

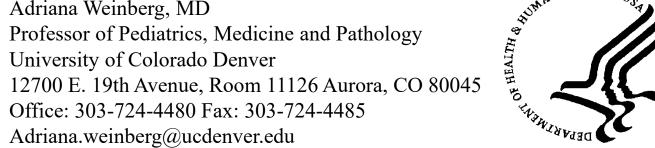


# Serologic Analysis of Severe Respiratory Infections in HIV-Exposed Uninfected Infants

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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

**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 $\le$ 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  $\geq 1$ , 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, bu 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 cellmediated 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
- 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  $\geq 2500g$  and  $\geq 37$  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)

#### • 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.

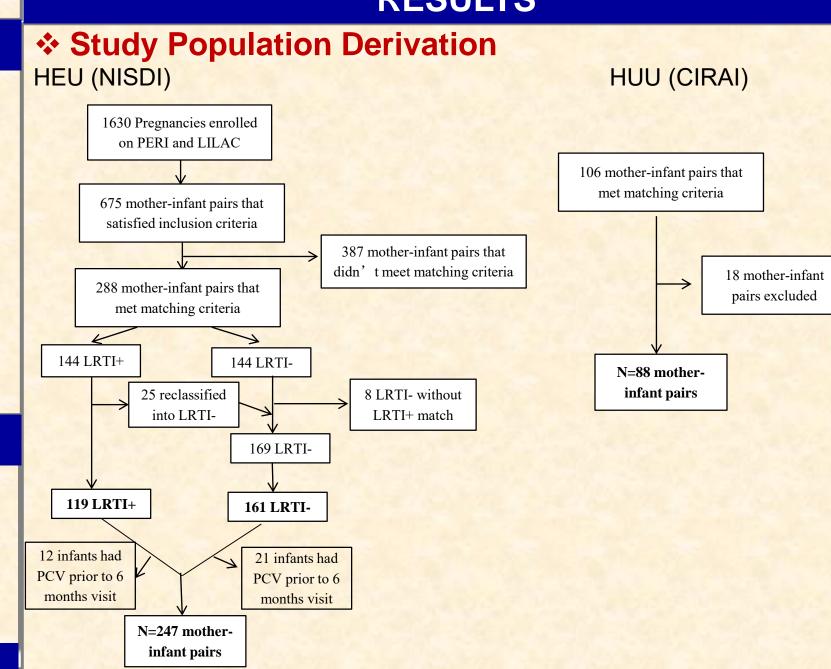
• Antibodies against pneumococci (PNC) 1, 5, 6 and 14 were performed as previously described by Quataert et al. 2001 Immunol Invest 30: 191 and Inostroza et al. 2005 Lab Immunol 12:722.

#### Statistical Analysis:

Definitions:

- Univariate statistics used to describe population characteristics
- Mantel-Haenszel estimate stratified by matched set used to obtain odds ratio for mathced 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

### RESULTS

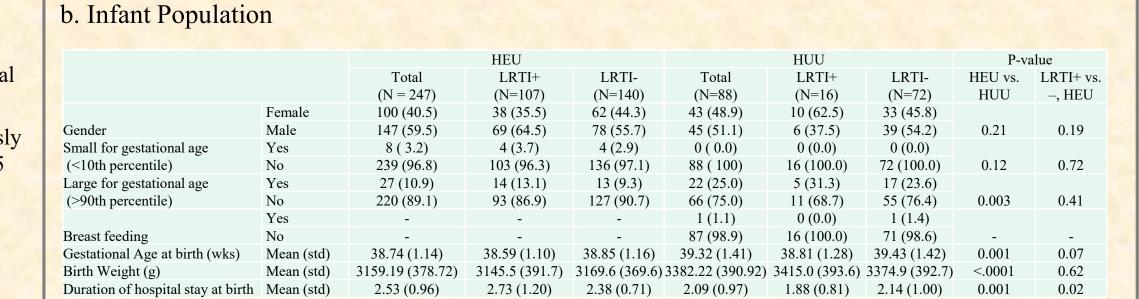


### Population Characteristics

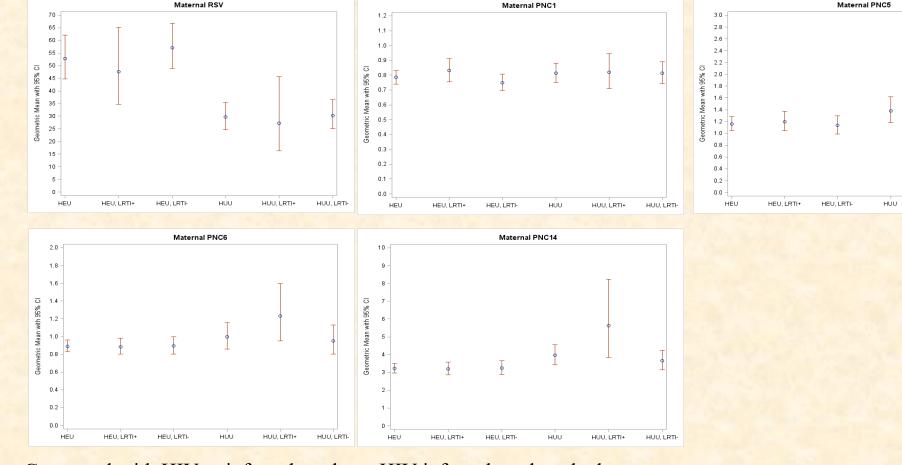
#### a. Maternal Population

			HEU			HUU		P-v	alue
		Total	LRTI+	LRTI-	Total	LRTI+	LRTI-	HEU vs.	LRTI+ vs.
		(N = 247)	(N=107)	(N=140)	(N=88)	(N=16)	(N=72)	HUU	, HEU
RACE	Black	37 (16.4)	14 (14.7)	23 (17.7)	7 (8.0)	0(0.0)	7 (9.7)		
	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)	<.0001	0.24
Alcohol during pregnancy	Yes	49 (20.0)	19 (17.8)	30 (21.7)	9 (10.2)	0(0.0)	9 (12.5)		
	No	196 (80.0)	88 (82.2)	108 (78.3)	79 (89.8)	16 (100.0)	63 (87.5)	0.05	0.52
Tobacco during	Yes	58 (23.8)	21 (19.6)	37 (27.0)	12 (13.6)	2 (12.5)	10 (13.9)		
pregnancy	No	186 (76.2)	86 (80.4)	100 (73.0)	76 (86.4)	14 (87.5)	62 (86.1)	0.05	0.23
Marijuana during	Yes	9 (3.8)	3 (2.8)	6 (4.5)	0 ( 0.0)	0(0.0)	0(0.0)		
pregnancy	No	229 (96.2)	103 (97.2)	126 (95.5)	88 ( 100)	16 (100.0)	72 (100.0)	0.12	0.73
Crack/Cocaine during pre	Yes	8 ( 3.4)	4 (3.8)	4 (3.0)	0 ( 0.0)	0(0.0)	0(0.0)		
gnancy	No	230 (96.6)	102 (96.2)	128 (97.0)	88 ( 100)	16 (100.0)	72 (100.0)	0.11	1.00
Other recreational drugs									
during pregnancy	No	37 (100)	12 (100)	25 (100)	88 ( 100)	16 (100.0)	72 (100.0)	-	-
Maternal age at	Mean			27.37 (5.84)	25.69	23.94	26.08		
enrollment	(std)	26.92 (5.49)	26.32 (4.97)		(5.82)	(4.70)	(6.00)	0.08	0.14
Years of education	Mean			7.74 (3.20)		8.44 (2.53)	9.47 (2.38)		
	(std)	7.36 (3.21)	6.87 (3.17)		9.28 (2.43)			<.0001	0.04
# prior pregnancies	Mean			2.29 (1.93)		1.13 (0.96)	1.11 (1.25)		
	(std)	2.28 (2.05)	2.28 (2.21)		1.11 (1.20)			<.0001	0.98
Parity	Mean			1.73 (1.71)		1.06(0.93)	0.92 (1.15)		
	(std)	1.73 (1.63)	1.74 (1.54)		0.94 (1.11)			<.0001	0.96
# of people in household	Mean			3.95 (1.89)		4.31 (1.58)	4.58 (1.68)		
	(std)	4.15 (2.46)	4.42 (3.03)		4.53 (1.65)			0.11	0.14

# RESULTS (CONTINUED)



# ❖ 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

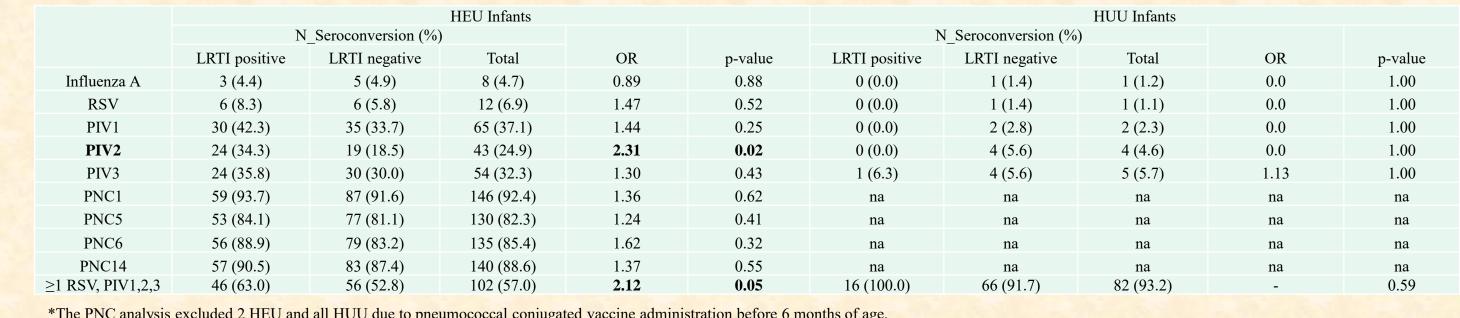
# Infant Antibody Titers Against Respiratory Pathogens

	transformed,								
	Mean (st. dev)	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)	-0.28 (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)	-0.22 (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-tr	- 4							_	
	Log-transformed,		HEU			HUU			alue*
	Log-transformed, Mean (st. err)	Total	HEU LRTI+	LRTI-	Total	HUU LRTI+	LRTI-		
	_	Total 0.91 (0.16)		LRTI- 0.50 (0.17)	Total -0.72 (0.40)	LRTI+	LRTI- 1.13 (0.29)		
	Mean (st. err)		LRTI+			LRTI+ 0.15 (0.66)		HEU vs. HUU	LRTI+ vs, HEU
	Mean (st. err) Influenza A	0.91 (0.16)	LRTI+ 0.89 (0.21)	0.50 (0.17)	-0.72 (0.40) 1.58 (0.25)	LRTI+ 0.15 (0.66)	1.13 (0.29)	HEU vs. HUU 0.0002	LRTI+ vs. –, HEU 0.16
	Mean (st. err) Influenza A RSV	0.91 (0.16) -0.46 (0.17)	LRTI+ 0.89 (0.21) 0.32 (0.31)	0.50 (0.17) -0.87 (0.26) -0.51 (0.24)	-0.72 (0.40) 1.58 (0.25) -3.62 (0.35)	LRTI+ 0.15 (0.66) 1.57 (0.06)	1.13 (0.29) 1.58 (0.03) -3.31 (0.25)	HEU vs. HUU 0.0002 <.0001	LRTI+ vs, HEU 0.16 0.004
	Mean (st. err) Influenza A RSV PIV1	0.91 (0.16) -0.46 (0.17) -0.13 (0.18)	LRTI+ 0.89 (0.21) 0.32 (0.31) 0.26 (0.30)	0.50 (0.17) -0.87 (0.26) -0.51 (0.24)	-0.72 (0.40) 1.58 (0.25) -3.62 (0.35) -3.63 (0.47)	LRTI+ 0.15 (0.66) 1.57 (0.06) -3.61 (0.56)	1.13 (0.29) 1.58 (0.03) -3.31 (0.25)	HEU vs. HUU 0.0002 <.0001 <.0001	LRTI+ vs, HEU 0.16 0.004 0.05
	Mean (st. err) Influenza A RSV PIV1 PIV2	0.91 (0.16) -0.46 (0.17) -0.13 (0.18) -1.83 (0.23)	LRTI+ 0.89 (0.21) 0.32 (0.31) 0.26 (0.30) -1.40 (0.39) 0.37 (0.38)	0.50 (0.17) -0.87 (0.26) -0.51 (0.24) -2.11 (0.32)	-0.72 (0.40) 1.58 (0.25) -3.62 (0.35) -3.63 (0.47)	LRTI+ 0.15 (0.66) 1.57 (0.06) -3.61 (0.56) -4.01 (0.58)	1.13 (0.29) 1.58 (0.03) -3.31 (0.25) -3.56 (0.27)	HEU vs. HUU 0.0002 <.0001 <.0001 0.001	LRTI+ vs, HEU 0.16 0.004 0.05 0.16
	Mean (st. err) Influenza A RSV PIV1 PIV2 PIV3	0.91 (0.16) -0.46 (0.17) -0.13 (0.18) -1.83 (0.23) -0.05 (0.24)	LRTI+ 0.89 (0.21) 0.32 (0.31) 0.26 (0.30) -1.40 (0.39) 0.37 (0.38) -0.37 (0.05)	0.50 (0.17) -0.87 (0.26) -0.51 (0.24) -2.11 (0.32) -0.28 (0.31)	-0.72 (0.40) 1.58 (0.25) -3.62 (0.35) -3.63 (0.47) -3.56 (0.54)	LRTI+ 0.15 (0.66) 1.57 (0.06) -3.61 (0.56) -4.01 (0.58) -2.68 (0.72) 0.11 (0.13)	1.13 (0.29) 1.58 (0.03) -3.31 (0.25) -3.56 (0.27) -2.68 (0.72)	HEU vs. HUU 0.0002 <.0001 <.0001 0.001 <.0001	LRTI+ vs, HEU 0.16 0.004 0.05 0.16 0.18
	Mean (st. err) Influenza A RSV PIV1 PIV2 PIV3 PNC1	0.91 (0.16) -0.46 (0.17) -0.13 (0.18) -1.83 (0.23) -0.05 (0.24) -0.38 (0.04)	LRTI+ 0.89 (0.21) 0.32 (0.31) 0.26 (0.30) -1.40 (0.39) 0.37 (0.38) -0.37 (0.05) -0.43 (0.04)	0.50 (0.17) -0.87 (0.26) -0.51 (0.24) -2.11 (0.32) -0.28 (0.31) -0.42 (0.04)	-0.72 (0.40) 1.58 (0.25) -3.62 (0.35) -3.63 (0.47) -3.56 (0.54) 0.11 (0.05)	LRTI+ 0.15 (0.66) 1.57 (0.06) -3.61 (0.56) -4.01 (0.58) -2.68 (0.72) 0.11 (0.13) 0.07 (0.14)	1.13 (0.29) 1.58 (0.03) -3.31 (0.25) -3.56 (0.27) -2.68 (0.72) 0.12 (0.06)	HEU vs. HUU 0.0002 <.0001 <.0001 0.001 <.0001 <.0001	LRTI+ vs, HEU 0.16 0.004 0.05 0.16 0.18 0.51
	Mean (st. err) Influenza A RSV PIV1 PIV2 PIV3 PNC1 PNC5	0.91 (0.16) -0.46 (0.17) -0.13 (0.18) -1.83 (0.23) -0.05 (0.24) -0.38 (0.04) -0.38 (0.03)	LRTI+ 0.89 (0.21) 0.32 (0.31) 0.26 (0.30) -1.40 (0.39) 0.37 (0.38) -0.37 (0.05) -0.43 (0.04)	0.50 (0.17) -0.87 (0.26) -0.51 (0.24) -2.11 (0.32) -0.28 (0.31) -0.42 (0.04) -0.34 (0.03)	-0.72 (0.40) 1.58 (0.25) -3.62 (0.35) -3.63 (0.47) -3.56 (0.54) 0.11 (0.05) -0.02 (0.04)	LRTI+ 0.15 (0.66) 1.57 (0.06) -3.61 (0.56) -4.01 (0.58) -2.68 (0.72) 0.11 (0.13) 0.07 (0.14)	1.13 (0.29) 1.58 (0.03) -3.31 (0.25) -3.56 (0.27) -2.68 (0.72) 0.12 (0.06) -0.03 (0.06)	HEU vs. HUU 0.0002 <.0001 <.0001 0.001 <.0001 <.0001 <.0001	LRTI+ vs, HEU 0.16 0.004 0.05 0.16 0.18 0.51 0.11

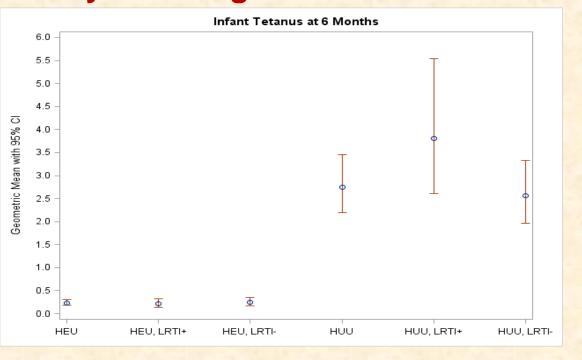
P-value

# RESULTS (CONTINUED)

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



# Infant Antibody Titers Against Tetanus Toxoid at 6 Months of Age



Data were derived from infants who received >2 doses of tetanus toxoid vaccine. Compared with HUU, HEU had significantly lower tetanus antibody titers (p<0.0001), but there were no differences between LRTI+ and LRTI- HEUs

## CONCLUSIONS

- LRTI+ HEU had higher frequency of paramyxovirus infections in the first 6 months of life when compared with LRTI- HEU, but not of influenza or PNC. This finding suggests that paramyxoviruses are responsible for initiating LRTI in HEU.
- Overall, HEU had lower antibody titers at birth than HUU against common respiratory pathogens. However, this difference did not seem to predispose the HEU to respiratory viral infections since the rate of seroconversion in the first 6 months of life to common respiratory viruses was similar in HEU and HUU. Furthermore, there were no differences in antibody titers at birth between LRTI+ and LRTI- HEU.
- The low antibody titers at birth in HEU compared with HUU were mostly due to lower transplacental transfer in HIV+ mothers, since antibody titers against RSV were higher in HIV-infected compared with uninfected mothers.
- However, mothers of LRTI+ HEU had significantly higher titers against PNC compared with mothers of LRTI- HUU, which suggests a higher rate of PNC carriage and/or infection in mothers of LRTI+ compared with those of LRTI- HEU. Compared with HUU, HEU had lower antibody responses to tetanus vaccine and lower antibody titers against respiratory viruses at 6 months. This
- suggests that antibody responses may be diminished in HEU compared with HUU and needs to be further investigated. Nevertheless, there were no differences in antibody titers against respiratory pathogens at birth or at 6 months or in tetanus antibody titers at 6
- months between LRTI+ and LRTI- HEU Taken together, the data that we present suggest that a relatively high incidence of paramyxoviral infections, unrelated to maternal antibody transfer or infant antibody production, and exposure to maternal PNC contribute to the high incidence of severe LRTI in HEU.

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