

Original Research Article

ANTI-BORDETELLA PERTUSSIS TOXIN IGG SEROPOSITIVITY AMONG CLINICALLY SUSPECTED PEDIATRIC CASES: A HOSPITAL-BASED OBSERVATIONAL STUDY

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ABSTRACT

Background: Detection of immunoglobulin G (IgG) antibodies against pertussis toxin (PT) by enzyme-linked immunosorbent assay (ELISA) may provide supportive serological evidence in the evaluation of clinically suspected pertussis, particularly when direct detection of the organism is less sensitive. The objective is to determine the seropositivity of anti-pertussis toxin IgG antibodies among clinically suspected pediatric cases of pertussis and to assess their distribution according to demographic characteristics, clinical presentation, and immunization status.

Materials and Methods: This hospital-based observational study included 100 serum samples collected from clinically suspected cases of pertussis among neonates and children aged 1 day to 12 years attending Sir Padampat Mother and Child Hospital, attached to SMS Medical College, Jaipur. Serum samples were tested for anti-Bordetella pertussis toxin IgG antibodies using a commercial ELISA kit. Results were interpreted according to the manufacturer's recommended criteria. Data were analyzed with respect to age and gender, and appropriate statistical tests were applied.

Results: Among the 100 clinically suspected pediatric cases tested, 7 (7%) were positive for anti-Bordetella pertussis toxin IgG, while 12 (12%) showed borderline results and 81 (81%) were negative. No statistically significant association was observed between anti-PT IgG serological status and gender ($P = 0.630$) or age group ($P = 0.921$). A statistically significant association was observed between immunization status and anti-PT IgG serological category ($\chi^2 = 34.379$, $P = 0.001$), with higher positivity among incompletely immunized participants.

Conclusion: Anti-pertussis toxin IgG seropositivity was detected in a proportion of clinically suspected pediatric pertussis cases, while a further subset showed borderline antibody levels. Serological testing using anti-PT IgG ELISA may provide supportive evidence in the evaluation of suspected pertussis cases, particularly when clinical diagnosis is difficult. Larger studies incorporating detailed vaccination history and complementary diagnostic methods are warranted to better define the epidemiology of pertussis in this population.

Keywords: Bordetella pertussis; pertussis; anti-pertussis toxin IgG; seropositivity; ELISA; children; serology.

INTRODUCTION

Pertussis, commonly known as whooping cough, is a highly contagious respiratory infection caused

predominantly by *Bordetella pertussis*, a Gram-negative coccobacillus belonging to the genus *Bordetella*.^[1] The infection is transmitted mainly through respiratory droplets and is particularly

important in infants and young children, in whom it may result in serious complications including apnea, pneumonia and, in severe cases, death.^[1,2]

The clinical course of pertussis is traditionally divided into catarrhal, paroxysmal and convalescent stages. During the early catarrhal stage, symptoms may resemble those of other common respiratory infections, making clinical diagnosis difficult. The characteristic paroxysmal cough, inspiratory whoop and post-tussive vomiting may develop later in the course of illness.^[2,3] However, classical manifestations may be absent, particularly in young infants and previously vaccinated individuals, further complicating diagnosis.

Several virulence factors contribute to the pathogenicity of *B. pertussis*. Among these, pertussis toxin (PT) is a major virulence factor and an important immunological marker of infection. Other important components include filamentous haemagglutinin, pertactin, fimbriae and adenylate cyclase toxin, which contribute to bacterial adhesion, colonization and modulation of the host immune response.^[3,4] The ability of *B. pertussis* to cause disease despite vaccination and the occurrence of atypical presentations highlight the continuing importance of accurate laboratory diagnosis.

Laboratory confirmation of pertussis can be achieved using culture, molecular methods and serological assays. Culture is highly specific but its sensitivity decreases with increasing duration of illness and prior antibiotic exposure. Polymerase chain reaction (PCR) provides more rapid detection but may have limited availability in some healthcare settings. Serological detection of antibodies against pertussis toxin, particularly immunoglobulin G (IgG), can be useful in patients presenting later in the course of illness, when direct detection of the organism becomes less sensitive.^[5,6]

Anti-PT IgG measurement by enzyme-linked immunosorbent assay (ELISA) has been widely investigated as a serological approach for assessing recent exposure to *B. pertussis*. Elevated anti-PT IgG concentrations may indicate recent infection, although interpretation can be influenced by previous vaccination and the interval between vaccination, infection and sample collection.^[5,7] Previous seroepidemiological studies have demonstrated detectable anti-PT antibodies in different age groups and have highlighted the persistence of pertussis circulation despite widespread vaccination.^[7-9]

In children with clinically suspected pertussis, determination of anti-PT IgG may therefore provide useful supportive evidence, particularly when clinical features are atypical or when patients present after the optimal period for bacterial detection. Assessment of seropositivity according to demographic and clinical characteristics may also contribute to a better understanding of pertussis epidemiology in the local population.

Therefore, the present study was undertaken to determine the seropositivity of anti-Bordetella pertussis toxin IgG antibodies among clinically

suspected pediatric cases of pertussis using ELISA and to evaluate the distribution of serological results according to age and gender.

MATERIALS AND METHODS

Study Design and Setting: This hospital-based observational study was conducted in the Department of Microbiology, Sawai Man Singh Medical College and Attached Hospitals, Jaipur, Rajasthan. The study was carried out over a period of 18 months following approval from the Institutional Ethics Committee. The study included clinically suspected cases of pertussis among neonates and children attending the hospital.

Study Population and Sample Size: A total of 100 serum samples were collected from clinically suspected cases of pertussis. The study population comprised neonates and children aged 1 day to 12 years. Patients were included on the basis of clinical suspicion of pertussis, including features such as prolonged or paroxysmal cough, inspiratory whoop, post-tussive vomiting and other compatible respiratory symptoms.

Inclusion Criteria

Patients were included if they were:

1. Neonates or children aged 1 day to 12 years.
2. Clinically suspected of having pertussis.
3. Presenting with symptoms compatible with pertussis, including persistent or paroxysmal cough.
4. Accompanied by a parent or guardian who provided informed consent for participation.

Exclusion Criteria

Patients were excluded if:

1. Adequate clinical information was unavailable.
2. The serum sample was insufficient or unsuitable for analysis.
3. The patient or guardian did not provide consent for participation.

Sample Collection and Processing: Approximately 2–3 mL of venous blood was collected aseptically from each participant using a sterile disposable syringe. Blood samples were transferred into appropriately labelled sterile tubes and transported to the microbiology laboratory. Samples were allowed to clot and were subsequently centrifuged to separate serum. The separated serum was transferred into labelled sterile containers and stored at the recommended temperature until analysis.

Demographic and relevant clinical information, including age, gender and available vaccination history, was recorded using a structured data collection form.

Serological Testing: Serum samples were tested for anti-Bordetella pertussis toxin (anti-PT) IgG antibodies using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN Medizinische Labordiagnostika AG). The results were interpreted according to the manufacturer's recommended cut-off values: anti-PT

IgG levels ≥ 100 IU/mL were considered positive, levels of 40 to <100 IU/mL were considered borderline, and levels <40 IU/mL were considered negative.

The assay was based on the detection of specific IgG antibodies against pertussis toxin in patient serum. Briefly, serum samples and appropriate controls were added to antigen-coated microplate wells. Following incubation, unbound antibodies were removed by washing. An enzyme-labelled anti-human IgG conjugate was subsequently added. After further incubation and washing, substrate was added and the enzymatic reaction produced a measurable colour change. The optical density was measured using an ELISA reader at the wavelength specified by the manufacturer.

Interpretation of Results

Anti-PT IgG results were interpreted according to the manufacturer's recommended cut-off values and categorized as:

- Positive
- Borderline
- Negative

The serological status of each participant was subsequently evaluated according to demographic characteristics.

Statistical Analysis: Data were entered into a computerized database and analyzed using appropriate statistical software. Categorical variables were expressed as frequencies and percentages. Associations between anti-PT IgG serological categories and categorical variables, including gender, age group, and immunization status, were assessed using the chi-square test. Clinical symptoms were summarized descriptively because individual participants could report more than one symptom and the symptom categories were therefore not mutually exclusive. A P value <0.05 was considered statistically significant.

Ethical Considerations: The study was conducted after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from the parent or legal guardian of each participant before sample collection. Participant confidentiality was maintained throughout the study, and the collected information was used exclusively for research purposes.

RESULTS

Overall Seropositivity of Anti-Bordetella pertussis Toxin IgG:

A total of 100 serum samples obtained from clinically suspected cases of pertussis among neonates and children aged 1 day to 12 years were analyzed for anti-Bordetella pertussis toxin IgG antibodies. Of the 100 samples tested, 7 (7%) were positive, 12 (12%) were borderline, and 81 (81%) were negative for anti-PT IgG antibodies.

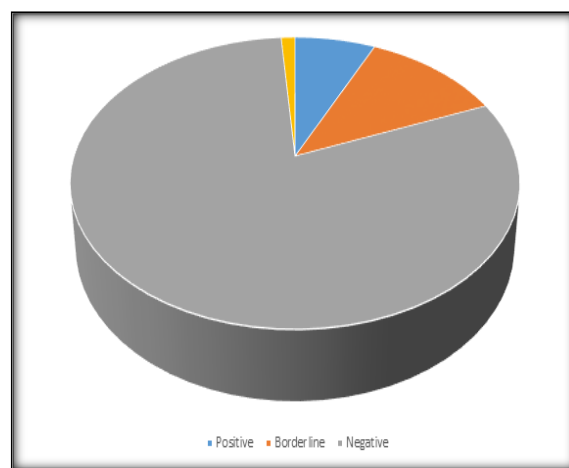


Figure 1: Pie chart showing the overall distribution of anti-Bordetella pertussis toxin IgG status among the study participants.

Table 1: Distribution of anti-Bordetella pertussis toxin IgG antibodies (N = 100)

Anti-PT IgG status	Number of samples (n)	Percentage (%)
Positive	07	07
Borderline	12	12
Negative	81	81
Total	100	100

Gender-wise Distribution of Anti-Bordetella pertussis Toxin IgG: Among the 100 participants, 52 (52%) were males and 48 (48%) were females. Anti-PT IgG positivity was higher among females (4/48, 8.33%) than males (3/52, 5.76%). Similarly, borderline positivity was higher among females

(7/48, 14.58%) than males (5/52, 9.61%). Among males, 44 (84.61%) were negative, whereas 37 (77.08%) females were negative. The association between gender and anti-PT IgG categories was not statistically significant ($\chi^2 = 0.923$, $df = 2$, $P = 0.630$).

Table 2: Gender-wise distribution of anti-Bordetella pertussis toxin IgG antibodies (N = 100)

Gender	Positive, n (%)	Borderline, n (%)	Negative, n (%)	Total
Male	03 (5.76%)	05 (9.61%)	44 (84.61%)	52
Female	04 (8.33%)	07 (14.58%)	37 (77.08%)	48
Total	07 (7%)	12 (12%)	81 (81%)	100

$\chi^2 = 0.923$; $df = 2$; $P = 0.630$.

Age-wise Distribution of Anti-Bordetella pertussis Toxin IgG: The age-wise distribution of anti-PT IgG

antibodies is presented in [Table 3]. The highest proportion of positivity was observed among children

aged 6–7 years (10.52%), followed by those aged 2–3 years (7.69%), 4–5 years (7.40%), and 0–1 and 8–9 years (7.14% each). No positive cases were observed among children aged 10–11 years or ≥12 years.

Borderline results were observed in the 2–3-year (23.07%), 4–5-year (11.11%), 6–7-year (10.52%), 8–9-year (14.28%), and 10–11-year (20%) age groups. No borderline cases were observed in the 0–1-year or ≥12-year age groups.

There was no statistically significant association between age group and anti-PT IgG category ($\chi^2 = 5.900$, $P = 0.921$).

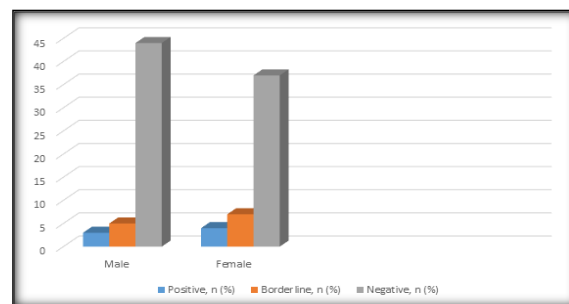


Figure 2: Clustered bar graph showing the distribution of positive and borderline anti-Bordetella pertussis toxin IgG results according to gender.

Table 3: Age-wise distribution of anti-Bordetella pertussis toxin IgG antibodies (N = 100)

Age group (years)	Positive, n (%)	Borderline, n (%)	Negative, n (%)	Total
0–1	01 (7.14%)	00 (0%)	13 (92.85%)	14
2–3	01 (7.69%)	03 (23.07%)	09 (69.23%)	13
4–5	02 (7.40%)	03 (11.11%)	22 (81.48%)	27
6–7	02 (10.52%)	02 (10.52%)	15 (78.94%)	19
8–9	01 (7.14%)	02 (14.28%)	11 (78.57%)	14
10–11	00 (0%)	02 (20%)	08 (80%)	10
≥12	00 (0%)	00 (0%)	03 (100%)	3
Total	07 (7%)	12 (12%)	81 (81%)	100

$\chi^2 = 5.900$; $P = 0.921$.

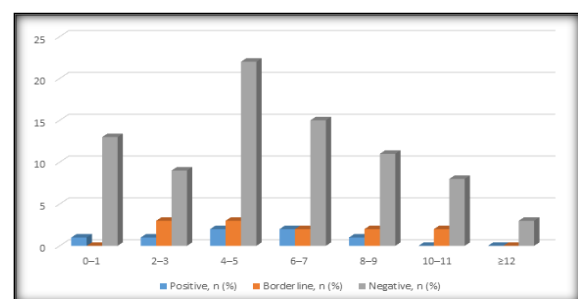


Figure 3: Bar graph showing the distribution of positive and borderline anti-Bordetella pertussis toxin IgG results according to age group.

Distribution According to Associated Symptoms:

The distribution of clinical symptoms is presented in [Table 4]. Fever was reported in all 100 participants (100%). Cough with whoop was present in 70 (70%) cases, while cough without whoop was reported in 30 (30%) cases. Post-tussive vomiting was observed in 32 (32%) participants, and apnea was reported in 9 (9%) participants. Since individual participants could present with more than one symptom, these clinical manifestations were not mutually exclusive.

Table 4: Distribution of anti-Bordetella pertussis toxin IgG according to associated symptoms (N = 100)

Clinical symptom	n (%)
Fever	100 (100%)
Cough with whoop	70 (70%)
Cough without whoop	30 (30%)
Post-tussive vomiting	32 (32%)
Apnea	9 (9%)

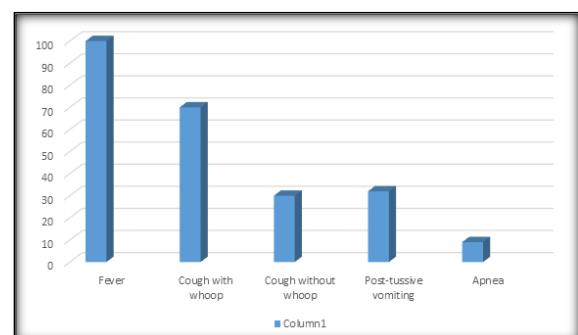


Figure 4: Bar graph showing the distribution of clinical symptoms among the study participants.

Distribution According to Immunization Status:

The distribution of anti-PT IgG according to

immunization status is presented in Table 5. Among participants with complete immunization, 2 (3.38%) were positive, none were borderline, and 57 (96.61%) were negative. Among participants with incomplete immunization, 5 (12.82%) were positive, 10 (25.64%) were borderline, and 24 (61.54%) were negative. Of the two participants who were unable to recall their immunization status, both (100%) showed borderline anti-PT IgG results.

A statistically significant association was observed between immunization status and anti-Bordetella pertussis toxin IgG serological category ($\chi^2 = 34.379$, $P = 0.001$). Anti-PT IgG positivity was higher among incompletely immunized participants (5/39, 12.82%) than among completely immunized participants (2/59, 3.38%). However, this association should not

be interpreted as a causal relationship because of the observational study design. Incomplete

immunization was associated with higher anti-PT IgG positivity.”

Table 5: Distribution of anti-Bordetella pertussis toxin IgG according to immunization status (N = 100)

Immunization status	Positive, n (%)	Borderline, n (%)	Negative, n (%)	Total
Complete	2 (3.38%)	0 (0%)	57 (96.61%)	59
Incomplete	5 (12.82%)	10 (25.64%)	24 (61.54%)	39
Unable to recall	0 (0%)	2 (100%)	0 (0%)	2
Total	7 (7%)	12 (12%)	81 (81%)	100

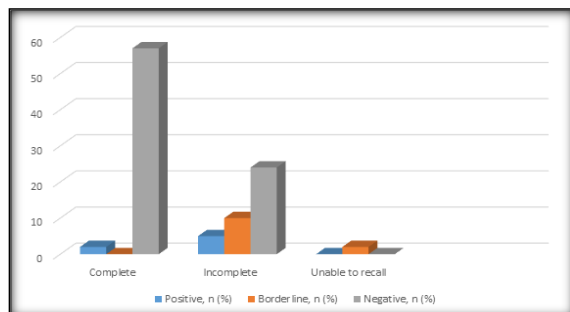


Figure 5: Clustered bar graph showing the distribution of positive, borderline, and negative anti-Bordetella pertussis toxin IgG results according to immunization status.

DISCUSSION

Pertussis is an important vaccine-preventable respiratory infection, particularly among infants and young children. Diagnosis may be challenging because its clinical manifestations can overlap with those of other respiratory infections, while the sensitivity of direct detection methods may vary according to the stage of illness. Detection of antibodies against pertussis toxin (PT), particularly anti-PT IgG, can provide supportive serological evidence of *Bordetella pertussis* exposure or infection in appropriate clinical settings.^[1,2]

In the present study, 7 (7%) of the 100 clinically suspected pediatric cases were positive for anti-Bordetella pertussis toxin IgG, while 12 (12%) showed borderline results. The observed seropositivity indicates the presence of detectable anti-PT IgG among clinically suspected cases; however, anti-PT IgG results should be interpreted in the context of vaccination history and clinical presentation, as serological positivity may reflect previous exposure or vaccination. The relatively low proportion of positive cases in the present study may also be related to differences in the study population, vaccination status, timing of sample collection, and serological testing methodology compared with other studies.^[10-12]

The relatively low proportion of seropositive cases may be influenced by several factors. The study included clinically suspected rather than laboratory-confirmed cases, and the timing of serum collection may influence the detection of antibody responses. In addition, previous vaccination may affect anti-PT IgG levels and complicate the distinction between vaccine-induced antibodies and antibodies associated with natural infection.^[2,3] Therefore, anti-PT IgG

results should be interpreted in conjunction with clinical findings, vaccination history and the timing of specimen collection.^[13]

Gender-wise findings: Gender-wise distribution showed a slightly higher anti-Bordetella pertussis toxin IgG positivity among females (4/48, 8.33%) than males (3/52, 5.76%); however, the difference was not statistically significant ($P = 0.630$). This finding indicates that gender was not significantly associated with anti-PT IgG serological status in the present study. The observed female predominance should therefore be interpreted cautiously, particularly considering the small number of seropositive participants.^[14]

Age-wise, the highest proportion of anti-PT IgG positivity was observed among children aged 6–7 years (2/19, 10.52%), followed by those aged 2–3 years (1/13, 7.69%), 4–5 years (2/27, 7.40%), and 0–1 and 8–9 years (7.14% each). No positive cases were observed among children aged 10–11 years or ≥ 12 years. However, the association between age group and anti-PT IgG serological category was not statistically significant ($\chi^2 = 5.900$, $P = 0.921$). These findings suggest that the differences in seropositivity across age groups in this study may represent variation within the sample rather than a statistically demonstrable age-related association.^[15]

Clinical presentation: Fever was reported in all 100 participants (100%), followed by cough with whoop in 70 (70%), post-tussive vomiting in 32 (32%), cough without whoop in 30 (30%), and apnea in 9 (9%). Since individual participants could present with more than one symptom, these clinical manifestations were not mutually exclusive and were therefore interpreted descriptively. The presence of classical symptoms such as cough with whoop and post-tussive vomiting may support clinical suspicion of pertussis; however, clinical manifestations alone may not reliably distinguish pertussis from other respiratory illnesses. Laboratory testing may therefore provide useful supportive information in clinically suspected cases.^[16]

Immunization status and anti-PT IgG positivity: A statistically significant association was observed between immunization status and anti-Bordetella pertussis toxin IgG serological category ($\chi^2 = 34.379$, $P = 0.001$). Anti-PT IgG positivity was higher among incompletely immunized participants (12.82%) than among completely immunized participants (3.38%). This finding may indicate a greater likelihood of detectable anti-PT IgG among children with incomplete immunization; however, the association

should not be interpreted as causal. Differences in previous exposure to *B. pertussis*, vaccination history, timing of vaccination, and the interval between vaccination and serum collection may have contributed to the observed pattern.^[17]

The finding emphasizes the importance of maintaining complete age-appropriate immunization among children. Nevertheless, anti-PT IgG serological results should be interpreted together with clinical history and vaccination status, as antibody detection alone cannot distinguish all cases of previous infection from vaccine-related antibody responses. The observed association indicates that anti-PT IgG positivity was higher among incompletely immunized participants; however, this finding should be interpreted cautiously and does not establish a causal relationship between incomplete immunization and seropositivity.^[18]

Borderline anti-PT IgG results: In the present study, 12 (12%) samples showed borderline anti-Bordetella pertussis toxin IgG results. According to the manufacturer's recommendations, borderline results should be verified by testing a second blood sample collected after 7–10 days. However, repeat sampling could not be performed because the participants could not be traced for follow-up. Therefore, these borderline results were not classified as seropositive and were retained as a separate category in the analysis. The clinical significance of borderline anti-PT IgG results should consequently be interpreted with caution.^[19]

Clinical and epidemiological implications: The findings of this study demonstrate that a proportion of children clinically suspected of having pertussis had detectable anti-PT IgG antibodies. The significant association observed with immunization status further highlights the importance of maintaining complete childhood vaccination. At the same time, the absence of significant associations with age, gender and clinical symptoms indicates that demographic and clinical characteristics alone may not adequately identify serologically positive cases.^[20]

Anti-PT IgG ELISA may therefore be useful as an adjunctive diagnostic approach in selected clinically suspected cases. However, serological testing should be interpreted within the appropriate clinical and epidemiological context and should not replace molecular or culture-based diagnostic methods when these are available and clinically indicated.^[1,2]

Strengths and Limitations: The strength of the present study lies in the assessment of anti-PT IgG antibodies among clinically suspected pediatric pertussis cases together with evaluation of demographic characteristics, clinical manifestations and immunization status. The inclusion of immunization status provides an additional epidemiological perspective on serological findings. The study has several limitations. First, the sample size was relatively small and the study was conducted at a single tertiary-care center, which may limit the generalizability of the findings. Second, the study

included clinically suspected cases without parallel confirmation by culture or PCR; therefore, the diagnostic accuracy of anti-PT IgG ELISA could not be determined. Third, interpretation of anti-PT IgG may be influenced by previous vaccination and the interval between vaccination, infection and serum collection. Finally, because of the observational study design, the significant association between immunization status and anti-PT IgG positivity should not be interpreted as a causal relationship.

Further multicentric studies with larger sample sizes, documented vaccination histories and complementary molecular diagnostic methods are required to better characterize the epidemiology of pertussis and the role of serological testing in pediatric populations.

CONCLUSION

The present study demonstrated anti-Bordetella pertussis toxin IgG seropositivity in 7% of clinically suspected pediatric cases, while 12% showed borderline antibody levels. No statistically significant association was observed between anti-PT IgG serological status and gender or age group. In contrast, immunization status was significantly associated with anti-PT IgG serological category, with higher positivity observed among incompletely immunized participants. However, this association should not be interpreted as causal because of the observational nature of the study.

The findings highlight the importance of complete age-appropriate immunization and support the potential role of anti-PT IgG ELISA as an adjunctive investigation in clinically suspected pertussis. Serological findings should be interpreted together with clinical history and vaccination status, and where available, supported by molecular or culture-based diagnostic methods. Further multicentric studies with larger pediatric populations, reliable vaccination records, and complementary diagnostic methods are warranted.

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