Maternal Influenza Vaccination and Effect on Influenza Virus Infection in Young Infants

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Objective: To assess the effect of seasonal influenza vaccination during pregnancy on laboratory-confirmed influenza in infants to 6 months of age.

Design: Nonrandomized, prospective, observational cohort study.

Setting: Navajo and White Mountain Apache Indian reservations, including 6 hospitals on the Navajo reservation and 1 on the White Mountain Apache reservation.

Participants: A total of 1169 mother-infant pairs with mothers who delivered an infant during 1 of 3 influenza seasons.

Main Exposure: Maternal seasonal influenza vaccination.

Main Outcome Measures: In infants, laboratory-confirmed influenza, influenzalike illness (ILI), ILI hospitalization, and influenza hemagglutinin inhibition antibody titers.

Results: A total of 1160 mother-infant pairs had serum collected and were included in the analysis. Among infants, 193 (17%) had an ILI hospitalization, 412 (36%) had only an ILI outpatient visit, and 555 (48%) had no ILI episodes. The ILI incidence rate was 7.2 and 6.7 per 1000 person-days for infants born to unvaccinated and vaccinated women, respectively. There was a 41% reduction in the risk of laboratory-confirmed influenza virus infection (relative risk, 0.59; 95% confidence interval, 0.37-0.93) and a 39% reduction in the risk of ILI hospitalization (relative risk, 0.61; 95% confidence interval, 0.45-0.84) for infants born to influenza-vaccinated women compared with infants born to unvaccinated mothers. Infants born to influenza-vaccinated women had significantly higher hemagglutinin inhibition antibody titers at birth and at 2 to 3 months of age than infants of unvaccinated mothers for all 8 influenza virus strains investigated.

Conclusions: Maternal influenza vaccination was significantly associated with reduced risk of influenza virus infection and hospitalization for an ILI up to 6 months of age and increased influenza antibody titers in infants through 2 to 3 months of age.


INFuenza virus activity and disease severity varies from season to season; however, studies have consistently identified young children to be at high risk of influenza complications. Among healthy children younger than 5 years, annual influenza-associated hospitalization rates are approximately 100 per 100,000, while children with underlying medical conditions have rates of approximately 500 per 100,000 in the United States.1,4

Influenza virus infection in infants is generally more frequent among those aged 6 to 12 months than in the first 6 months of life, potentially owing to the protection conferred by maternal influenza antibodies acquired transplacentally or through breastfeeding.2 However, during severe influenza seasons, morbidity and mortality rates among infants younger than 6 months have been reported to exceed those of older infants.6-11

The US Advisory Committee on Immunization Practices recommends that pregnant women receive influenza vaccine because of the increased risk of influenza complications in pregnant women.4 Maternal influenza vaccination may also confer a benefit to infants born during influenza season, as they are ineligible for vaccination until 6 months of age.

Several studies have investigated the ability of maternally derived influenza-specific antibodies to protect infants from influenza virus infection and/or to reduce severity of illness.12,13 Findings among infants attributable to maternal influenza vaccination have included delayed onset of in-
fever, older age when infected, and 1 randomized trial showing a reduction in laboratory-confirmed influenza.

Navajo and White Mountain Apache children have rates of acute respiratory infection that are significantly higher than in the general US population.15-17 Because influenza vaccination of all pregnant women is recommended, a randomized clinical trial was not possible in the United States. Therefore, we conducted a nonrandomized, prospective, observational cohort study to evaluate the effect of seasonal influenza vaccination during pregnancy on influenza virus infection in infants compared with that of infants born to unvaccinated women.

METHODS

SETTING

The study was conducted on the Navajo and White Mountain Apache Indian reservations in the Southwest region of the United States. Health care is administered through the Indian Health Service, an agency of the federal government, or through the tribe; services are provided without fees to registered tribal members. Six Navajo hospitals (Chinle, Gallup, Fort Defiance, Shiprock, Tuba City, and Winslow) and 1 (Whiteriver) on the White Mountain Apache reservation participated in this study.

DESIGN

The study was conducted during 3 influenza seasons from November 2002 to September 2005. The enrollment periods for each year were December 1, 2002, to March 15, 2003; November 1, 2003, to March 8, 2004; and November 1, 2004, to March 15, 2005. Inclusion was restricted to mothers who delivered a healthy infant at 36 weeks' or later gestation during the enrollment periods. Eligible infants were aged 2 weeks or younger at enrollment.

RECRUITMENT

Mother-infant pairs were recruited after delivery at Indian Health Service hospitals on the Navajo or White Mountain Apache reservation either at the hospital or by home visit. Written informed consent was obtained from all mothers. For all subjects, the study began following delivery. The decision for influenza vaccination was made by the treating clinician and the pregnant woman; study personnel had no involvement in these decisions. We provided annual training/education to medical providers and the community regarding influenza vaccine recommendations and the purpose of the study. Prior to the study, less than 10% of eligible pregnant women received influenza vaccine. Donations of thimerosal-reduced inactivated influenza vaccine were made annually to the Indian Health Service hospitals to assure adequate supply for pregnant women.

DATA COLLECTION

Questionnaires were administered verbally at study enrollment and the end of the influenza season to collect information on demographics, breastfeeding status, potential influenza risk factors, and influenza vaccination status of all household members. The mother's medical record was reviewed for the period from first prenatal care visit to delivery for all prenatal visits, history, birth information, and the administration and timing of an influenza vaccine. Surveillance for all medically attended illnesses in enrolled infants was conducted at Indian Health Service and nearby private facilities during the influenza season, defined as November through April, for each year of the study or until the child reached 6 months of age (whichever came first). The primary outcome of interest was medically attended influenzalike illness (ILI). This outcome was defined as a medical visit with at least 1 of the following signs or symptoms: fever of 38.0°C or higher, diarrhea, or respiratory symptoms (including cough, runny nose, or difficulty breathing). Diarrhea was included in this definition because young infants can experience diarrhea with influenza virus infection.18,19 Daily active surveillance for ILI episodes included review of the clinic, emergency department, and inpatient pediatric ward logs. Parents were asked to contact research staff whenever their child had a medical visit for a respiratory, diarrheal, or febrile illness. Medical record reviews were conducted to identify missed episodes. When an ILI was documented in the infant's chart, information was collected on demographics, clinical symptoms, laboratory data including rapid influenza diagnostic testing and respiratory syncytial virus antigen testing, severity of illness, and diagnosis. A nasopharyngeal aspirate specimen for viral culture was obtained from infants with ILI within 72 hours of the medical visit.

Postpartum blood was obtained from the mother at or within 14 days of delivery. Umbilical cord blood was collected at delivery or, if unavailable, an infant blood specimen was collected within 14 days of delivery. Venous blood was obtained from the infant at 2 to 3 months and 6 months of age.

LABORATORY TESTING

Blood specimens were centrifuged at 3000 rpm for 10 minutes. The collected serum was divided into 2 aliquots of at least 0.5 mL each, frozen at –70°C, and shipped on dry ice to the Influenza Division, Centers for Disease Control and Prevention, for serological testing. Serum specimens were tested for influenza virus strain–specific antibodies using a standard hemagglutinin inhibition (HI) antibody assay. Specimens were tested for antibodies to influenza A and B vaccine strains and predominant circulating strains (if antigenically distinct from vaccine strains) for the corresponding season of interest: for the 2002-2003 season, A/New Caledonia/20/99 IVR-116 (H1N1) (A/New Cal; vaccine strain), A/Panama/2007/99 respiratory syncytial virus 17 (H3N2) (A/Panama; vaccine strain), A/Wyoming/3/2003 X-147 (H3N2) (A/Wyoming; predominant strain), B/Hong Kong/330/2001 (B/HK, vaccine strain from B/Victoria/2/87 lineage), and B/Sichuan/379/99 (B/Sichuan, circulating virus from B/Yamagata/16/88 lineage) were used; for the 2003-2004 season, the same as the 2002-2003 season were used except B/Br/2002/02 (B/Br) instead of B/HK; B/Br was dominant in B/Victoria/2/87 lineage virus during the season); and for the 2004-2005 season, A/New Cal (H1N1 vaccine strain), A/Wisconsin/67/2005 (H3N2) (A/Wisconsin; predominant virus), B/Shanghai/361/02 (B/Shanghai, vaccine strain from B/Yamagata/16/88 lineage), and B/HK.

Nasopharyngeal aspirate specimens were mixed with viral transport media (Remel, Lenexa, Kansas), transported on wet ice, frozen and stored at –70°C, and shipped on dry ice to the University of Iowa for viral culture. Nasopharyngeal aspirate specimens were inoculated into Madin-Darby canine kidney cell culture, and viruses were detected by cytopathic effect and hemadsorption. Influenza virus infection was confirmed by indirect fluorescent antibody staining of infected cells.

STATISTICAL ANALYSIS

Maternal influenza vaccination status was based on medical record review or, if missing, by maternal report at enrollment. Hemagglutinin inhibition antibody titers of 1:40 or higher were
considered evidence of immunity. Serum specimens with undetectable HI antibody titers were assigned a titer of 5.

The primary analytical outcome, laboratory-confirmed influenza, was defined as the first episode of ILI with (1) isolation of influenza virus from the nasopharyngeal aspirate specimen, (2) a 4-fold or greater rise in HI antibody in serum collected at 2 to 3 or 6 months compared with the previous serum specimen, indicating influenza virus infection during the time interval, or (3) a positive rapid influenza diagnostic test result with a medical diagnosis of influenza. The secondary analytic outcome of interest was ILI. Logistic regression was used to investigate the relationship between maternal influenza vaccination status, cord blood influenza HI antibody titers, and the occurrence of ILI or laboratory-confirmed influenza. In addition, ILI was further stratified by hospitalization status. Potential confounders such as daycare attendance, household smoking, wood or coal burning stove in the home, and breastfeeding were investigated. Relative risks with 95% confidence intervals not including 1.0 were considered significant.

To describe and compare influenza-specific antibodies, HI antibody titers were converted to a logarithmic scale. Geometric mean titers (GMTs) of influenza-specific antibody in postpartum, cord, 2 to 3-month, and 6-month serum specimens were calculated for influenza-vaccinated and unvaccinated mother-infant pairs. Comparisons between these 2 groups used a 2-sided t test. For each virus strain, the proportion of infants in each group with cord blood HI antibody titers of 1:40 or greater were calculated and compared using the Fisher exact test. Cord blood GMTs and the proportion of infants with cord blood HI antibody titers of 1:40 or greater were compared between mothers vaccinated in the second and third trimester using Wilcoxon rank sum and χ² tests, respectively.

Stata 8.1 (Stata Corp, College Station, Texas) software was used for the statistical analyses. The study was approved by the Institutional review boards of the Navajo Nation, Phoenix Area Indian Health Service, and the Johns Hopkins Bloomberg School of Public Health.

### RESULTS

Overall, 1169 mother-infant pairs were enrolled in the study for 2002-2003 (n=241), 2003-2004 (n=574), and 2004-2005 (n=354) influenza seasons. Of these, 1160 (99%) mother-infant pairs had at least 1 blood specimen collected and were included in the analysis. Forty-nine percent of infants (n=573) were born to mothers who received influenza vaccine during their pregnancy. Infants born to influenza-vaccinated and unvaccinated mothers were similar except that those born to vaccinated mothers were more likely to have a wood or coal burning stove in the house and to be breastfed (Table 1).

Of the 1160 infants, there were 908 ILI episodes; 193 children (17%) were hospitalized for ILI (84 had at least...
We found a 41% reduction in the risk of laboratory-confirmed influenza virus infection (relative risk [RR], 0.59; 95% confidence interval, 0.37-0.93) for infants of influenza-vaccinated mothers compared with infants of unvaccinated mothers (Table 2). A significant reduction in influenza virus infection risk was also observed with increasing cord blood HI antibody titers (log scale) (RR range, 0.42-0.68) and among children with HI antibody titers of 1:40 or greater compared with those with titers of less than 1:40 (RR range, 0.14-0.54) for all 8 virus strain antigens (Table 2). When the analysis was restricted to serologically confirmed influenza virus infection, there was a significant association between increasing cord blood titers and decreasing risk of influenza virus infection (Table 3). This finding was observed for all 8 virus strains, although only statistically significant for A/NewCal (H1N1), A/Panama (H3N2), A/Wyoming (H3N2), B/Sichuan, and B/Brisbane.

No statistically significant associations were found between maternal influenza vaccination status or influenza strain–specific cord blood HI antibody titers and ILI occurrence (Table 2). However, when the outcome was restricted to ILI requiring hospitalization, a 39% reduction in risk was found (RR, 0.61; 95% confidence interval, 0.45-0.84) for infants born to influenza-vaccinated mothers compared with those of unvaccinated mothers. Among infants who had an ILI, those born to influenza-vaccinated mothers had a 42% reduction in the risk of the ILI resulting in hospitalization (RR, 0.58; 95% confidence interval, 0.41-0.83) compared with infants born to unvaccinated mothers.

We found no statistically significant associations between smoking in the household, daycare attendance of household children, a wood- or coal-burning stove, or increase in influenza virus infection risk was also observed with increasing cord blood HI antibody titers (log scale) (RR range, 0.42-0.68) and among children with HI antibody titers of 1:40 or greater compared with those with titers of less than 1:40 (RR range, 0.14-0.54) for all 8 virus strain antigens (Table 2). When the analysis was restricted to serologically confirmed influenza virus infection, there was a significant association between increasing cord blood titers and decreasing risk of influenza virus infection (Table 3). This finding was observed for all 8 virus strains, although only statistically significant for A/NewCal (H1N1), A/Panama (H3N2), A/Wyoming (H3N2), B/Sichuan, and B/Brisbane. No statistically significant associations were found between maternal influenza vaccination status or influenza strain–specific cord blood HI antibody titers and ILI occurrence (Table 2). However, when the outcome was restricted to ILI requiring hospitalization, a 39% reduction in risk was found (RR, 0.61; 95% confidence interval, 0.45-0.84) for infants born to influenza-vaccinated mothers compared with those of unvaccinated mothers. Among infants who had an ILI, those born to influenza-vaccinated mothers had a 42% reduction in the risk of the ILI resulting in hospitalization (RR, 0.58; 95% confidence interval, 0.41-0.83) compared with infants born to unvaccinated mothers.

We found no statistically significant associations between smoking in the household, daycare attendance of household children, a wood- or coal-burning stove, or infant breastfeeding and the occurrence of any of the outcomes (data not shown). Therefore, we did not adjust the univariate regression models by these factors as originally planned.

### Table 3. Relative Risk of Serologically Confirmed Influenza Virus Infection by Influenza-Specific Cord Blood HI Antibody Titera

<table>
<thead>
<tr>
<th>Cord Blood HI Antibody Titer</th>
<th>Influenza HI Antibody Titer With a 4-Fold Rise in Titer During the 2-3– or 6-mo Blood Draw</th>
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<tbody>
<tr>
<td>B/Sichuan (n=14)</td>
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<tr>
<td>Log scale</td>
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<tr>
<td>Titer ≥1:40</td>
<td>0.36 (0.18-0.72)</td>
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<tr>
<td>Titer ≥1:40</td>
<td>0.28 (0.03-2.59)</td>
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<tr>
<td>A/New Cal, H1N1 (n=14)</td>
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<tr>
<td>A/Panama, H3N2 (n=33)</td>
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<tr>
<td>A/Wyoming, H3N2 (n=10)</td>
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<tr>
<td>A/Wisconsin, H3N2 (n=6)</td>
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<tr>
<td>B/Shanghai (n=10)</td>
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<tr>
<td>B/Brisbane (n=7)</td>
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<tr>
<td>Log scale</td>
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<tr>
<td>Titer ≥1:40</td>
<td>0.25 (0.10-0.63)</td>
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<tr>
<td>Table 2</td>
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<td>Maternal Influenza Vaccination and Protection From Infection</td>
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| Fifty-two percent (n=605) of infants had an ILI, of which 14% (83) were laboratory-confirmed influenza. Ten of the laboratory-confirmed influenza virus infections were confirmed by virus culture, 71 by serology, and 2 by rapid influenza diagnostic testing. The mean infant age at first ILI was 47 days (median, 41; range, 0-175) and did not differ between infants born to vaccinated and unvaccinated mothers. We found a 41% reduction in the risk of laboratory-confirmed influenza virus infection (relative risk [RR], 0.59; 95% confidence interval, 0.37-0.93) for infants of influenza-vaccinated mothers compared with infants of unvaccinated mothers (Table 2). A significant reduction in influenza virus infection risk was also observed with increasing cord blood HI antibody titers (log scale) (RR range, 0.42-0.68) and among children with HI antibody titers of 1:40 or greater compared with those with titers of less than 1:40 (RR range, 0.14-0.54) for all 8 virus strain antigens (Table 2). When the analysis was restricted to serologically confirmed influenza virus infection, there was a significant association between increasing cord blood titers and decreasing risk of influenza virus infection (Table 3). This finding was observed for all 8 virus strains, although only statistically significant for A/NewCal (H1N1), A/Panama (H3N2), A/Wyoming (H3N2), B/Sichuan, and B/Brisbane. No statistically significant associations were found between maternal influenza vaccination status or influenza strain–specific cord blood HI antibody titers and ILI occurrence (Table 2). However, when the outcome was restricted to ILI requiring hospitalization, a 39% reduction in risk was found (RR, 0.61; 95% confidence interval, 0.45-0.84) for infants born to influenza-vaccinated mothers compared with those of unvaccinated mothers. Among infants who had an ILI, those born to influenza-vaccinated mothers had a 42% reduction in the risk of the ILI resulting in hospitalization (RR, 0.58; 95% confidence interval, 0.41-0.83) compared with infants born to unvaccinated mothers. We found no statistically significant associations between smoking in the household, daycare attendance of household children, a wood- or coal-burning stove, or infant breastfeeding and the occurrence of any of the outcomes (data not shown). Therefore, we did not adjust the univariate regression models by these factors as originally planned.

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(REPRINTED) ARCH PEDIATR ADOLESC MED/VOL 165 (NO. 2), FEB 2011 WWW.ARCHPEDIATRICS.COM
Hemagglutinin inhibition antibody GMTs to each of the 8 influenza virus antigens tested in the postpartum, cord, and 2- to 3-month infant blood were significantly higher in infants born to influenza-vaccinated compared with unvaccinated mothers. However, no differences were found in the GMTs of HI antibody titers at 6 months of age between the 2 groups, with the exception of B/Sichuan and B/Brisbane (Figure).

A significantly higher proportion of subjects in the vaccinated group had HI antibody titers of 1:40 or greater against each of the 8 influenza viruses than the unvaccinated group for postpartum and cord blood (eTable;
MATERNAL INFLUENZA VACCINATION TIMING

Of the 522 influenza-vaccinated mothers with a cord blood specimen, 123 were vaccinated in the second trimester and 390 in the third trimester. For all 8 influenza virus strains, there was no difference in the cord blood titers (GMTs or proportion with titers 1:40 or greater) according to trimester of vaccination (Table 4).

This prospective study demonstrated that maternal influenza vaccination was associated with a 41% reduced risk of laboratory-confirmed influenza virus infection and a 39% reduced risk of ILLI hospitalization among the infants born to these mothers. The ILLI incidence rate among infants of unimmunized mothers was 7.2 per 1000 person-days of observation; 9% of infants born to unimmunized mothers had laboratory-confirmed influenza virus infection in the first 6 months of life, a proportion comparable with the 10% seen in a randomized trial of maternal influenza vaccination in Bangladesh.14

The mechanism of reduced influenza virus infection risk in infants of vaccinated mothers could include reduced influenza exposure of the infant from the mother, increased protection of the infant following exposure through increased transplacentally acquired antibody titers, or residual confounding not controlled for in the study. We did not evaluate maternal illness patterns during or following pregnancy but did evaluate influenza strain–specific antibody status of the infants and the correlation between these antibodies and influenza virus infection. We found significantly higher influenza HI antibody titers in infants born to women vaccinated during pregnancy compared with infants of unvaccinated mothers at 2 to 3 months of age and demonstrated a correlation with a reduced risk of influenza virus infection in young infants with higher antibody titers. These findings are consistent with the serologic data from the Bangladesh clinical trial.20

Findings from previous studies are inconsistent about the effects of maternal influenza antibody levels or vaccination on infant outcomes. Together, 3 prospective US-based observational studies concluded that higher infant influenza antibody titers delay the age at first infection and decrease the severity of influenza illness but could not identify a reduced risk of illness episodes.12,13,14 This last observation, which was also seen in a cohort study by France et al,22 may be attributable to the lack of laboratory confirmation and use of nonspecific clinical outcomes for influenza. A recent prospective, randomized study conducted in Bangladesh investigated the association between maternal influenza vaccination and protection from influenza illness in infants. Infants born to influenza-vaccinated mothers were 63% and 29% less likely to have illness defined by a positive result on a rapid
influenza diagnostic test and febrile respiratory illness, respectively, compared with infants in the control group.\(^\text{14}\)

There are several limitations to our study. We investigated mother-infant characteristics that could confound the findings and found no associations with vaccination or outcome; however, there is always the possibility of uncontrolled residual confounding. Furthermore, the study was conducted during 3 relatively mild influenza seasons. Although we only identified a small number of culture-confirmed influenza illnesses, we serologically identified many additional infants with influenza. Serologic detection is not 100% sensitive for influenza virus infection; however, assuming the sensitivity does not vary according to maternal vaccination status, any lack of sensitivity would reduce the power for detecting a difference. If the sensitivity of detecting rising antibody titers is lower in infants of vaccinated than unvaccinated mothers, which could occur as a result of falling passively acquired antibodies, then the odds ratio would be overestimated. We do not believe this is a significant issue because of the interval of blood drawing and the rate of declining antibodies. Serological data strengthened our findings and indicated that influenza HI antibodies in cord blood and infant sera up to 2 to 3 months of age provided protection against laboratory-confirmed, clinically apparent, mild, and asymptomatic influenza virus infections. Investigation of these associations during more severe seasonal or pandemic epidemics may add to and further enhance these associations.

This study found that influenza vaccination of pregnant women was significantly associated with increased influenza antibody titers in their infants through 2 to 3 months of age and protection from influenza virus infection and ILI hospitalization during the first 6 months of life. Although influenza vaccination is recommended for pregnant women to reduce their risk of influenza complications, these findings provide support for the added benefit of protecting infants from influenza virus infection up to 6 months, the period when infants are not eligible for influenza vaccination but are at highest risk of severe influenza illness. These findings are particularly relevant with the emergence of 2009 pandemic influenza A (H1N1) virus, which had a substantial effect on pregnant women and high hospitalization rates among young infants.\(^\text{23,24}\) The 2009 H1N1 virus strain is included as a component of the 2010 Southern and 2010-2011 Northern Hemisphere seasonal trivalent influenza vaccines.\(^\text{25-27}\) The findings of this study underscore the public health importance of maternal influenza vaccination to prevent influenza in both pregnant women and their infants.\(^\text{23,24}\)

**Accepted for Publication:** July 29, 2010.  
**Published Online:** October 4, 2010. doi:10.1001/archpediatrics.2010.192. This article was corrected on October 5, 2010.

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**Author Contributions:** Drs O’Brien and Eick had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Uyeki, Klimov, Santosham, and O’Brien. **Acquisition of data:** Eick, Uyeki, Klimov, and Reid. **Analysis and Interpretation of data:** Eick, Uyeki, Klimov, Hall, and O’Brien. **Drafting of the manuscript:** Eick, Uyeki, and O’Brien. **Critical revision of the manuscript for important intellectual content:** Eick, Uyeki, Klimov, Hall, Reid, Santosham, and O’Brien. **Statistical analysis:** Eick. **Obtained funding:** Uyeki and O’Brien. **Administrative, technical, or material support:** Eick, Uyeki, Hall, Reid, and O’Brien. **Study supervision:** Eick, Klimov, and O’Brien.

**Financial Disclosure:** Dr O’Brien reports receiving grants for research projects unrelated to this article from MedImmune and Pfizer and serving on expert advisory committees for pneumococcal vaccines from Sanofi-Pasteur; all 3 manufacture influenza vaccine.

**Funding/Support:** The study was funded by the National Vaccine Program Office, Department of Health and Human Services, the Office of Minority Women’s Health, Centers for Disease Control and Prevention, Aventis-Pasteur, and Evans-Powerject.

**Role of the Sponsor:** The funding organizations had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

**Disclaimer:** The views expressed are those of the authors and do not necessarily reflect the policies of the Centers for Disease Control and Prevention or the Indian Health Service.

**Online-Only Material:** The eTable is available at http://www.archpediatrics.com.

**Additional Contributions:** The authors thank the field workers, nurses and staff of the Center for American Indian Health for their dedicated work on the Maternal Influenza Study from which these data were collected, as well as the families who participated in this study for their commitment to this project and to the health of their tribes.

## REFERENCES

10. Schrag SJ, Shay DK, Gershman K, et al; Emerging Infections Program Respira-


Correction

Error in Expansion of NOURISH Trial Acronym. In the Review Article titled “Interventions Aimed at Decreasing Obesity in Children Younger Than 2 Years: A Systematic Review,” by Ciampa et al, published in the December issue of the Archives (2010;164[12]:1098-1104), an error occurred in expanding the acronym NOURISH. On page 1102, right-hand column, “Ongoing Studies” subsection, lines 6 through 8, the NOURISH trial (ACTRN12608000056392, reference 43) acronym has no expansion.