Susceptibility to Diphtheria among Vaccinated Children Aged 1–14 Years: A Serosurvey in a Rural Subdistrict, Bangladesh, 2019

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Abstract

In Bangladesh, diphtheria vaccine is administered to children at six, ten and fourteen weeks of age, with no booster dose given up to 14 years. As antibody levels decrease over time, over five years may become susceptible to diphtheria. We conducted a serosurvey in a rural subdistrict among children aged 1–14 years to estimate the proportion of protected children, to determine the mean anti-diphtheria antibody concentration, and to identify factors associated with being unprotected in vaccinated children. We enrolled children who had completed their primary diphtheria vaccination and selected three age groups: 1–4 (n=422), 5–9 (n=518), and 10–14 years (n=402), using cluster sampling across 96 locations in a selected rural subdistrict. We collected information on socio-demographic, environmental, maternal and birth-related characteristics, and vaccination history. Serum diphtheria antibody levels were measured by ELISA, with ≥0.1 IU/mL considered protective. We used chi-squared test and odds ratio to identify associated factors and applied multiple logistic regression to adjust for confounding. The proportion of protected children in the three age groups was 76% (1–4), 48% (5–9) and 36% (10–14). The geometric mean titer (GMT) of diphtheria antibody was highest in 1–4 years (0.18 IU/mL) and lowest 10–14 years (0.08 IU/mL). Being in older age groups were significantly associated with being unprotected. Over half of children aged 5–9 years and two-thirds of children aged 10–14 years were unprotected. We recommend introducing a booster dose of diphtheria vaccine for children over five years.

Keywords: diphtheria, immunity, serosurvey, vaccine, booster

Introduction

Diphtheria, an acute infection caused by the exotoxin producing bacteria *Corynebacterium diphtheria*, spreads from person to person via respiratory route or direct contact.[1] The crude case-fatality is 5–10% and increases to 20% for children <5 years of age.[2] In the pre-vaccine era, diphtheria was a major cause of childhood death. With vaccination, global incidence of diphtheria decreased rapidly. However, spikes in incidence show diphtheria's potential for outbreaks in under vaccinated populations with no or waning immunity.[3] Vaccination is the most effective prevention strategy with 87% effectiveness after three doses.[4] Diphtheria immunity depends on presence of Immunoglobulin G (IgG) antibodies against diphtheria toxoid, known as anti-diphtheria toxoid antibodies (IgG-DTAb) or diphtheria antitoxin. Antitoxin levels

≥0.1 international units per milliliter (IU/mL) confer full protection and ≥1.0 IU/mL indicate long-term protection.[5]

Bangladesh initiated a weekly passive hospital-based surveillance for diphtheria and other vaccine preventable diseases in 1995. The surveillance case definition for diphtheria is a person with a respiratory tract illness characterized by sore throat, fever, adherent membrane of tonsils, pharynx or nose without other apparent cause determined by the physician. This is a clinical diagnosis and reported to the National Expanded Programme on Immunization (EPI).

No diphtheria outbreak was reported from 2012–2016 in Bangladesh.[6,7] In 2017, an outbreak of >7,000 cases occurred when Forcibly Displaced Myanmar Nationals entered Bangladesh from Myanmar, a diphtheria endemic neighboring country.[6] Median age of cases was 10 years.[6] However, their vaccination status remains unknown due to unavailability of vaccination cards.[8] From 2018–2023, the Institute of Epidemiology, Disease Control and Research (IEDCR) investigated 16 diphtheria notifications from different parts of Bangladesh. Reports from these investigations show that, among 25 clinically confirmed cases with a typical pseudo-membrane, 65% were between 5–14 years of age. Of cases with known vaccination status, 79% were fully vaccinated as per EPI schedule. Case fatality rate was 8% (2/25). Although only sporadic cases have been reported, diphtheria remains a threat to children with low sero-protection.

Bangladesh EPI vaccinates children with diphtheria toxoid in combination with pertussis, tetanus, hepatitis-B and Haemophilus influenza-B as DPT-HepB-Hib (pentavalent) vaccine at ages 6, 10 and 14 weeks.[9,10] The third dose of DTP-Hep B-Hib vaccine has 98% coverage.[11] Since 2019, EPI recommends tetanus-diphtheria (Td) vaccine only for women aged 15–49 years.[12] However, no diphtheria booster is recommended in Bangladesh for children aged 1–14 years.

Immunity to diphtheria following 3-dose primary schedule wanes over time without a boosting effect from natural exposure in the environment.[13] At 5–6 years after vaccination, antibody titers drop to pre-vaccination level.[14] World Health Organization recommends three diphtheria booster after completing 3-dose primary schedule, to be administered at 12–23 months, 4–7 years and 9–15 years of age.[15]

However, the number and timing of boosters vary in different countries, depending on local epidemiologic patterns linked to regional diphtheria endemicity (endemic, low-endemic or non-endemic), local incidence of respiratory diphtheria, prevalence of cutaneous diphtheria as a source of natural immunity, herd immunity, and seroprevalence of antitoxin in different age groups.[1] In India, two boosters are administered at 16–24 months and 5–6 years.[2] In Indonesia, four boosters are administered at 18 months of age and at 1st, 2nd and 5th grade of elementary school.[16] However, socioeconomic changes, especially migration and sociocultural changes, may change local epidemiologic patterns of diphtheria.[1]

The objectives of our study were to estimate the proportion of vaccinated children having a protective level of anti-diphtheria antibody in different age groups, determine the mean anti-diphtheria antibody concentration in vaccinated children of different age groups, and determine factors associated with being unprotected against diphtheria in vaccinated children.

Methods

Study Design and Site

A cross-sectional serosurvey was conducted from July–September 2019 in Belabo Subdistrict of Narshingdi District, Bangladesh. We selected Narshingdi District due to high coverage of pentavalent vaccine (94.1%) and high vaccination card retention rate (98.3%).[17] Belabo is a rural subdistrict of Narshingdi District with 190,086 population.[18] According to EPI (internal report, 2019), Belabo Subdistrict had an administrative coverage of the third dose of pentavalent vaccine of 96.4% in 2018.

Study Population

We included children from three age groups: 1–4; 5–9; and 10–14 years, who had completed all three doses of pentavalent vaccine. Age was determined by the date of birth on the EPI vaccination card. We excluded children who had received the third dose of pentavalent vaccine within one month of data collection, children with known bleeding disorder or with a clinical diagnosis of diphtheria, and children without a vaccination card.

Sample Size and Assumptions

The sample size for this study was 483 participants per age group, totaling 1,449 individuals. This estimation was based on an assumed 70% protection rate among children, drawn from findings in a similar study, with a 5% margin of error and a design effect of 1.5.[19,20]

Recruitment

We recruited children by household cluster sampling. Belabo Subdistrict has eight administrative unions, each has three wards with four blocks. Each block has

two vaccination centers. We conveniently selected one of two vaccination sites in each block (total 96 vaccination centers) as the starting point of a location. Thus, total 96 locations were selected across Belabo. From the selected starting point (vaccination center) in each location, the survey sampling direction was randomly determined by spinning a pen. In that direction, the nearest house was visited first and then the next house, until a sample of five eligible children were recruited from each age group. If there were more than one child in the same age group in a house, one of them was randomly selected by lottery.

Collection of Socio-demographic Information

Eight teams conducted face-to-face interviews of parents of eligible children using a pretested semi-structured questionnaire. Data were collected on socio-demographic, socio-economic and environmental conditions, gestation and birth related history, clinical information and vaccination history of the children.

Collection and Transportation of Biological Samples

Recruited medical technologists aseptically collected two milliliters (mL) of venus blood from eligible children. Serum was daily transported under cold-chain to the IEDCR laboratory, where it was stored at -20 °C.

Determination of Diphtheria Toxoid IgG Antibody

Diphtheria antitoxin (IgG type) was measured in IU/mL of serum by enzyme linked immuno-sorbent assay (ELISA) kit (diphtheria toxoid IgG ELISA, Demeditec Diagnostics GmbH, Lise-Meitner, Kiel, Germany).[21] The ELISA kit is calibrated using international standards (WHO reference preparation 00/496). This assay has been shown to correlate well with other ELISAs (coefficient of correlation of 0.94 with Immunolab diphtheria IgG test, Virotech, Germany [Cat No. EC129.00]).[22] There is good correlation between clinical protection and serum antitoxin level; a level of 0.1 IU/mL is considered protective.[23] For this analysis, we considered children with titers $\geq$0.1 IU/mL as protected and children with titers <0.1 IU/mL as unprotected.[19,24,25]

Data Analysis

We calculated the proportion of protected children in each age group by dividing the number of protected children by the number of all children in that age group. To examine association between antibody titer and age, log antibody titers were compared with age using correlation and linear regression and by calculating the correlation coefficient. Geometric mean titer (GMT) of diphtheria antitoxin with corresponding 95% confidence interval (CI) was calculated for each age group and for both genders. Differences in GMT among three age groups were assessed by one-way ANOVA. Differences in GMT between males and females were assessed using a t-test. A p-value of <0.05 was considered significant.

We compared unprotected to protected children to analyze the association of socio-demographic characteristics, environmental conditions, gestational and birth-related characteristics. We calculated odds ratio (OR) and chi-square to determine statistical significance. To adjust for confounding effects, multiple logistic regression was performed.

The following independent variables were tested for a 15% change in odds ratio of the main variable age group to form a model: age group, gender, maternal diabetes during pregnancy, birthplace, mode of delivery, completion of pneumococcal conjugate vaccine (PCV) and color-marking of tube-wells representing the arsenic content in water.[26] Completion of PCV was excluded, as it was applicable to only age group 1–4 years. During model formation, the following variables were excluded by forward selection: gender, maternal diabetes during pregnancy, birthplace and mode of delivery. Finally, the final model comprised of two variables: age group and colour of tube-wells. Adjusted odds ratio with 95% confidence intervals (CI) were calculated.

Results

We interviewed and collected samples from children in three age groups: 1–4 years (422), 5–9 years (518) and 10–14 years (402), totaling 1,342 children. Among participants, 674 (50.22%) were male and 668 (49.78%) were female (Table 1).

Mean maternal age at birth was 22.65±5.52 years, and the median was 22.14 years. Among different age groups, the mean maternal age at birth was highest in youngest age group and lowest in oldest age group. Only 262 (19.52%) mothers of the participating children attended at least four antenatal visits while pregnant with that child (Table 2).

Immunity Status of Study Participants and Associated Factors

Of 1,342 children, 713 (53.13%) children were protected, and 629 (46.87%) children were unprotected. Overall GMT of diphtheria antitoxin was 0.11 IU/mL (95% CI 0.11–0.12) in all 1,342 children (Table 3).

Table 1. Participant characteristics of vaccinated children aged 1–14 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

Characteristics	n (%)
Age distribution, years (n=1,342)	
1–4	422 (31.45)
5–9	518 (38.60)
10–14	402 (29.96)
Gender distribution (n=1,342)	
Male	674 (50.22)
Female	668 (49.78)
Educational status (n=915)	
Attending educational institute	839 (91.70)
Not attending educational institute	76 (08.30)
Family size of the participant children (n=1,342)	
Up to five family members	887 (66.10)
More than five family members	455 (33.90)
Monthly family expenditure in BDT (n=1,342)	
Up to 12,000 BDT	739 (55.07)
More than 12,000 BDT	603 (44.93)
House-hold floor structure (n=1,337)	
Clay made	934 (69.85)
Cemented	387 (28.95)
Other materials (woods, tiles etc.)	16 (01.20)
Use of tube well water for drinking (n=1,323)	
Yes	1,284 (97.05)
No	39 (02.95)
High arsenic content in tube well water (n=1,265)	
Yes	14 (01.11)
No	355 (28.06)
Unknown	896 (70.83)
PCV vaccination in 1–4 years age group (n=422)	
PCV-1	277 (65.64)
PCV-2	268 (63.51)
PCV-3	247 (58.53)

Based on 1 US dollar (USD) was equivalent to 82.91 Bangladesh Taka (BDT). PCV: pneumococcal conjugate vaccine.

Table 2. Maternal and birth related characteristics of vaccinated children aged 1–14 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

Characteristics	n (%)
Maternal age at birth	
Mean±SD (years)	22.65±5.52
Median	22.14
Number of antenatal visits	
At least four visits	262 (19.52)
Less than four visits	1,080 (80.48)
Gestational period (n=1,329)	
Pre-term	103 (07.75)
Term completed	1,226 (92.25)
Known diabetes mellitus during pregnancy (n=1,273)	
Absent	1,257 (98.74)
Present	16 (01.26)
History of hypertension during pregnancy (n=1,208)	
Absent	1,148 (95.03)
Present	60 (04.97)
Active smoking during pregnancy (n=1333)	
Smoked	18 (01.35)
Did not smoke	1,315 (98.65)
History of passive smoking during pregnancy (n=1,256)	
Present	506 (40.29)
Absent	750 (59.71)
Place of birth (n=1,330)	
Health Facility	447 (33.61)
Home	883 (66.39)
Mode of delivery (n=1,320)	
Vaginal	1,040 (78.79)
Caesarian	280 (21.21)
Reported birth weight (kilograms)(n=980)	
Less than 2.5	205 (20.92)
More than 2.5	775 (79.08)

SD: standard deviation.

Table 3. Immunity status against diphtheria among participant vaccinated children aged 1–14 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

Immunity status	Do not have protected level of diphtheria IgG n (%)	Have protected level of diphtheria IgG n (%)	P-value	GMT in IU/mL (95% CI)
Overall (n=1,342)	713 (53.13)	629 (46.87)		0.11 (0.11–0.12)
Age (years)				
1–4 (n=422)	100 (23.65)	322 (76.35)	<0.0001	0.18 (0.16–0.19)
5–9 (n=518)	270 (52.12)	248 (47.88)		0.10 (0.09–0.11)
10–14 (n=402)	259 (64.48)	143 (35.52)		0.08 (0.08–0.09)
Gender				
Male	300 (44.51)	374 (55.49)	0.08	0.12 (0.11–0.13)
Female	329 (49.25)	339 (50.75)		0.11 (0.10–0.12)

GMT: geometric mean titer. IgG: Immunoglobulin G. IU/mL: international units per milliliter. CI: confidence interval.
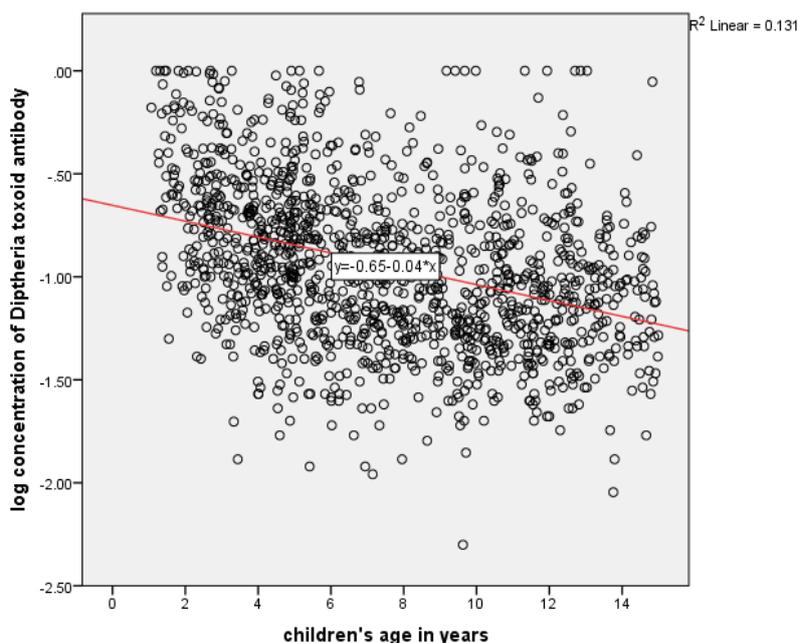
The proportion of unprotected children gradually increased with age and was lowest in children aged one year and highest in children aged 11 years. At age four years, more than 30% of children were unprotected (Figure 1).

The correlation coefficient between age (x) and ELISA titer (y) was -0.362 (p 0.01) and the regression equation was: y=-0.65–0.04x (Figure 2).
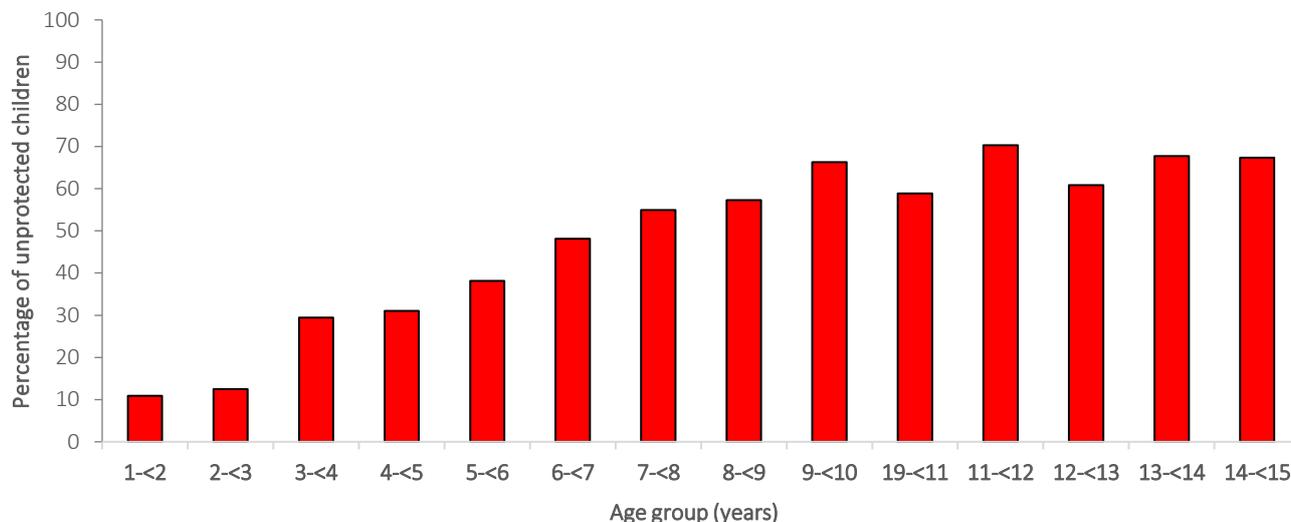
Figure 1. Proportion of unprotected children in different age groups among participant vaccinated children of 1-14 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

Figure 2. Log diphtheria toxoid antibody concentration and age of participant vaccinated children aged 1–14 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

The proportion of protected children was 76.35% in age group 1–4 years, 47.88% in age group 5–9 years, and 35.52% in age group 10–14 years, which was highly significant (p <0.0001) (Table 3).

GMT varied in three age groups and was highest in age group 1–4 years and lowest in age group 10–14 years. GMT of anti-diphtheria in age group 1–4 years was 0.18 IU/mL (95% CI 0.16–0.19), in age group 5–9 years was 0.10 IU/mL (95% CI 0.09–0.11) and in age group 10–14 years was 0.08 IU/mL (95% CI 0.08–0.09). GMT was significantly lower in older age groups, compared to 1–4 years (Table 3).

Among males, 55.49% (374/674) and among females 50.75% (339/668) were protected against diphtheria, which did not vary significantly (p 0.08). GMT of anti-diphtheria toxin antibody in males and females were 0.12 IU/mL (95% CI 0.11–0.13 IU/mL) and 0.11 IU/mL (95% CI 0.10–0.12 IU/mL) respectively (Table 3).

Among children who drank water from tube-wells marked arsenic-free by green paint, 47.59% were unprotected. However, only 7.14% of children drinking water from tube-wells containing arsenic marked by red paint were unprotected. Drinking water from tube-wells marked green (arsenic-free) was found to be a risk factor for being unprotected both in univariate (OR 11.80, 95% CI 1.52–90.99) (Table 4) and multivariate analysis (OR 17.42, 95% CI 2.17–139.90) (Table 5).

Table 4. Univariate analysis for factors associated with not having protective level of diphtheria antibody (IgG) in participant vaccinated children aged 1–14 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

Immunity status	Do not have protected level of diphtheria IgG n (%)	Have protected level of diphtheria IgG n (%)	OR (95% CI)
Age (years)			
1–4 (n=422)	100 (23.65)	322 (76.35)	Reference
5–9 (n=518)	270 (52.12)	248 (47.88)	3.51 (2.65–4.66)
10–14 (n=402)	259 (64.48)	143 (35.52)	5.83 (4.30–7.88)
Gender			
Female	329 (49.25)	339 (50.75)	1.21 (0.98–1.50)
Male	300 (44.51)	374 (55.49)	
Colour of tube-well used for drinking water			
Green	167 (47.59)	184 (52.41)	11.80 (1.52–90.99)
Red	1 (07.14)	13 (92.86)	
Maternal diabetes			
Absent	588 (46.77)	669 (53.23)	3.81 (1.08–13.41)
Present	3 (18.75)	13 (81.25)	
Maternal hypertension			
Absent	542 (47.19)	606 (52.81)	1.25 (0.74–2.11)
Present	25 (41.67)	35 (58.33)	
Birthplace			
Home	445 (50.34)	438 (49.66)	1.55 (1.23–1.95)
Hospital	177 (39.60)	270 (60.40)	
Mode of delivery			
Vaginal	523 (50.27)	517 (49.73)	2.00 (1.52–2.64)
C-section	94 (33.60)	186 (66.40)	
Gestational period			
Pre-term	54 (52.38)	49 (47.62)	1.28 (0.86–1.91)
Term completed	568 (46.34)	658 (53.66)	
Birth weight (kilograms)			
<2.5	98 (47.83)	107 (52.17)	1.10 (0.82–1.48)
≥2.5	353 (45.54)	422 (54.46)	

IgG: Immunoglobulin G. OR: odds ratio. CI: confidence interval.

Table 5. Multivariate analysis of characteristics associated with not having protective level of diphtheria antibody (IgG) in participant vaccinated children aged 1–14 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

Characteristics	Crude OR	Adjusted OR	95% CI
Age 1–4 years			Reference group
Age 5–9 years	3.51	4.17	2.38–7.14
Age 10–14 years	5.83	7.69	4.00–14.29
Drinking water from arsenic-free tube-well	11.80	17.42	2.17–139.90

Multivariate model included all variables presented in the table. IgG: Immunoglobulin G. OR: odds ratio. CI: confidence interval.

Protection against diphtheria was not associated with known maternal diabetes mellitus during pregnancy, known maternal hypertension during pregnancy, gestational period, child's birthplace, mode of delivery and interviewee-reported birthweight of participant children. Among children aged 1–4 years, not completing of three doses of PCV was found to be associated with being unprotected (OR 2.06, 95% CI 1.31–2.40). However, in the same age group, when the effect of age was adjusted, this was not found to be associated with being unprotected (OR 1.50, 95% CI 0.90–2.50) (Table 6).

Using multivariate analysis, age group was significantly associated with immunity status. Children of age group 5–9 years were 4 times more likely to be unprotected than children of age group 1–4 years (OR 4.17, 95% CI 2.38–7.14). Also, children of age group 10–14 years were seven times more likely to be unprotected than children of 1–4 years (OR 7.69, 95% CI 4.00–14.29) (Table 5).

Table 6. Association between incomplete PCV vaccination and being unprotected among children aged 1–4 years in Belabo Subdistrict, Narshingdi District, Bangladesh, 2019

Completion of PCV	Do not have protected level of diphtheria IgG n (%)	Have protected level of diphtheria IgG n (%)	Crude OR (95% CI)	Adjusted OR* (95% CI)
Incomplete PCV	55 (31.43)	120 (68.57)	2.06 (1.31–2.40)	1.5 (0.9–2.5)
Completed PCV	45 (18.22)	202 (82.78)		

Adjusted for age. PCV: pneumococcal conjugate vaccine. IgG: Immunoglobulin G. OR: odds ratio.

Discussion

In this study, we found that immunity decreased with increasing age in children who completed primary vaccination against diphtheria. Proportion of protection was highest in age group 1–4 years, and lowest in age group 10–14 years. The GMT of diphtheria antibody also varied significantly in three age groups, being highest in age group 1–4 years and lowest in age group 10–14 years. Being in an older age group was significantly associated with being unprotected.

Studies show that although antibody titer gradually decreased with increasing age, when there was a booster dose, titer increased.[19] Protective levels of antibody were found in 70% of Indian children of 4–6 years of age who had completed a total of four doses of diphtheria vaccine, including one booster by age 2 years. After a second booster was administered at age 4–6 years, 98% of these children had protective antibody levels after 28 days.[27] In our study, 60–70% of children aged 4–6 years were protected with just the 3-dose primary schedule, similar to Indian children before the second booster.

A prospective study conducted in Bangladesh between 2008–2017 examined association between drinking water arsenic concentrations and serum diphtheria antitoxin concentrations (IgG antibodies). Among vaccinated children, water arsenic concentrations measured during toddlerhood and early childhood were not associated with antibody levels.[28] In contrast, another study in Bangladesh, found significantly higher ($p < 0.001$) diphtheria specific IgG in serum of vaccinated children from a high arsenic area compared to a low arsenic area.[29] According to the authors, arsenic metabolites may upregulate factors that are responsible for isotype switching and immunoglobulin production boosting both total IgG and protein specific isotype antibody responses. In our study, drinking water from tube-wells with a high arsenic content was also found to be positively associated with protection. However, this association was not strongly established in multivariate analysis, as the range of 95% confidence interval was very wide. This result needs careful interpretation.

Our study did not show any association between children's immunity status and gender, maternal characteristics during pregnancy, or receiving three doses of PCV. PCV contains carrier protein cross-reacting material 197 (CRM-197) for conjugation. CRM-197 is an ideal carrier protein that is globally used in conjugated vaccines including vaccines for Haemophilus influenzae, pneumococcus and meningococcus. CRM-197 is structurally identical to diphtheria toxoid and is an enzymatically inactive and non-toxic form of diphtheria toxoid.[30] As a result it has immunogenic properties similar to diphtheria toxoid and is immunologically indistinguishable from diphtheria toxoid.[31] Bangladesh EPI routinely administers PCV (containing CRM-197) along with pentavalent vaccine (containing diphtheria toxoid) simultaneously, since 2015. Whether this simultaneous administration of diphtheria toxoid and structurally similar CRM-197 has any boosting effect or interfering effect on inducing immunity needs further study. Completion of three PCV doses was excluded from multivariate analysis, as this variable was applicable to only a single age group 1–4 years. In age group 1–4 years, completion of three PCV doses was not found significantly associated with protection, when the effect of age was adjusted within the single age group.

Studies including systematic reviews and meta-analyses show post-vaccination diphtheria antibody titer was persistently higher than pre-vaccination levels up to five years, then declined toward pre-vaccination levels. At 5–6 years post-vaccination, antibody titers were not significantly different from pre-vaccination level and dropped to pre-vaccination level.[10,14] Therefore, boosters are needed to ensure long-term protection in older children.

Limitations

We only performed ELISA test for estimating diphtheria antibody titers. We did not perform the "gold standard" in vitro toxin neutralization assay. Though there is a good correlation between ELISA and neutralization assay, ELISA may overestimate some sera below 0.1 IU/mL.[32] Misclassification of history of maternal diabetes mellitus, maternal hypertension, gestational period, and birthweight may have occurred as this was self-reported. Only color-marking of tube-well was used to indicate Arsenic content; current Arsenic content was not measured. Thus, examination for an association between immunity and these factors may not have been adequately assessed. We also did not apply weighting for age-specific population size per site, as age-group stratified population size by site was not available.

Recommendations

Our findings suggest that the Bangladesh national EPI should introduce and routinely administer a booster vaccine for diphtheria to children <5 years old to ensure sufficient level of protection among the high-risk children. This could be implemented by introducing a routine booster at the age of school entry. A campaign for booster vaccination against diphtheria should be conducted for children 10–14 years old to avert any potential outbreak of diphtheria. A similar study should be conducted to assess the persistence of immunity after booster vaccination and to decide if another booster should be introduced for older age groups. Also, vaccination programs with only primary schedule should evaluate the need and optimal timing for a booster.

Conclusion

In Bangladesh, about half of children aged 5–9 years, and two-thirds of children aged 10–14 years were unprotected against diphtheria. GMT of diphtheria antibody also varied in three age groups and was highest in age group 1–4 years and lowest in age group 10–14 years. Being unprotected was significantly associated with age group of the vaccinated children.

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Author Contributions

Farzana Islam Khan: Conceptualization, methodology, investigation, project administration, data curation, formal analysis, visualization, writing—original draft, writing—review & editing. **Mallick Masum Billah:** Methodology, visualization, writing—review & editing. **Alden Keith Henderson:** Validation, visualization, writing—review & editing. **Sharifuddin Hasnat:** Data curation, formal analysis, software, visualization. **Arifa Akram:** Validation. **Ashek Ahammed Shahid Reza:** Methodology. **Mohammad Rashidul Alam:** Conceptualization. **Tahmina Shirin:** Resources. **Meerjady Sabrina Flora:** Conceptualization, funding acquisition, resources, supervision.

Ethical Approval

The study was approved by the Institutional Review Board of IEDCR before commencement of the study (protocol number: IEDCR/IRB/2019/09; date of approval: 20 Jun 2019). Participant identities and results of the serum sample tests remained confidential and were anonymously used solely for analysis purpose.

Informed Consent

We obtained written consent from parents and assent from children >5 years.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

No conflicts of interest by all authors.

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No generative AI or AI assisted technologies were used in writing.

Suggested Citation

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