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Pertussis seroprevalence in mother–infant pairs from India: role of maternal immunisation

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ABSTRACT

Objective To evaluate pertussis antibody status of pregnant women and their newborns, and the impact of antenatal immunisation.

Design Observational study.

Setting Hospitals in urban western India.

Participants Pregnant women and their newborns.

Methods Pertussis antibody titres in mothers and their newborns were determined. Vaccinated and unvaccinated mothers and their newborns were compared for baseline characteristics, geometric mean titres (GMTs) and placental transfer ratio of antibodies. Multivariate logistic regression was performed to understand the influence of different factors on protective antibody titres.

Results Of 284 mother–infant pairs, 75 mothers and 73 of their newborns were seropositive for anti-pertussis toxin (PT) IgG antibodies. 94 women were vaccinated in pregnancy; 51 (54.3%) of these mothers and newborns were PT IgG positive, compared with 24 (12.3%) of the women (and 22 newborns) not vaccinated in pregnancy. Women vaccinated in pregnancy and their newborns had higher GMT (30.88 and 32.54 IU/mL), compared with women who were not vaccinated (12.63%, 2.24 IU/mL) and their newborns (11.58%, 2.53 IU/mL). Placental transfer ratios in newborns of mothers vaccinated in pregnancy and those who had childhood immunisation or natural immunity were similar (1.05 and 1.12, respectively). Protective titres of antibodies at birth (>20 IU/mL) were observed in 72.3% vs 21% of newborns of vaccinated and unvaccinated pregnant women, respectively; influenced by mother's vaccination status and seropositivity.

Conclusion Protection against pertussis is low in newborns of mothers who are only immunised during childhood. Vaccination early in pregnancy boosts maternal and neonatal immunity.

INTRODUCTION

Despite decades of worldwide immunisation, pertussis remains poorly controlled¹ with a high disease burden in India.² The whole cellular pertussis (wP) vaccine was introduced under the expanded programme of immunisation in 1978. The primary immunisation with the wP vaccine at 6, 10 and 14 weeks of age is followed by boosters at 16–24 months and 4–5 years of age.³ There is no national policy for booster immunisation of pregnant women or any specific age group beyond 5 years. Limited usage of the acellular pertussis (aP) vaccine booster at 10 years and during pregnancy exists in the private sector. The wP vaccine is not offered to older children and adults including pregnant women due to the risk of adverse effects.

What is already known on this topic?

- ▶ Neonatal pertussis is potentially life-threatening.
- ▶ Maternal immunisation can help to reduce infant susceptibility to pertussis.

What this study adds?

- ▶ The study reports for the first time the maternal and infant immune status for pertussis at birth from India.
- ▶ Protection is low in infants born to mothers who received whole cellular vaccine in childhood compared with women immunised during pregnancy.
- ▶ Circulation of *Bordetella pertussis* in the community is suggested by seropositivity and high antibody titres in women who have not been vaccinated recently.

Evidence suggests that the wP vaccine-induced protection is reduced to half in a period of 6–12 years.⁴ This implies that women reaching child-bearing age are unlikely to have sufficient protection against pertussis, impacting infant susceptibility to the disease. The immune system of newborns, especially if preterm, is immature and does not protect them actively against vaccine-preventable infections.^{5 6} These infants remain dependent on maternally transferred antibodies for protection until primary vaccination is completed.⁷ It is important to estimate the maternal immune status for pertussis as well as the transmission of antibodies to newborns, to understand the need for and effectiveness of booster immunisation among pregnant women.

The present study was carried out at two hospitals in urban western India to evaluate pertussis antibody status of pregnant women, their newborns, impact of antenatal immunisation and factors influencing placental transfer of antibodies. A subset of newborns were followed up for 3 months for pertussis-like illness.

MATERIALS AND METHODS

Study design, participant enrolment, sample and data collection

A prospective study enrolled participants from two tertiary care hospitals located in Pune, Maharashtra, western India, between December 2019 and January 2021. Both hospitals have an average of 80–120 deliveries per month. At one hospital, the antenatal care



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policy provides an option of aP vaccination during pregnancy, an informed choice that mothers can make.

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The sample size was calculated as 289, considering prevalence of anti-pertussis antibodies at 25% with margin of error of 5% at 95% confidence level. Healthy pregnant women between 18 and 45 years of age were eligible. At least one participant was enrolled per day to avoid selection bias and maintain data quality. Exclusion criteria included high-risk pregnancy, acute infection and severe underlying disease. For twin pregnancies, each twin and their mother were considered individual dyads. Sociodemographic data, information on gestation, health status of baby at birth and maternal history of cough illness and vaccination for pertussis during pregnancy were recorded on a standard predesigned form using Epi Info V.7.2. Cord blood (5 mL) of the newborn was obtained from the placenta directly after birth. Maternal blood (3–5 mL) was collected shortly before delivery.

Laboratory investigations

All laboratory investigations were performed at the bacteriology group of the ICMR Indian Council of Medical Research-National Institute of Virology, Pune, India. Samples were transported in cold chain within 24 hours of collection to the laboratory. Sera from all the enrolled participants were aliquoted and held at 4°C prior to testing within 1 week of collection. Anti-pertussis toxin (PT) IgG antibodies were determined using Euroimmun ELISA (Euroimmun, Luebeck, Germany). Standard calibration sera provided with the kit were used to estimate the antibody titre. Using MS Excel, point-to-point curves were made, plotting the optical density (OD) values measured for these sera against the corresponding units (linear/linear). Using the corresponding line equations, the titres of anti-PT IgG antibodies in test sera were calculated and expressed in international units per millilitre (IU/mL). The lower limit of detection (LLOD) of the assay was 0.2 IU/mL. Values lower than the LLOD were halved. For OD values higher than the highest calibrator (200 IU/mL), the samples were retested in serial twofold dilution and the result multiplied by the dilution factor to estimate the antibody titre. Internal standards calibrated to the WHO International Standard 06/140 (NIBSC National Institute for Biological Standards and Control) were used in each assay.⁸ An antibody titre of ≥ 100 IU/mL was considered indicative of either acute infection within the past 1 year or recent vaccination for the mothers, ≥ 40 IU/mL as seropositive and < 40 IU/mL as seronegative.⁹ In comparison with other commercially available ELISAs, the test had good sensitivity (95.5%–97.8%) and specificity (100%) (https://www.euroimmun.com/documents/Indications/Infections/Bordetella/EI_2050_D_UK_A.pdf).¹⁰

Studies consider protective titre of PT antibodies as > 5 IU/mL, for children.^{11 12} Considering a slightly higher titre of 10 IU/mL¹³ and known half life of PT antibodies as 36 days,¹⁴ newborns with a cord titre of 20 IU/mL at birth were predicted to be protected before receiving primary pertussis immunisation.

In a subset of participants positive for anti-PT IgG antibodies in the absence of recent vaccination, anti-fimbrial haemagglutinin (FHA) IgG antibodies were estimated. Also, anti-pertactin (PRN) IgG antibodies were estimated in a subset positive for anti-PT IgG antibodies, irrespective of vaccination status. The assays were performed using Euroimmun ELISA with estimation of antibody titres as described above.

Statistical analysis

The data were entered in Epi Info V.7.2 and analysed using STATA V.16.1 (STATA Corp, Texas, USA). Categorical variables were

Table 1 Baseline characteristics of study population

Characteristic	Vaccinated (n=94)	Unvaccinated (n=190)	P value
Maternal age	165	87	0.0044*
N (mean \pm SD)	30.18 (3.76)	28.70 (4.02)	
Parity			0.124
N	94	190	
Primiparous n(%)	59 (62.76)	101 (52.06)	
Multiparous n(%)	35 (37.23)	89 (46.8)	
Gestational age (weeks)	94	170	0.06
N (Mean \pm SD)	38.32 (1.47)	37.90 (2.15)	
Birth weight (grams)	90	178	
N (mean \pm SD)	2934.92 (431.447)	2809.09 (493.98)	0.0331*
Length (cm)	83	169	
N (mean \pm SD)	49.44 (2.47)	49.88 (2.98)	0.222
Prolonged cough illness in pregnancy			
N (n%)	17(18.09)	32 (16.84)	0.794

N=numbers available for analysis.

*P<0.05 considered as significant.

compared by Pearson's χ^2 test. Seroprevalence of anti-PT IgG antibodies with 95% CIs was determined in infant–mother dyads. Geometric mean titres (GMTs) along with their 95% CI were calculated. Antibody titres were compared by two-sample t-test with unequal variances and two-sample Kolmogorov-Smirnov test. Statistical significance ($p < 0.05$) is reported from the latter test. Placental transfer ratio of anti-PT IgG antibodies was defined as the ratio of cord to maternal geometric mean concentrations (GMCs). Univariate logistic regression was carried out with five variables classified as categorical variables (maternal age, vaccination status, maternal seropositivity for anti-PT antibodies, birth weight and gestational age of infant) to understand the influence on cord PT antibody titres > 20 IU/mL. Unadjusted ORs were recorded and significant variables considered for multivariate logistic regression.

RESULTS

Baseline characteristics of the participants

During the study period, 375 pregnant women were eligible, 9 of whom declined participation. The 366 participants enrolled included 284 mother–newborn dyads (including three twin pregnancies). Twenty-four unpaired mothers and 58 unpaired infants were excluded because of inadequate sample in terms of quality or quantity. The remaining 284 dyads were considered for final analysis. Ninety-four of the 284 women (33%) had received immunisation with the aP vaccine during pregnancy. Baseline characteristics were compared between the vaccinated and unvaccinated subsets of the study population (table 1). Maternal age and birth weight were significantly higher in the vaccinated group, while parity, gestational age and infant sex ratio were similar.

Laboratory results

Seropositivity and GMT of anti-PT IgG antibodies are summarised in table 2. Overall, 26.4% of mothers and 25.7% of newborns were seropositive for anti-PT IgG antibodies. Seropositivity was significantly higher among vaccinated mothers (54.3% vs 12.6%). The median interval (IQR) between immunisation and sampling estimated for 43 seronegative vaccinated women was 49.5 days (21.5–66.5). Significantly higher seropositivity was noted in infants born to vaccinated women (54.3% vs 11.6%).

A small proportion of women who were not vaccinated during pregnancy (24 of 190, 12.6%) showed seropositivity. These included 10 women (5.26%) who had evidence of recent infection (titre > 100 IU/mL). Seronegativity was observed in 6 of 51 (11.7%)

Table 2 Seropositivity and GMT of anti-PT IgG antibodies in vaccinated and unvaccinated subsets

	Vaccinated (n=94)	Unvaccinated (n=190)	P value
Seropositive mothers (anti-PT IgG), n (%)	51 (54.26)	24 (12.63)	<0.001*
Seropositive infants, (n)	51 (54.26)	22 (11.58)	<0.001*
Maternal anti-PT IgG	30.88	2.24	<0.001*
GMT (IU/mL) (95% CI)	21.23 to 44.91	1.47 to 3.91	
Cord anti-PT IgG	32.54	2.53	<0.001*
GMT (IU/mL) (95% CI)	22.85 to 46.33	1.68 to 3.81	
Placental transfer ratio	1.05	1.12	0.555
Maternal anti-PT IgG titre >100 (IU/mL), n (%)	22 (23.4)	10 (5.26)	0.0417*
Cord anti-PT IgG titre >20 (IU/mL), n (%)	68 (72.3)	40 (21.0)	<0.001*

*P<0.05 considered significant
anti-PT, anti-pertussis toxin; GMT, geometric mean titre.

and 5 of 24 (20.8%) infants born to seropositive vaccinated and unvaccinated women, respectively.

Maternal and cord GMTs for anti-PT IgG antibodies were significantly higher in the vaccinated subset (table 2). Good correlation of maternal and cord GMT was observed in both vaccinated (r=0.92) and unvaccinated (r=0.88) groups. Placental transfer ratios were similar in both groups (table 2).

Significantly more infants born to vaccinated mothers had cord anti-PT IgG titres of more than 20 IU/mL (72.3% vs 21%). Considering protective titre to be 10 IU/mL,¹³ and half life of antibodies to be 36 days,¹⁴ this gives an estimate of the infants having protective titres before they receive first dose of wP vaccine at 6 weeks of age.

Univariate regression analysis to understand the effect of maternal age, seropositivity and vaccination status, gestational age, and birth weight on protective cord anti-PT IgG titres (>20 IU/mL) is represented in table 3. All the factors except maternal age were significant. Multivariate logistic regression of the four significant variables showed that mother's vaccination status (adjusted OR 4.9; 95% CI 2.5 to 10.0) and seropositivity (adjusted OR 30.4; 95% CI 12.2 to 76.1) influenced the cord anti-PT IgG titres of >20 IU/mL.

Sera of 18 of 24 unvaccinated but seropositive women were available for anti-FHA IgG estimation. Eight of 18 were positive with GMT of 234.1 IU/mL (95% CI 99.1 to 608.2). The anti-PRN IgG was estimated for 104 women whose sera were available. Significantly, more vaccinated women were positive for anti-PRN antibodies (table 4). Two of 32 unvaccinated women with cough illness in pregnancy had anti-PT IgG titres >100 IU/mL and were positive for anti-FHA and anti-PRN IgG antibodies, suggestive of recent infection.

During the follow-up period of 3 months, none of the infants (n=194) developed pertussis-like illness.

Table 3 Univariate regression analysis—effect on cord anti-PT IgG titres >20 IU/mL

Variable	OR (95% CI)	P value
Maternal age	1.60 (0.88 to 2.91)	0.118
Vaccinated in pregnancy	0.80 (5.54 to 17.35)	<0.001*
Maternal seropositivity	41.04 (17.52 to 96.11)	<0.001*
Term gestational age	2.58 (1.32 to 5.05)	0.005*
Birth weight	2.26 (1.10 to 4.67)	0.026*

*P<0.05 considered significant
anti-PT, anti-pertussis toxin.

Table 4 Anti-PRN antibodies in vaccinated and unvaccinated women

	Vaccinated (n=67)	Unvaccinated (n=37)	P value
Anti-PRN IgG positive, n (%)	42 (62.6)	20 (54.05)	0.0377*

*P<0.05 considered significant
anti-PRN, anti-pertactin.

DISCUSSION

Unimmunised neonates and partially immunised infants remain the most vulnerable to pertussis and at risk of life-threatening complications. Maternal immune status plays an important role in determining neonatal susceptibility to pertussis.¹⁵ In the present study, we estimated the seroprevalence of antibodies to PT in pregnant women, their newborns, and studied the impact of antenatal pertussis vaccination in a subset of women and their babies. The antibody, anti-PT IgG, is specific to *Bordetella pertussis*, commonly evaluated in infection and vaccine efficacy studies and correlated with clinical protection against pertussis infection.^{4 16} *B. pertussis*-specific antibodies can be detected by ELISAs or multiplex immunoassays. As recommended by the European Union Pertstrain group, the ELISA kit in our study uses purified non-detoxified PT as an antigen, has a broad linear range and expresses results quantitatively in IU/mL.¹⁷ We overcame the limitation of standardisation of pertussis antibody estimation by using the recommended WHO reference serum, which is widely used to assist in the standardisation of immunoassays for measurement of human antibodies.⁸

Vaccinated women and their newborns had higher seroprevalence and GMT of antibodies as compared with unvaccinated women. The low seroprevalence of antibodies in the dyads which included women unvaccinated in pregnancy indicates that these women and their babies remain susceptible to pertussis. Around half (54%) of the vaccinated women showed seropositivity. We explored the cause of seronegativity among the vaccinated women and found the majority (75%) had been immunised less than 67 days before delivery. The recommended timing for vaccination in pregnancy by the Centers for Disease Control¹⁸ is between 27 and 36 weeks. Recent studies suggest that antibody concentrations are highest if the vaccine is administered between week 27th and 30th.¹⁹ Our results corroborate these findings with better seroconversion observed for earlier vaccination.

Vaccinated women and their infants possessed significantly higher GMT of antibodies. Women unvaccinated during pregnancy were assumed to have received the wP vaccine during childhood, as childhood immunisation records were not available. It is possible that some of them did not receive the vaccine or that some had natural infection-induced immunity. Waning of childhood vaccine-induced immunity is confirmed by low GMT in these participants and their infants. Although we did not attempt to explore population immunity in this study, the data support current concerns regarding adult susceptibility to pertussis. The atypical clinical presentation of pertussis in adults can result in the diagnosis being missed,²⁰ while the adult host remains an important source of infection.²¹

Irrespective of the quantity of antibodies in maternal sera, efficient transplacental transfer was demonstrated in both groups of study participants. This phenomenon could be effectively used to improve neonatal immunity to pertussis.

High antibody titres of >100 IU/mL in around 5% of the unvaccinated women indicate recent natural pertussis infection.²² Two unvaccinated women with a history of prolonged cough illness in pregnancy were positive for all three antibodies (PT, PRN, FHA) studied. This indirectly corroborates the persistent circulation of *B.*

pertussis in the community. *B. pertussis* is strictly a human pathogen without an animal or environmental reservoir,²³ which continues to circulate in the natural environment.

Although protective titres of antibodies are not absolutely quantified,^{24–26} earlier studies have considered different titres as protective for PT, such as 5 IU/mL based on vaccine efficacy data^{11 12} and 10IU/mL.¹³ The effective half life for anti-PT IgG antibodies is calculated to be 36 days.¹⁴ We hypothesised the antibody titre of 20IU/mL at birth as leading to predicted antibody titres of 10IU/mL at 5 weeks. Primary immunisation begins at 6 weeks in India. Considering poor immunogenicity of pertussis vaccine, good protection can be achieved only after three primary doses⁴ completed by 14 weeks of age in India. In our study, protective titres were observed in 72.3% babies in maternal vaccinated group compared with 21% in newborns of mothers who were not vaccinated in pregnancy. Therefore, most babies born to mothers not vaccinated in pregnancy are susceptible to pertussis in the first few months of life. Pertussis is more severe and can be fatal in neonatal period,¹ highlighting the need for boosting maternal immunity.

Only maternal vaccination and seropositivity influenced cord GMT of anti-PT antibodies, underscoring the importance of boosting maternal immunity to protect neonates against pertussis.

The exploration of PRN antibodies in a subset of available sera showed more vaccinated women produced antibodies against PRN than against PT. The role of PRN is important in relation to susceptibility to pertussis, with higher PRN antibodies offering moderate protection against disease.¹²

Our study has some limitations. It was conducted in one urban site and results may vary in other regions. Expansion of the study to additional sites in India is in process. Decay of antibodies in infants was extrapolated and not directly estimated. However, as the half life of pertussis antibody is well defined through previous studies, the extrapolation would provide a reasonably accurate indication of susceptibility to pertussis among these infants.^{14 21 26} Follow-up was short and is being extended.

Recent studies have highlighted the potential for blunting of infant immune response to wP vaccine, in the background of maternal immunisation.²⁷ Follow-up studies need to be carried out to explore this aspect further.

CONCLUSION

Our study highlights for the first time from India, the maternal and infant immune status to pertussis at birth. Protection against pertussis is low in mothers immunised during childhood. Circulation of *B. pertussis* in the community is suggested. Infants born to unvaccinated mothers have low neonatal protection. Vaccination given early in pregnancy boosts both maternal and neonatal immunity. Efficient antibody transfer may be effectively used for providing neonatal protection against pertussis. The impact on immune response to infant vaccination needs to be studied further.

Correction notice This article has been updated since it was first published. A small typographical error has been corrected.

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Contributors RV—concept, planning laboratory investigation, data analysis, first and final draft. SB and HD—planning, clinical data collection and interpretation. SJ—statistical analysis. SK and ShrM—laboratory investigations and data interpretation. KP and ShwM—clinical sample and data collection and verification. All authors read and approved the final draft.

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