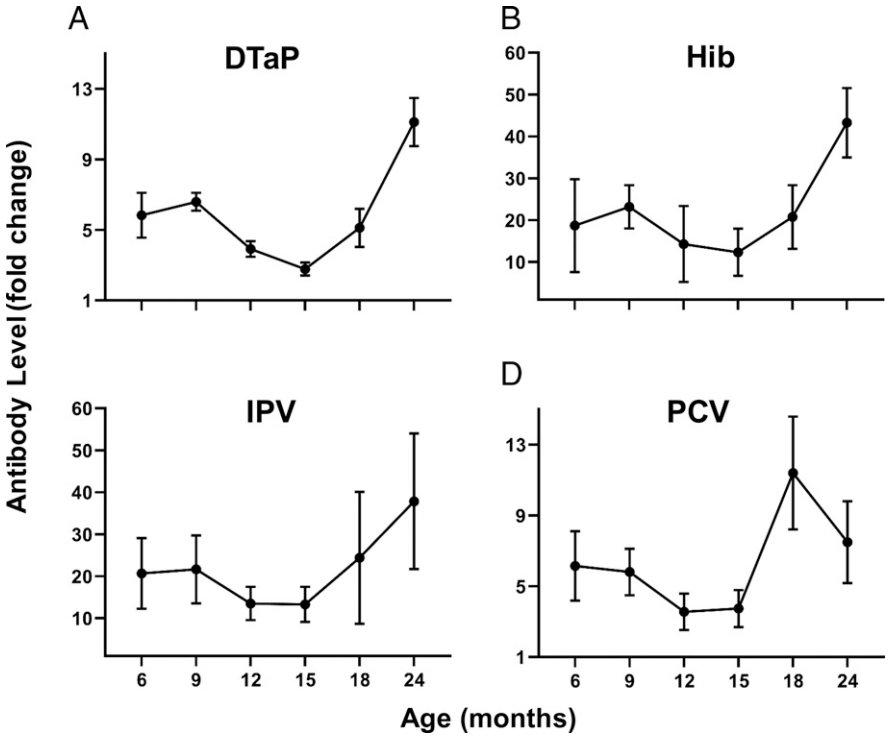
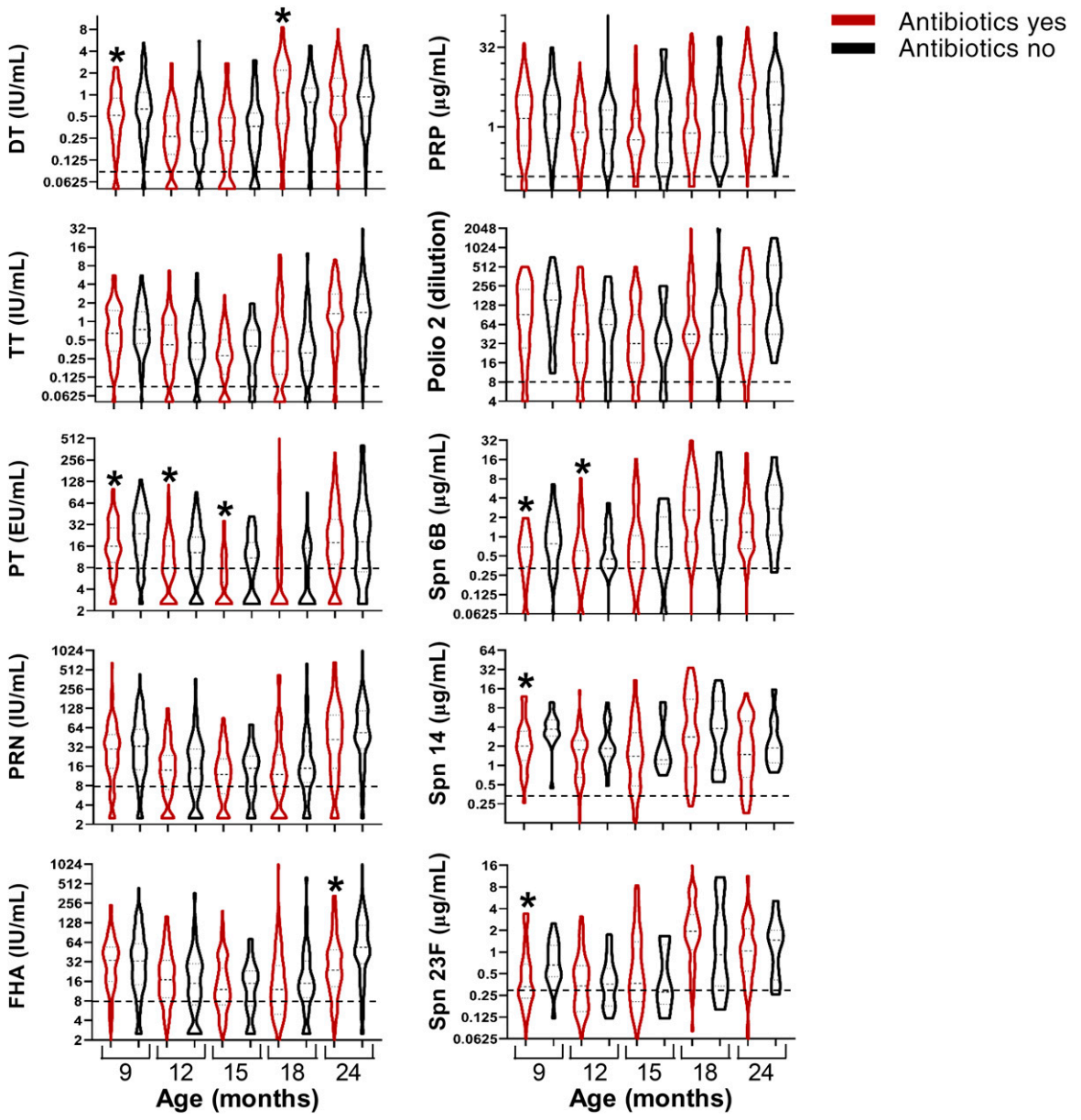
Supplemental Information

A question might be raised about whether observed effects on vaccine-induced antibody levels were caused by the antibiotics or the underlying illness that prompted antibiotic treatment. The American Association of Pediatrics[41] and Advisory Committee on Immunization Practices[42] have recommended for many years that mild illness should not be considered a contraindication to childhood immunization. In the 1970s, a theoretical concern that induction of interferon by a viral illness might interfere with seroconversion or attained antibody levels after viral vaccination against measles. Studies published in 1985 and 1988 from Haiti[43] and Rwanda[44] both revealed no difference in seroconversion or geometric mean titers after vaccination for measles given to 9-month-old children who were either sick with a variety of illnesses or healthy. One study conducted in the United States revealed that afebrile subjects with symptoms of upper respiratory tract infection had a significantly lower seroconversion rate to measles vaccine than asymptomatic children in whom no illness was reported during the previous month.[45] Authors of subsequent studies did not document reduced rates of seroconversion to measles vaccine in children with concurrent illness, including upper respiratory tract infection, otitis media, and diarrhea, or reduced rates due to fever at the time of vaccination.[46–48] Therefore, the body of data, in line with recommendations by the American Association of Pediatrics and Advisory Committee on Immunization Practices, do not support the notion that milder illness, as had occurred in our study population, reduces vaccine-induced antibody levels significantly. Moreover, no theoretical concerns have been raised about the impact of illness on vaccine-induced antibody levels, as we predominantly measured in the current study. In contrast, a mechanism for reduced antibody levels caused by antibiotic effects on the gut microbiome has been revealed not only in animal models[14–16] but also in a study of adults receiving influenza vaccine.[17] Specifically, the identification of TLR5 (a toll-like receptor capable of recognizing bacterial flagellin among bacteria in the gut) expression correlated with the magnitude of the antibody response to influenza vaccine.[17] This potential role of the gut flora influencing antibody response was subsequently directly tested in adults in whom profound antibiotics-driven perturbation of gut microbiome resulted in a significantly reduced impact of H1N1-specific antibody responses in adults with low preexisting immunity to influenza.[17]



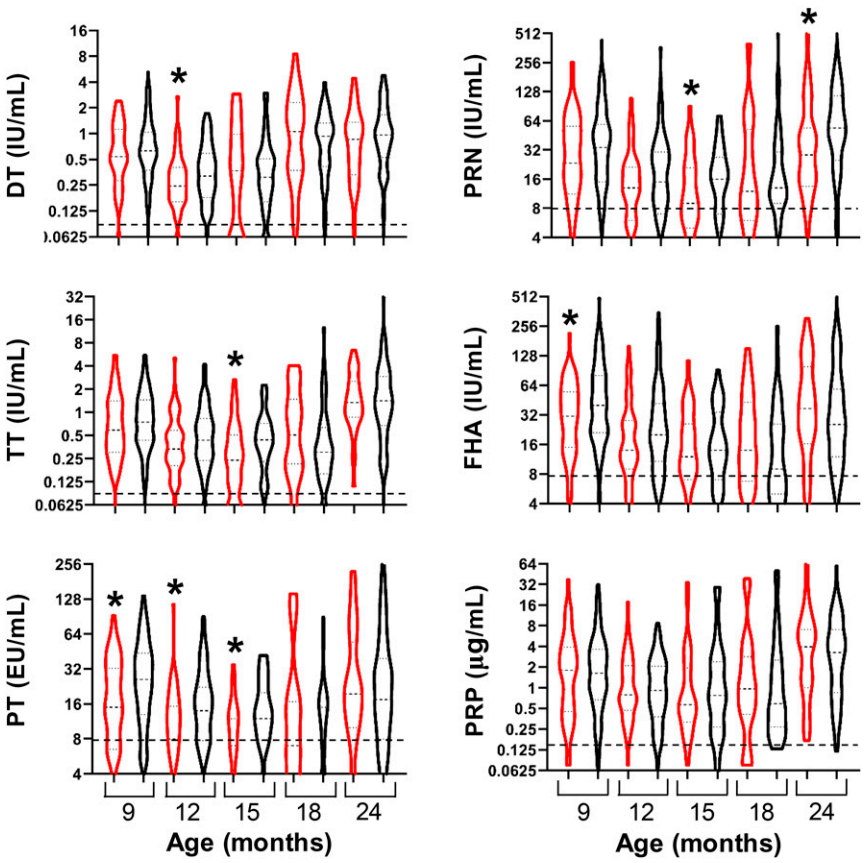
SUPPLEMENTAL FIGURE 3

Vaccine response at healthy visits. Serum vaccine antibody levels from 6 to 24 months of age are shown as mean ± 1 SD at each age time point for each vaccine type. A, DTaP combines antibody levels to DT, TT, PT, FHA, and PRN into a single value using the known or putative minimum threshold of protection as baseline and fold change above baseline to derive an overall antibody level. B, Hib PRP antibody levels. C, IPV neutralization levels to serotype 2. D, PCV antibody levels to serotypes 6B, 14, and 23F combined. One-way analysis of variance was used to determine the association of child age with normalized vaccine-induced antibody level at each age time point.



SUPPLEMENTAL FIGURE 4

Serum vaccine antibody levels from 9 to 24 months of age. Vaccine-induced antibody levels to vaccine components were determined at each age for children with antibiotic use and no antibiotic use. Vaccine antibody levels are shown as violin plots in $\log_2$ scale for each vaccine antigen. Median and 95% confidence intervals are shown as dotted lines within plots. Dotted line across each graph represents approximate protective threshold level. Number of measurements for each antigen (antibiotics yes and antibiotics no): DT, 717 and 449; TT, 720 and 448; PT, 729 and 449; PRN, 721 and 447; FHA, 718 and 449; PRP, 699 and 432; IPV serotype 2, 277 and 86; *Streptococcus pneumoniae* (Spn) serotype 6B, 250 and 75; Spn 14, 245 and 75; and Spn 23F, 247 and 75. *$P < .05$ by Mann-Whitney test.



SUPPLEMENTAL FIGURE 5

Serum vaccine antibody levels from 9 to 24 months of age when antibiotics were prescribed within 30 days compared to no antibiotic prescriptions. Vaccine-induced antibody levels to vaccine components were determined at each age for children with antibiotic use and no antibiotic use. Vaccine antibody levels are shown as violin plots in $\log_2$ scale for each vaccine antigen. Median and 95% confidence intervals shown as dotted lines within plots. Dotted line across each graph represents approximate protective threshold level. Number of measurements for each antigen (antibiotics yes and antibiotics no): DT, 233 and 449; TT, 235 and 448; PT, 236 and 449; PRN, 235 and 447; FHA, 235 and 449; and PRP, 224 and 432. *$P < .05$ by Mann-Whitney test.

SUPPLEMENTAL TABLE 2 Coefficients for Fig 2A

Vaccine	Intercept	Slope	Percent Change	SE	*P*
DTaP	1.13	−0.06	−5.8	0.02	.01
Hib	1.73	−0.07	−6.8	0.04	.05
IPV	2.25	−0.12	−11.3	0.06	.04
PCV	1.11	−0.11	−10.4	0.05	.01

SUPPLEMENTAL TABLE 3 Coefficients for Fig 2B

Vaccine	Intercept	Slope	Percent Change	SE	*P*
DTaP	2.17	−0.2	−18.1	0.06	.001
Hib	2.76	−0.24	−21.3	0.10	.020
IPV	3	−0.21	−18.9	0.08	.020
PCV	1.98	−0.13	−12.2	0.06	.030

SUPPLEMENTAL TABLE 4 *P* Values for Covariate Analysis

	Race						Feeding			
	Multiracial	White	Other	Male Sex	Siblings Yes	Day Care Yes	Breastfed	Formula	Smoking Yes	Atopy Yes
Prebooster										
DTaP	0.54	0.35	0.01*	0.25	0.17	0.55	0.90	0.76	0.19	0.69
Hib	0.77	0.41	0.16	0.60	0.39	0.82	0.40	0.73	0.67	0.25
IPV	0.62	0.84	0.35	0.54	0.74	0.74	0.17	0.33	0.95	0.10
PCV	0.16	0.02	0.001*	0.72	0.85	0.12	0.86	0.87	0.29	0.75
Postbooster										
DTaP	0.09	0.32	0.93	0.73	0.04*	0.29	0.27	0.11	0.07	0.01*
Hib	0.77	0.03*	0.76	0.10	0.84	0.70	0.29	0.40	0.52	0.43
IPV	0.02*	0.50	0.50	0.20	0.26	0.34	0.60	0.84	0.95	0.01*
PCV	0.57	0.11	0.04*	0.07	0.05*	0.66	0.77	0.87	0.10	0.15

*Significant at $P < .05$.

SUPPLEMENTAL TABLE 5 Percent Reduction in the Median Antibody Levels 45–100 Days After the Baseline Antibody Measurement

	FHA	PRN	PT	PRP	TT	DT
Antibiotic group, %	25.8	25.0	34.7	41.7	21.4	11.5
No antibiotic group, %	12.3	9.8	13.4	18.9	16.4	5.4
P value for the difference	.10	.02	.004	.02	.27	.55
Sample sizes in the antibiotic group, *n*	23	24	25	24	24	23
Sample sizes in the no antibiotic group, *n*	36	36	37	35	34	33

For this analysis, we used as baseline the antibody level for each child at the time of antibiotic prescription when an antibody level was measured at that time. This approach was conservative because the antibody level before antibiotic prescription would have been higher than at the time of antibiotic prescription because of natural antibody decay. Comparing the higher preantibiotic level with the postantibiotic level would lead to measurement of larger differences associated with adverse effects attributed to antibiotic prescription. In the no antibiotic group, the change was calculated over 2 measurements 45–100 days apart. The percentages were calculated on the basis of the means of logarithms of the ratios (follow-up/baseline). A *t* test was used to compare the antibiotic group with the no antibiotic group, and *P* values are as reported in the table.

SUPPLEMENTAL TABLE 6 Demographics for the Group of Children Included Versus Those Excluded Because of the Lack of Crucial Data

	Children Included (n = 560), n (%)		Children Excluded (n = 296), n (%)		
Variable	Yes	Not Recorded	Yes	Not Recorded	P
Day care	191 (34.1)	0 (0)	62 (20.9)	0 (0)	<.001
Siblings	348 (62.1)	18 (3.2)	159 (53.7)	3 (1.0)	.003
Feeding	—	92 (16.4)	—	63 (21.3)	.290
Breastfed	115 (20.5)	—	61 (20.6)	—	—
Formula	270 (48.2)	—	127 (42.9)	—	—
Both	83 (14.8)	—	45 (15.2)	—	—
Smoking	61 (10.9)	0 (0)	43 (14.5)	0 (0)	.150
Atopy	161 (28.8)	0 (0)	68 (23.0)	0 (0)	.080
Sex	—	0 (0)	—	0 (0)	.130
Male	285 (50.9)	—	134 (45.3)	—	—
Female	275 (49.1)	—	162 (54.7)	—	—
Race	—	1 (0.2)	—	0 (0)	<.001
Black	38 (6.8)	—	37 (12.5)	—	—
Multiracial	63 (11.2)	—	53 (17.9)	—	—
Other	18 (3.2)	—	14 (4.7)	—	—
White	440 (78.6)	—	192 (64.9)	—	—

SUPPLEMENTAL TABLE 7 Counts of the Blood Samples Used for Antibody Testing in Children at Various Ages

	Age, months						
	6	9	12	15	18	24	Total
No antibiotic group	54	97	87	94	98	23	453
Antibiotic group	49	204	248	76	134	20	731
Total	103	301	335	170	232	43	1184

We included samples collected in proximity to the specified age, including samples when antibiotics were prescribed, usually ± 1 mo.

SUPPLEMENTAL REFERENCES

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