Pertussis seroprevalence in adults, post-partum women and umbilical cord blood

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ABSTRACT

Pertussis is a vaccine-preventable disease that affects people of all ages. Young adults who have lost their immunity to *pertussis* are the major source of infection in infants. Given the steady increase of *pertussis* cases, new prevention strategies are required.

Objective. To assess *pertussis* seroprevalence in adult blood donors, post-partum women, and umbilical cords.

Metod. Measurement of total titers of anti-*Bordetella spp.* (*Bordetella*) antibodies using an enzyme-linked immunosorbent assay. Serum samples from 103 donors, 101 post-partum women and 100 umbilical cords were analyzed. Titers ≤80 were considered of low impact against the disease. The assessment included transplacental transfer of antibodies and the umbilical cord / maternal ratio of antibody titers.

Results. Donors mean age was: 28 ± 6 years old. Median anti-*Bordetella* titers: 320; interquartile range (IQR):160-320; 10% had titers ≤80.

Post-partum women mean age was: 26 ± 6 years old. Median anti-*Bordetella* titers:160 (IQR:80-320), with titers significantly lower than in female donors (p= 0.00002). Titers ≤80 were found in 30% of post-partum women.

Median anti-*Bordetella* titers in umbilical cords: 160 (IQR: 80-160). Titers ≤80 were more frequently found in umbilical cords than in mothers (44% versus 30%, p= 0.04). Transplacental transfer was 0.83. Umbilical cord titers were equal to maternal titers in 54% of cases, lower in 37%, and higher only in 8%.

Conclusion. Titers of anti-*Bordetella* antibodies in post-partum women were significantly lower than in female blood donors. Titers ≤80 were found in 30% of post-partum women and 44% of umbilical cords. These data may account for the high rates of *pertussis* in young infants who have not yet completed their vaccination schedule.

Key words: *pertussis, prevalence, prevention and control.*

http://dx.doi.org/10.5546/aap.2014.eng.315

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Conflict of Interest:
None.

Received: 9-16-2013
Accepted: 1-27-2014

INTRODUCTION

Pertussis, also known as whooping cough, is an acute bacterial disease of the respiratory system caused by *Bordetella pertussis* (*Bp*). This disease mainly affects infants and toddlers. It is easily transmitted person-to-person, with an intra-family transmission rate of 75-90%, while school transmission is 50-80%, and can be endemic, with epidemic outbreaks every three to five years.[1]

The mass use of whole-cell vaccines reduced pertussis incidence by more than 95% in the 1980s, compared to the 1940s-1950s pre-vaccine era.

However, over the past years, a steady increase of cases has been observed, even in countries with high vaccination coverages.[2] The World Health Organizationestimates that 16 million cases of *pertussis* occur every year worldwide, with more than 194 000 deaths, which account for 13% of vaccine-preventable deaths.[3]

This is also the situation in Argentina, where a steady increase of cases has been observed since 2002 to date, with the highest morbidity and mortality rates observed among infants and toddlers. Ninety-two percent of hospitalized children are younger than one year old. In 2011, 70 infants died, 91% were younger than four months old.[4]

Over the past two decades, a change has been recorded in the epidemiological profile of pertussis, with cases also detected among adolescents and adults.[2] Although in these populations the disease is not so severe, it does have an epidemiological impact because adolescents and adults act as the main source of infection in infants.[3-7]

Different causes may epidemiological contribute to the present situation: greater surveillance, new diagnostic methods, a relatively low vaccine efficacy, short duration of vaccine-induced immunity, and/or the adaptability of the causative agent to the immunity conferred by vaccines.[3]

Regardless of causes, the significant increase in the number of cases has prompted health systems of different countries to review and implement new strategies aimed at reducing the incidence of pertussis in the most vulnerable population: infants younger than one year old. The precise mechanism that provides protective immunity to pertussis is not entirely known, but there is evidence of the role of both tumor and cellular response.[8] Protection in infants younger than two months old (before receiving their first vaccine dose) depends on the transfer of maternal antibodies. Studies have been conducted on the role of such anti-*Bordetella* antibodies on the prevention of mortality in this group.[9]

Having our own data on the seroprevalence of *pertussis* in our population of young adults and post-partum women and on transplacental transfer of antibodies would be important at the time of designing new strategies aimed at improving the condition of this disease.

OBJECTIVES

- To assess the seroprevalence of anti-*Bordetella* antibodies in a population of young adults, including post-partum women and their umbilical cords.
- To compare the titer of antibodies in post-partum women with that of umbilical cord blood of their newborns.

POPULATION AND METHODS

Prospective, observational and analytical study with blind analysis of samples. Study period: June 2009-June 2010.

Study population

Group A: voluntary blood donors aged 17-45 years old, called "donors."

Group B: women in their immediate post-partum period, called "post-partum women."

Group C:serum from their respective newborns collected from their umbilical cord blood, called "umbilical cords."

Inclusion criteria

Group A: healthy adult blood donors seen at the Hemotherapy Center of HNRG.

Group B: women with uneventful pregnancies reaching 37-41 weeks of gestation and giving birth at Hospital Santojanni.

Group C: umbilical cord blood serum from their infants, who were born healthy, at term and with an adequate weight for their gestational age.

Exclusion criteria

Adults who received gamma globulin or blood products in the past month.

Women with complicated pregnancies or deliveries.Umbilical cord blood from preterm newborn infants, those born with fetal anomalies or perinatal conditions.

Ethical aspects

The informed consent was obtained from all subjects included in the study, before blood donation in the case of donors, and before delivery in the case of mothers and umbilical cords.

The study was evaluated and approved by the ethics committees of hospitals Ricardo Gutiérrez and Santojanni.

Methods

For Groups A and B, questionnaires were completed in relation to demographic, epidemiological (cohabitation with children or adolescents, employment modality), and clinical data (history of prolonged cough, contact with confirmed cases of *pertussis*, concomitant conditions during pregnancy) and history of vaccination against *pertussis* and/or having had *pertussis*.

Serum samples were collected and stored at -20 ºC,then they were blindly sent to the laboratory, where they were all simultaneously processed.

The sample size was estimated using the EPI5 software for a population or descriptive study with a non-grouped randomized sampling and a 99.99% confidence interval (CI). It was expected that 50% of the general population and 45% of pregnant women had antibody titers ≥160. For the estimation of the voluntary blood donor sample, we considered a population of 4 000 000 inhabitants aged 15-49 years old, based on the National Statistics and Censuses Institute of Argentina, which resulted in a sample size of N= 79. For the estimation of the post-partum women sample, we considered a population of 700 000 deliveries/year, which resulted in a sample size of N= 79.

Titers of anti-*Bordetella* antibodies were determined using the enzyme-linked immunosorbent assay (ELISA), directed against whole-cell sonicates of *B. pertussis* strain Tohama I, which determines the level of total antibodies. Some studies have shown that titers ≥160 are related to a past *Bordetella* infection; therefore, titers ≤80 are considered to account for no evidence of protection against the disease.[10]

Transplacental transfer of antibodies was

estimated as the ratio of the antibody titer geometric mean for umbilical cords serum and maternal serum.

In the analyses, antibody titers were expressed using the median (of the reciprocal of dilution) and its corresponding interquartile range (IQR). The statistical analysis of antibody titer medians was conducted using the Wilcoxon test for non-parametric samples. The qualitative outcome measure analysis was done using the Chi-square test or Fisher's test. A value of $p < 0.05$ was considered significant.

RESULTS

Three-hundred and four samples were included and processed in the study, 103 were from voluntary donors, 101 from post-partum women, and 100 from umbilical cords. All included adults (donors and post-partum women) had received their last whole-cell vaccine dose at 6 years old (none had received the acellular vaccine). Group A consisted of 103 donors; 63 males and 40 females. Their mean age was 28 ± 6 years old (range: 17-44 years old), with no gender differences (males: 28 ± 6 years old; females: 28.5 ± 6 years old). Median titer of anti-*Bordetella* antibodies was 320 (IQR: 160-320), and 11 (10%) had antibody titers ≤80. When analyzing antibody titers by sex or age, no statistically significant differences were observed.

Donors who did not cohabitate with children tended to have lower titers than those who lived with children (15% versus 8.6%), but this was not a significant difference. In addition, no significant differences were detected between these two groups in terms of history of prolonged cough.

Group B consisted of 101 women in their immediate post-partum period. Their mean age was 26 ± 6 years old (range: 17-44 years old). Median titer of anti-*Bordetella* antibodies was 160 (IQR: 80-320); 30 (30%) of post-partum women had anti-*Bordetella* antibody titers ≤80.

The analysis of antibody titers of post-partum women by age, history of prolonged cough or cohabitation with children showed no significant differences.

When comparing antibody titers between female donors and post-partum women, the latter had significantly lower titers, with a median of 160 (IQR: 80-320) versus 320 (IQR: 160-320) in female donors (p= 0.00002). At the same time, 30/101 (30%) of post-partum women and only 2/40 (5%) of female donors had anti-*Bordetella* antibody titers ≤80, a remarkably significant difference (OR: 8.1; 95% CI: 1.8-35.4, p= 0.0015) (*Figure 1*).

FIGURE 1. *Comparison of pertussis antibody titers in female donors (N= 40) and post-partum women (N= 101)*

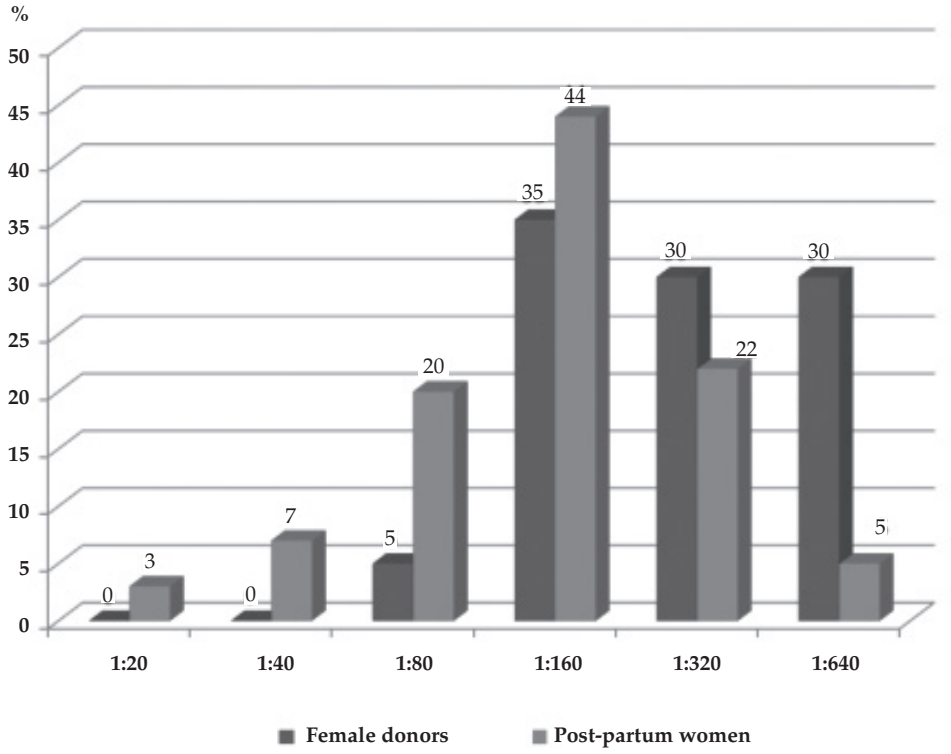


Figure 2 shows a comparison between titers of female donors and post-partum women by age, with the largest difference observed in young women.

The analysis of serum from 100 umbilical cordsshowed that the median titer of anti-*Bordetella* antibodies was 160 (IQR: 80-160), similar to maternal serum values, which showed a median of 160 (IQR: 80-320). However, 44% of umbilical cords showed antibody titers ≤80 versus 30% of post-partum women (OR 1.8; 95% CI: 1.01-3.22, p= 0.04).

Figure 3 shows antibody titers for each mother-newborn dyad, and demonstrates that umbilical cords titers were the same as in maternal serum in 54% of cases, lower in 37% of cases, and higher only in 8%. The transplacental transfer ratio was 0.83 for the overall population, and in most age groups, cords had lower titers than maternal serum samples (*Table 1*).

DISCUSSION

As in other countries, the increased number of cases in Argentina has had a major impact on the morbidity and mortality of infants who have not completed their primary vaccination schedule. An increase in the number of cases in young adults who have lost their vaccine-induced immunity has also been reported.[11-13]

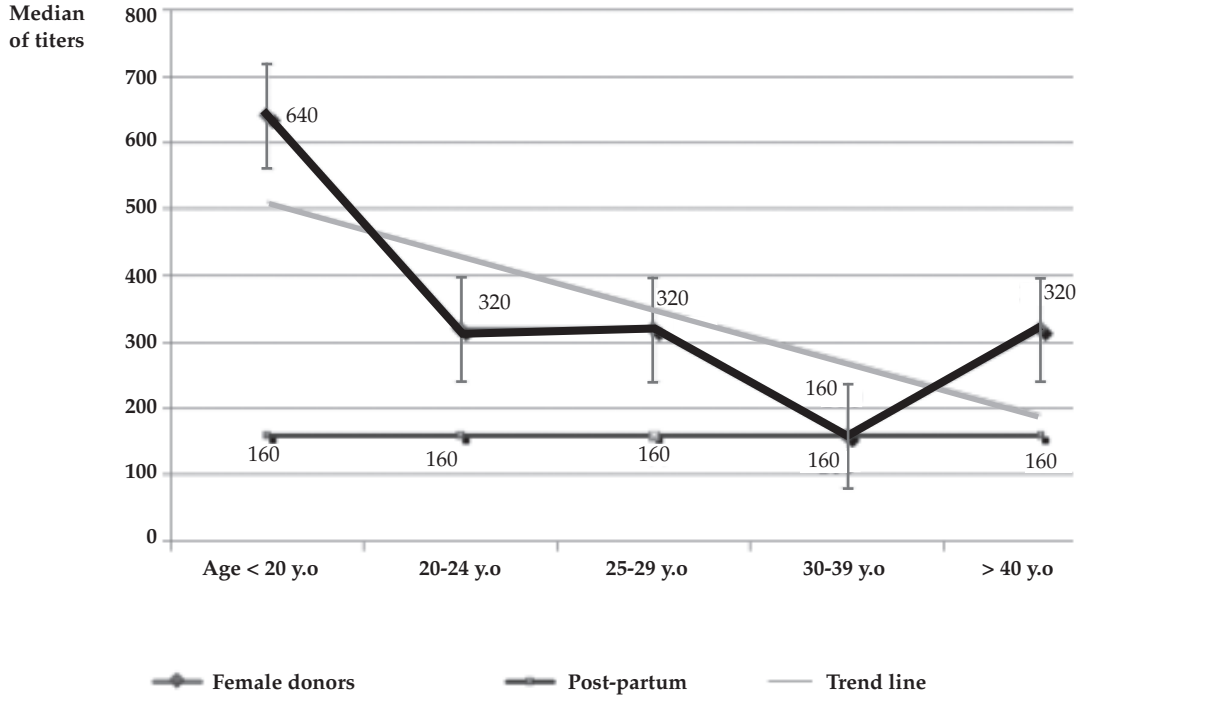
In a study conducted in our hospital during a *pertussis* outbreak (2007-2008) with 57 children hospitalized due to this condition, mean age of patients was 2.5 months old, with 80% younger than 6 months old and a 10% of deceased cases (all younger than 3 months old). The mean age of mothers was 24 years old, and 65% had a history of cough in the past month.[14]

The rate of pertussis cases in children aged 4-10 years old has remained stable. The fact that the highest rates occur in infants younger than six months old reflects that adults, especially young mothers, are the main reservoirs and sources of transmission, as demonstrated by several studies.[15,16]

This study provides the first data in Argentina regarding an important risk factor for *pertussis* in young infants, that is the serological "status" against *pertussis* in our young adult population (especially post-partum women) and at the time of birth. The methodology applied (total anti-*Bordetella* antibodies directed against whole-cell), the only one available in our country at the time of the study, allowed us to know the serological status of the assessed groups, with highly reproducible results and at an affordable cost.

At present, such methodology has been

FIGURE 2. *Comparison of pertussis seroprevalence in female donors (n= 40) and post-partum women (n= 101) by age grou*p



replaced by the measurement of *pertussis* antitoxin antibodies, which has a higher specificity, but is very expensive and still not available in our setting. Although no correlation has been established with protective immunity, there are sufficient data regarding the role of antibodies in infants younger than two months old.[17]

When analyzing the variation in titers in terms of participants' age, in the donor group it was observed that older adults had lower titers. However, in the post-partum women group, titers showed no variation in terms of age. Moreover, titers in this group were significantly lower than those of female donors.

In our cohort of mothers, 30% had low titers (≤80). A similar percentage was reported by Plans in Spain.[18] Although it is difficult to compare the results of different studies because antibodies are measured with different methodologies, most studies report very low titers during pregnancy, probably as a result of their immunosuppressive status.[17] This has also been reported in the response to other vaccines, including influenza.[19,20]

FIGURE 3. *Comparison of anti-Bordetella antibody titers in each mother-cord dyad (N= 100)*

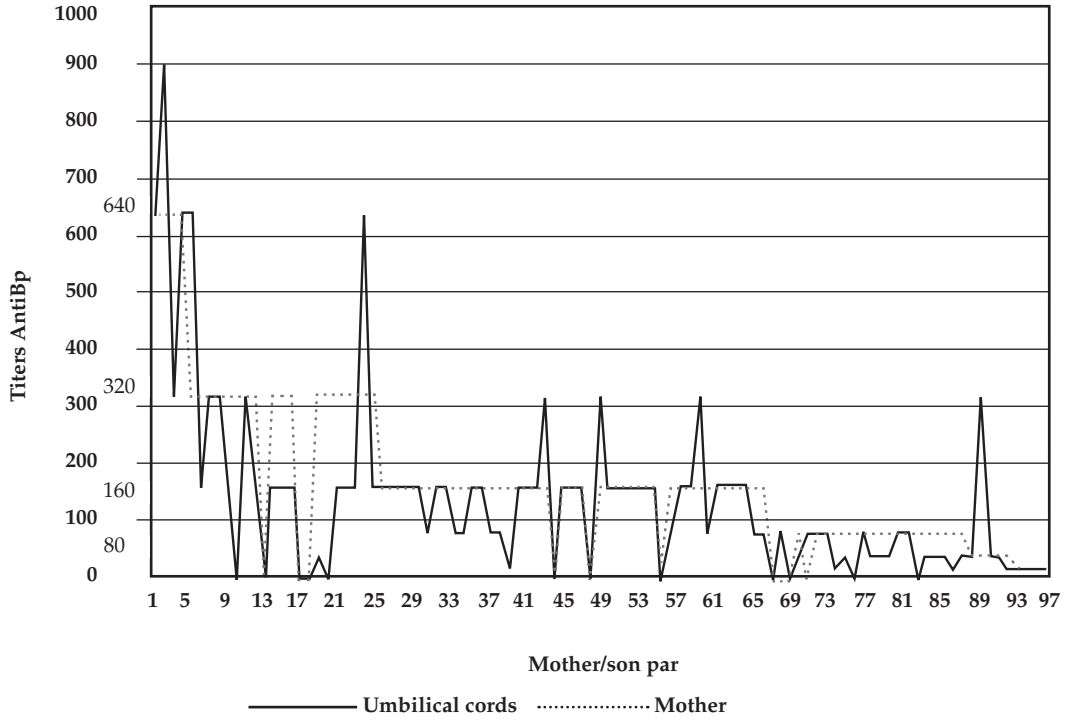


TABLE 1. *Distribution of anti-*Bordetella *antibody titers in mothers, umbilical cords and mother-cord ratio, by maternal age group*

Age of mothers	Geometric mean of anti-*Bordetella*[1] (range) Umbilical cords	Geometric mean of anti-*Bordetella*[1] (range) Mothers	Anti-*Bordetella* Mother/ umbilicalcords ratio[2]
<20 years old	121 (20-640)	166 (80-320)	0.65
20-24 years old	155 (20-2560)	157 (20-640)	0.99
25-29 years old	113 (40-640)	128 (40-640)	0.88
30-39 years old	103 (20-640)	141 (20-640)	0.73
>40 years old	67 (20-160)	95 (20-160)	0.71
All	**122 (20-2560)**	**146 (20-640)**	**0.83**

1. Anti-*Bordetella* antibodies.
2. Ratio between the geometric mean of umbilical cord antibody titers over the geometric mean of the maternal antibody titers.

The analysis of antibody titers in umbilical cords showed that 44% had titers above 80. Umbilical cord titers were the same as in the mothers in 54% of cases, lower in 37% and higher only in 8%. The umbilical cord serum/maternal serum ratio was 0.83. These results may suggest that placental transfer of antibodies is not as effective as proposed by other authors.[19,21]Heinenger proposes an active transport mechanism in the transplacental transfer of antibodies, which probably starts around 17 weeks of gestation and reaches levels 1.5-2 times higher than in the mother when infants are born at term.[19] Other studies reported similar maternal serum and umbilical cord serum levels.[22] In a study conducted in 2004, Healy, et al. found that titers were higher in umbilical cords than in mothers (1.7), but a study from 2006 in Hispanic mothers reported similar antibody levels in both mothers and umbilical cords.The reason for such conflicting results among the studies is unknown, but Healy suggests that there are probably ethnic differences that could influence results.[23-25]

This study was done before Argentina implemented a vaccine dose in pregnant women, and supports the need to assess new strategies so as to improve the protection of the most vulnerable group, that is, infants in their first months of life.

Different studies have been conducted worldwide with the purpose of preventing *pertussis* in the first months of life. Different strategies have been studied, which are not necessarily mutually exclusive, but some still pose questions on their clinical effectiveness or implementation problems. Clearly, newly designed strategies should be based on a basic point, such as being able to maintain adequate vaccination coverages from usual schedules (complete schedule at <18 months old and boosters at 6 and 11 years old) because not achieving such critical objective would certainly render any other strategy unsuccessful.

Attempted strategies included:

- A booster dose in adolescents and young adults: its objective is to prolong the duration of vaccine-induced protection. The most important difficulty faced by this strategy is achieving adequate coverages. Its impact on young infants does not appear to be high.[26]
- Vaccination during the immediate post-partum period (cocooning): its objective is to protect susceptible mothers and indirectly protect their children, considering that the mother is the main source of transmission (50-75%) during the first months of life.[11,17,27] Ideally, any other susceptible person living with the infant should also be vaccinated so as to increase the potential success of this strategy. The most important difficulty in this case is to achieve an adequate coverage and the costs resulting from vaccinating all household members.[25,28] Another item to be considered is that the peak serological response takes place fifteen days following the vaccination, leaving infants younger than two weeks old unprotected.[29]
- Neonatal vaccination: trials on neonatal vaccination with an acellular vaccine have generally demonstrated good results in terms of serological response as of fifteen days after immunization, except in Halasa's study.[30-33] In Wood's study, a good response against *pertussis* was observed, but response titers with the hepatitis B and *Haemophilus influenza* vaccines were lower than in the control group.[33] The fact that no monovalent vaccine has been authorized for use in the care environment should also be considered.
- Vaccination in pregnant women: this is potentially the only strategy that could provide protection since birth, and has been proposed after demonstrating that administering the vaccine to a pregnant woman after 20 weeks of gestation is safe and might convey protection.[25,28-30] Antibodies transferred to the newborn infant could last approximately two months, when the usual vaccination schedule starts.[34,35] The lack of data on clinical effectiveness and a possible interference with subsequent vaccinations have been reasons for concern. Heinenger's study reported a lower serological response to the usual vaccination schedule when whole-cell vaccines are used.[19]

United States and Argentina have been the first countries to implement this latter strategy (2012). In the recommendations of both countries, it is emphasized that if the mother did not receive the vaccination during the pregnancy, it should be administered immediately in the post-partum period.[28,35]

We believe that, in the future, it would be important to widen these studies, verify results with a more specific methodology and, given that vaccination in pregnant women has been implemented, make comparisons between vaccinated and non-vaccinated populations, as well as study the progress of their infants so as to determine the impact of the strategy.

CONCLUSIONS

The most relevant data obtained in this study are the low levels of antibodies detected in post-partum women and the even lower levels observed in umbilical cords, which might be consistent with the increased occurrence of *pertussis* in the first months of infants' life.■

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