV (p<0.001), higher PIV 1, 2, 3 (p<0.001) and similar Flu antibodies (p=0.11) compared with HUU at 6 months. At 6 months, LRTI+ HEU had higher anti-RSV antibody levels (p=0.08) and rates of seroconversion to ≥1, 2 or 3 paramyxoviruses (p=0.05, 0.02 and 0.06, respectively) than LRTI- HEU.

Conclusions: The incidence of LRTI in HEU infants correlated with the frequency of paramyxovirus infections, but not with low levels of transferred maternal antibodies or with infant failure to make antibodies in response to infections or vaccines. The higher maternal PNC antibody levels in LRTI+ vs. LRTI- infants suggested that mothers of LRTI+ infants had higher rates of PNC infection and/or carriage increasing infant exposure to PNC and possibly contributing to LRTI morbidity. Collectively, our data suggest that environmental factors and innate and/or cell-mediated immune defects predispose HEU to LRTI.

INTRODUCTION

- Interruption of HIV vertical transmission has been one of the major recent achievements in the area of HIV treatment and prevention. Due to the large contingent of women of reproductive age already infected with HIV, HIV-exposed uninfected (HEU) infants are a rapidly growing population. In utero exposure to HIV has deleterious consequences to the infant such as greater morbidity and mortality than HIV-unexposed uninfected (HUU) infants, especially due to infections.
- Lower respiratory tract infection (LRTI) has been the leading etiology of severe infections leading to hospitalization and death of HEU, followed by gastroenteritis, sepsis, and malaria.
- The mechanisms responsible for the increased risk of severe infections in HEU are not known, but they are likely to involve dysfunctional infant immune responses. In addition there is very little information on the causes of LRTI outside Europe.

OBJECTIVES

- To describe microbiologic agents associated with LRTI among HIV exposed uninfected infants and to describe transfer of specific maternal antibodies;
- To compare immune response of HIV exposed uninfected infants to infants born to healthy mothers.

METHODS

Participants:

□ Brazilian HEU enrolled in NISDI Perinatal and LILAC protocols between 2002 and 2009 who met the inclusion criteria listed below were identified.

- Infant from first on-study pregnancy
- Singleton
- Followed up to ≥6 months
- HIV negative
- Born with birth weight ≥2500g and ≥ 37 weeks of gestation
- Without birth defects, cardiovascular or pulmonary diseases

HEU who developed LRTI before 6 months of life (LRTI+) were matched with LRTI- HEU from the same site, with the same age (+/- 2 months).

□ A group of infants born to HIV-uninfected mothers who did not plan to breastfeed were enrolled in 2011 as HUU controls (CIRAI).

METHODS (CONTINUED)

Definitions:

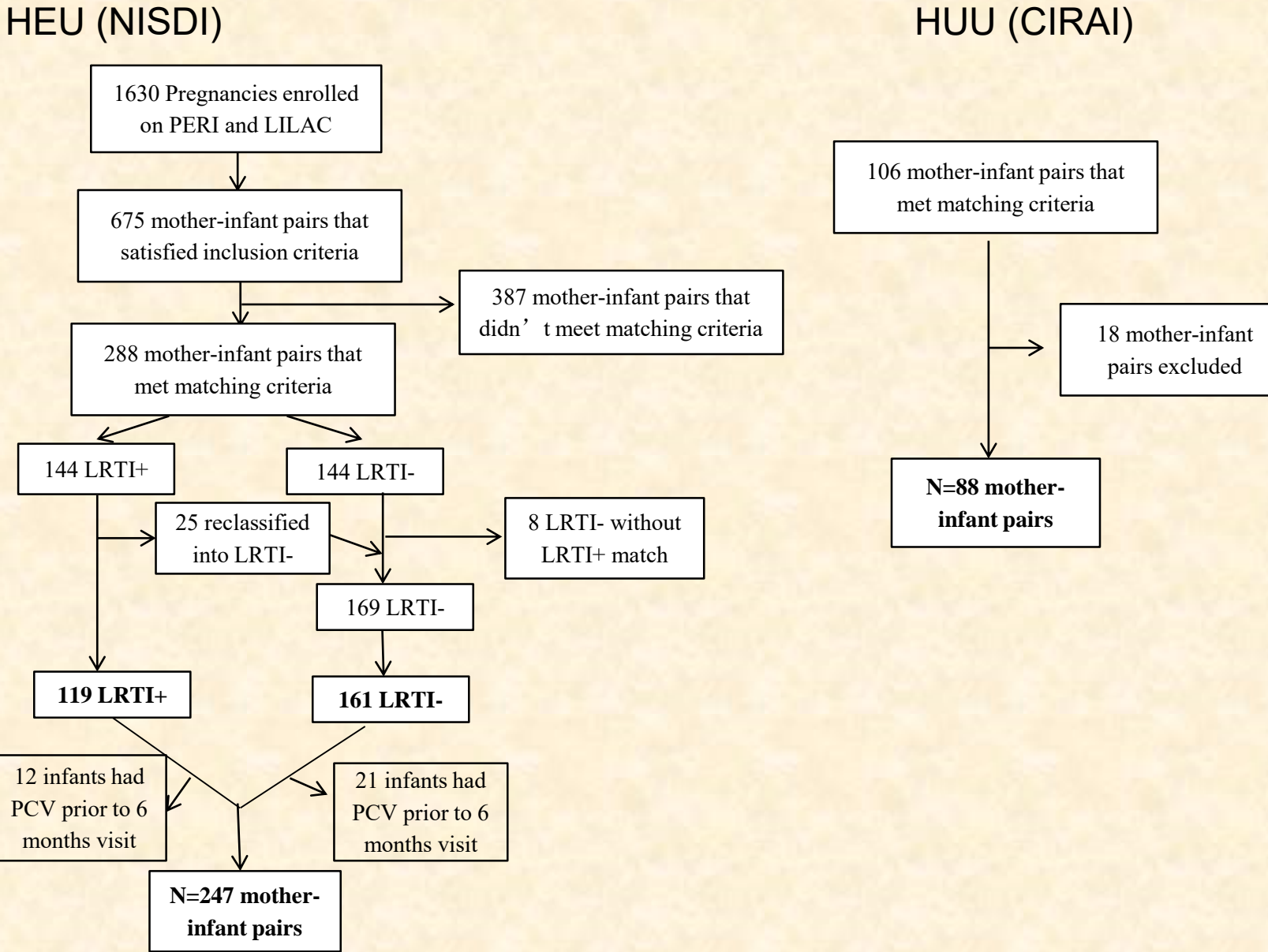
- Antibodies anti-influenza A, respiratory syncytial virus (RSV), parainfluenza 1, 2 and 3 (PIV 1, 2, and 3) and tetanus were performed quantitatively using commercial kits according to the manufacturer's instructions.
- Antibodies against pneumococci (PNC) 1, 5, 6 and 14 were performed as previously described by Quataert et al. 2001 Immunol Invest 30: 191 and Inostroza et al. 2005 Lab Immunol 12:722.

Statistical Analysis:

- Univariate statistics used to describe population characteristics
- Mantel-Haenszel estimate stratified by matched set used to obtain odds ratio for matched LRTI+/- subjects
- Chi-square/Fisher's exact test used for comparisons for categorical variables; T-test used for comparisons for continuous variables
- Linear regression model used to adjust for baseline values for antibody titers at 6 months.

RESULTS

Study Population Derivation



Population Characteristics

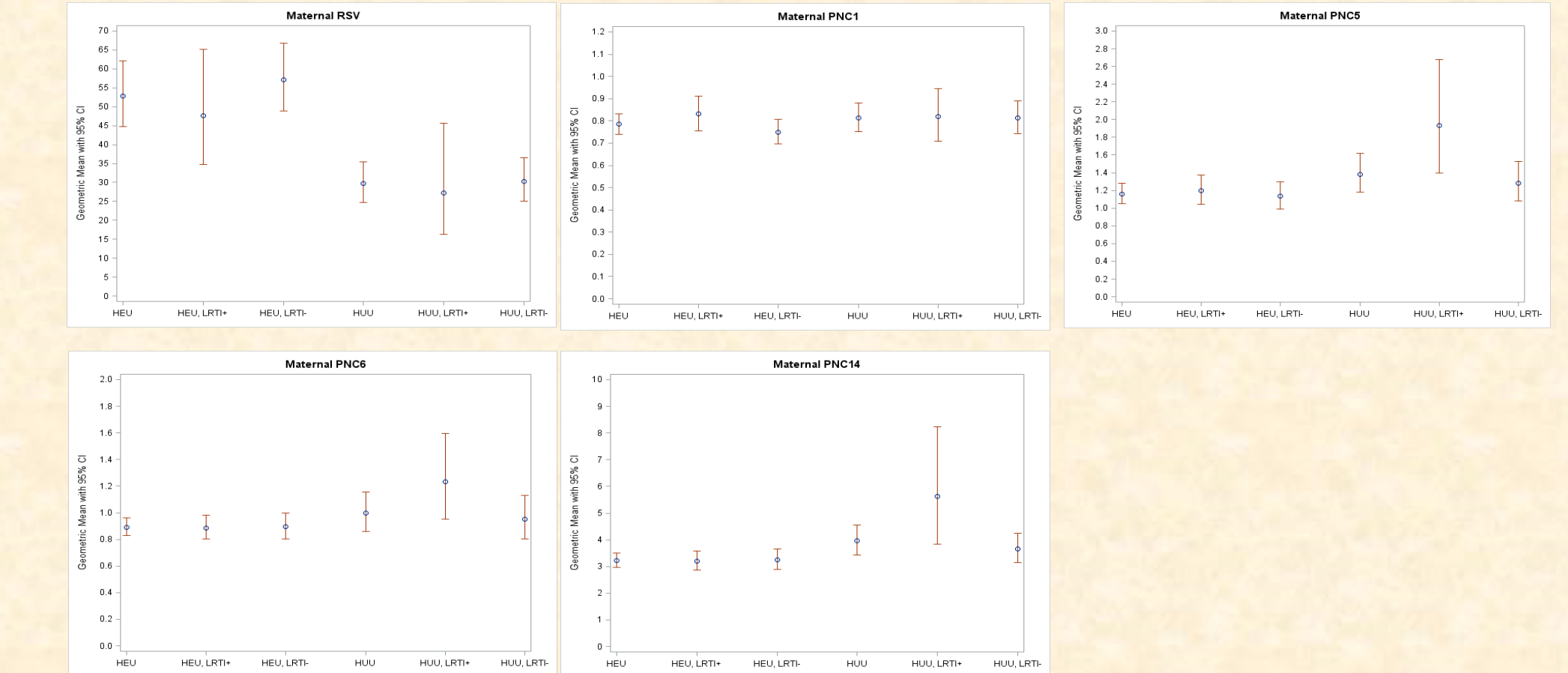
a. Maternal Population

		Total (N = 247)	HEU LRTI+ (N=107)	LRTI- (N=140)	Total (N=88)	HUU LRTI+ (N=16)	LRTI- (N=72)	P-value HEU vs. HUU LRTI+ vs. - HEU	
RACE	Black	37 (16.4)	14 (14.7)	23 (17.7)	7 (8.0)	0 (0.0)	7 (9.7)	<.0001	0.24
	Mestizo	25 (11.1)	6 (6.3)	19 (14.6)	0 (0.0)	0 (0.0)	0 (0.0)		
	Other	8 (3.6)	3 (3.2)	5 (3.8)	15 (17.0)	4 (25.0)	11 (15.3)		
	Unknown	55 (24.4)	26 (27.4)	29 (27.4)	0 (0.0)	0 (0.0)	0 (0.0)		
	White	100 (44.4)	46 (48.4)	54 (41.5)	66 (75.0)	12 (75.0)	66 (75.0)		
Alcohol during pregnancy	Yes	49 (20.0)	19 (17.8)	30 (21.7)	9 (10.2)	0 (0.0)	9 (12.5)	0.05	0.52
	No	196 (80.0)	88 (82.2)	108 (78.3)	79 (89.8)	16 (100.0)	63 (87.5)		
Tobacco during pregnancy	Yes	58 (23.8)	21 (19.6)	37 (27.0)	12 (13.6)	2 (12.5)	10 (13.9)	0.05	0.23
	No	186 (76.2)	86 (80.4)	100 (73.0)	76 (86.4)	14 (87.5)	62 (86.1)		
Marijuana during pregnancy	Yes	9 (3.8)	3 (2.8)	6 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0.12	0.73
	No	229 (96.2)	103 (97.2)	126 (95.5)	88 (100)	16 (100.0)	72 (100.0)		
Crack/Cocaine during pregnancy	Yes	8 (3.4)	4 (3.8)	4 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.11	1.00
	No	230 (96.6)	102 (96.2)	128 (97.0)	88 (100)	16 (100.0)	72 (100.0)		
Other recreational drugs during pregnancy	No	37 (100)	12 (100)	25 (100)	88 (100)	16 (100.0)	72 (100.0)	-	-
	Mean (std)	26.92 (5.49)	26.32 (4.97)	27.37 (5.84)	25.69 (5.82)	23.94 (4.70)	26.08 (6.00)	0.08	0.14
Maternal age at enrollment	Mean (std)	26.92 (5.49)	26.32 (4.97)	27.37 (5.84)	25.69 (5.82)	23.94 (4.70)	26.08 (6.00)	0.08	0.14
Years of education	Mean (std)	7.36 (3.21)	6.87 (3.17)	7.74 (3.20)	9.28 (2.43)	8.44 (2.53)	9.47 (2.38)	<.0001	0.04
	Mean (std)	2.28 (2.05)	2.28 (2.21)	2.29 (1.93)	1.11 (1.20)	1.13 (0.96)	1.11 (1.25)	<.0001	0.98
# prior pregnancies	Mean (std)	2.28 (2.05)	2.28 (2.21)	2.29 (1.93)	1.11 (1.20)	1.13 (0.96)	1.11 (1.25)	<.0001	0.98
	Mean (std)	1.73 (1.63)	1.74 (1.54)	1.73 (1.71)	1.06 (0.93)	0.92 (1.15)	0.92 (1.15)	<.0001	0.96
Parity	Mean (std)	1.73 (1.63)	1.74 (1.54)	1.73 (1.71)	1.06 (0.93)	0.92 (1.15)	0.92 (1.15)	<.0001	0.96
# of people in household	Mean (std)	4.15 (2.46)	4.42 (3.03)	3.95 (1.89)	4.53 (1.65)	4.31 (1.58)	4.58 (1.68)	0.11	0.14

b. Infant Population

		Total (N = 247)	HEU LRTI+ (N=107)	LRTI- (N=140)	Total (N=88)	HUU LRTI+ (N=16)	LRTI- (N=72)	P-value HEU vs. HUU LRTI+ vs. - HEU	
Gender	Female	100 (40.5)	38 (35.5)	62 (44.3)	43 (48.9)	10 (62.5)	33 (45.8)	0.21	0.19
	Male	147 (59.5)	69 (64.5)	78 (55.7)	45 (51.1)	6 (37.5)	39 (54.2)		
Small for gestational age (<10th percentile)	Yes	8 (3.2)	4 (3.7)	4 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0.12	0.72
	No	239 (96.8)	103 (96.3)	136 (97.1)	88 (100)	16 (100.0)	72 (100.0)		
Large for gestational age (>90th percentile)	Yes	27 (10.9)	14 (13.1)	13 (9.3)	22 (25.0)	5 (31.3)	17 (23.6)	0.003	0.41
	No	220 (89.1)	93 (86.9)	127 (90.7)	66 (75.0)	11 (68.7)	55 (76.4)		
Breast feeding	Yes	-	-	-	1 (1.1)	0 (0.0)	1 (1.4)	-	-
	No	-	-	-	87 (98.9)	16 (100.0)	71 (98.6)		
Gestational Age at birth (wks)	Mean (std)	38.74 (1.14)	38.59 (1.10)	38.85 (1.16)	39.32 (1.41)	38.81 (1.28)	39.43 (1.42)	0.001	0.07
	Mean (std)	3159.19 (378.72)	3145.5 (391.7)	3169.6 (369.6)	3382.22 (390.92)	3415.0 (393.6)	3374.9 (392.7)	<.0001	0.62
Duration of hospital stay at birth	Mean (std)	2.53 (0.96)	2.73 (1.20)	2.38 (0.71)	2.09 (0.97)	1.88 (0.81)	2.14 (1.00)	0.001	0.02

Maternal Antibody Titers at Delivery



Compared with HIV-uninfected mothers, HIV-infected mothers had:

- Significantly higher titers for RSV (p<0.0001); significantly lower titers for PNC14 (p=0.01); marginally lower titers for PNC5 (p=0.06).

There were no significant differences in antibody titers between mothers of LRTI+ and LRTI- HEU.

Transplacental Transfer of Maternal Antibodies

Antibody ratio at birth, mean (st.dev)	HEU (N=247)	HUU (N=88)	Difference (95% CI)	p-value
PNC1	0.93 (0.27)	0.95 (0.18)	-0.02 (-0.08, 0.04)	0.49
PNC5	0.78 (0.41)	0.86 (0.32)	-0.08 (-0.18, 0.02)	0.12
PNC6	0.82 (0.33)	0.88 (0.21)	-0.06 (-0.14, 0.01)	0.11
PNC14	0.94 (0.28)	0.99 (0.23)	-0.05 (-0.12, 0.02)	0.16
RSV	1.30 (3.51)	1.81 (0.77)	-0.51 (-1.03, 0.01)	0.05

Infant Antibody Titers Against Respiratory Pathogens

At Birth

Log-transformed, Mean (st. dev)	HEU			HUU			P-value	
	Total	LRTI+	LRTI-	Total	LRTI+	LRTI-	HEU vs. HUU	LRTI+ vs. - HEU
Influenza A	3.96 (1.27)	4.00 (1.07)	3.92 (1.41)	5.28 (0.56)	5.40 (0.42)	5.25 (0.59)	<.0001	0.67
RSV	4.35 (1.08)	4.25 (1.15)	4.42 (1.02)	3.90 (0.83)	3.77 (1.07)	3.93 (0.78)	0.0002	0.28
PIV1	1.38 (2.35)	1.24 (2.52)	1.48 (2.21)	2.42 (0.81)	2.24 (0.74)	2.46 (0.82)	<.0001	0.47
PIV2	2.04 (2.24)	1.89 (2.33)	2.16 (2.17)	3.25 (0.72)	3.26 (0.92)	3.24 (0.67)	<.0001	0.40
PIV3	2.41 (1.96)	2.44 (1.94)	2.40 (1.98)	3.27 (0.44)	3.24 (0.59)	3.28 (0.41)	<.0001	0.89
PNC1	-0.36 (0.48)	-0.22 (0.49)	-0.42 (0.46)	-0.28 (0.34)	-0.29 (0.33)	-0.28 (0.34)	0.12	0.03
PNC5	-0.21 (0.50)	-0.28 (0.47)	-0.20 (0.52)	0.08 (0.60)	0.32 (0.59)	0.03 (0.60)	<.0001	0.82
PNC6	-0.40 (0.45)	-0.38 (0.41)	-0.42 (0.48)	-0.16 (0.64)	-0.02 (0.47)	-0.19 (0.67)	0.002	0.54
PNC14	1.05 (0.55)	1.07 (0.50)	1.03 (0.58)	1.34 (0.66)	1.62 (0.71)	1.28 (0.64)	0.0004	0.67

At 6 months

Log-transformed, Mean (st. err)	HEU			HUU			P-value*	
	Total	LRTI+	LRTI-	Total	LRTI+	LRTI-	HEU vs. HUU	LRTI+ vs. - HEU
Influenza A	0.91 (0.16)	0.89 (0.21)	0.50 (0.17)	-0.72 (0.40)	0.15 (0.66)	1.13 (0.29)	0.0002	0.16
RSV	-0.46 (0.17)	0.32 (0.31)	-0.87 (0.26)	1.58 (0.25)	1.57 (0.06)	1.58 (0.03)	<.0001	0.004
PIV1	-0.13 (0.18)	0.26 (0.30)	-0.51 (0.24)	-3.63 (0.47)	-3.61 (0.56)	-3.31 (0.25)	<.0001	0.05
PIV2	-1.83 (0.23)	-1.40 (0.39)	-2.11 (0.32)	-3.62 (0.35)	-4.01 (0.58)	-3.56 (0.27)	0.001	0.16
PIV3	-0.05 (0.24)	0.37 (0.38)	-0.28 (0.31)	-3.56 (0.54)	-2.68 (0.72)	-2.68 (0.72)	<.0001	0.18
PNC1	-0.38 (0.04)	-0.37 (0.05)	-0.42 (0.04)	0.11 (0.05)	0.11 (0.13)	0.12 (0.06)	<.0001	0.51
PNC5	-0.38 (0.03)	-0.43 (0.04)	-0.34 (0.03)	-0.02 (0.04)	0.07 (0.14)	-0.03 (0.06)	<.0001	0.11
PNC6	-0.47 (0.05)	-0.54 (0.06)	-0.52 (0.05)	0.32 (0.07)	0.52 (0.21)	0.28 (0.10)	<.0001	0.87
PNC14	1.04 (0.04)	1.03 (0.06)	0.98 (0.05)	1.32 (0.05)	1.30 (0.12)	1.26 (0.05)	<.0001	0.49

*Both means and p-values were adjusted for antibody titers at birth.

RESULTS (CONTINUED)

Seroconversions to Respiratory Pathogens during the First 6 Months of Life*

	HEU Infants					HUU Infants				
	N_Seroconversion (%)			OR	p-value	N_Seroconversion (%)			OR	p-value
LRTI positive	LRTI negative	Total	LRTI positive			LRTI negative	Total			
Influenza A	3 (4.4)	5 (4.9)	8 (4.7)	0.89	0.88	0 (0.0)	1 (1.4)	1 (1.2)	0.0	1.00
RSV	6 (8.3)	6 (5.8)	12 (6.9)	1.47	0.52	0 (0.0)	1 (1.4)	1 (1.1)	0.0	1.00
PIV1	30 (42.3)	35 (33.7)	65 (37.1)	1.44	0.25	0 (0.0)	2 (2.8)	2 (2.3)	0.0	1.00
PIV2	24 (34.3)	19 (18.5)	43 (24.9)	2.31	0.02	0 (0.0)	4 (5.6)	4 (4.6)	0.0	1.00
PIV3	24 (35.8)	30 (30.0)	54 (32.3)	1.30	0.43	1 (6.3)	4 (5.6)	5 (5.7)	1.13	1.00
PNC1	59 (93.7)	87 (91.6)	146 (92.4)	1.36	0.62	na	na	na	na	na
PNC5	53 (84.1)	77 (81.1)	130 (82.3)	1.24	0.41	na	na	na	na	na
PNC6	56 (88.9)	79 (83.2)	135 (85.4)	1.62	0.32	na	na	na	na	na
PNC14	57 (90.5)	83 (87.4)	140 (88.6)	1.37	0.55	na	na	na	na	na
≥1 RSV, PIV1,2,3	46 (63.0)	56 (52.8)	102 (57.0)	2.12	0.05	16 (100.0)	66 (91.7)	82 (93.2)	-	0.59