Seroprevalence and Determinants of Immunity to Diphtheria for Children Living in Two Districts of Contrasting Incidence During an Outbreak in East Java, Indonesia

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Background: In 2012, an ongoing outbreak of diphtheria in Indonesia was focused in the province of East Java. There was a need to assess vaccine coverage and immunity gaps in children.

Methods: We conducted a cross-sectional seroprevalence and vaccine coverage survey of children 1–15 years of age in 2 districts of East Java: one of high incidence (on the island of Madura) and one of low incidence (on the mainland). From each district, we sampled 150 children (10 children per year of age). Sera and throat swabs were taken to determine immunity and carriage status. Immunity was defined as ≥0.1 international unit/mL of antibody to diphtheria toxin.

Results: A total of 297 children were selected to participate in the study. Coverage of three doses of combined vaccine for diphtheria, tetanus and pertussis was significantly lower ($P < 0.001$) in the high incidence district compared with the low [57%, 95% confidence interval (CI): 36–78 vs. 97%, 95% CI: 93–100]. Despite this higher vaccine coverage, seroprevalence of immunity was lower in the low incidence district compared with the high (71%, 95% CI: 63–80 vs. 83%, 95% CI: 76–90). Immunity in the high incidence district was associated with increased age, increased prevalence of toxigenic *Corynebacterium diphtheriae* carriers and with receipt of multiple (and likely more recent) boosters.

Conclusions: Significant variation exists in vaccine coverage and seroprevalence of immunity to diphtheria in East Java. Immunity in high incidence districts is likely because of natural immunity acquired through exposure to toxigenic *C. diphtheriae*. Booster vaccines are essential for achieving protective levels of immunity.

Key Words: diphtheria, *Corynebacterium diphtheriae*, serology, seroprevalence, Indonesia

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Diphtheria is an acute bacterial disease caused by *Corynebacterium diphtheriae*, associated with cyclical periods of high morbidity and mortality.[1] In the era of vaccines, effective vaccination programs have eliminated diphtheria from many countries, although sustained transmission does still occur in populations where immunity gaps exist.[2] Nasopharyngeal carriage acts as a reservoir for *C. diphtheriae* and can lead to skin and upper respiratory tract infections. These infections contribute to the development of natural immunity in endemic areas.[3] In the absence of immunization, younger children without naturally acquired immunity are at highest risk of infection.[3] Absorption of toxin after infection with toxigenic *C. diphtheriae* can lead to disseminated organ damage and death.[4]

Indonesia is the fourth most populated country in the world, with a culturally diverse population of over 240 million living in 33 different provinces, 52% of whom live in rural areas.[5] The childhood vaccination schedule for all provinces and districts of Indonesia recommends 3 doses of combined vaccine for diphtheria, pertussis and tetanus (DTP) at 2, 3 and 4 months of age, with boosters of high-dose diphtheria and tetanus vaccine (DT) on entry to school at 5 years of age, and a further booster of low-dose diphtheria and tetanus (Td) vaccine in school for those 6–7 years of age.[6] Boosters are intended to ensure levels of immunity do not wane below protective levels.[7,8]

In 2012, Indonesia (1192 cases) was second to India (2525 cases) in the number of diphtheria cases reported to the World Health Organization (WHO).[9] At this point, the annual incidence of diphtheria in Indonesia had increased more than 30-fold since 2001. By 2013, the province of East Java (population 37 million) had reported almost 650 cases of diphtheria (East Java Provincial Health Office, unpublished results), a substantial fraction of the total number of diphtheria cases (775) reported to WHO for Indonesia in that year. A focus of the outbreak in East Java was in Madura, an island off the north east coast of the mainland whose population is culturally different to that of the mainland and which has a higher than average level of deprivation compared with the province as a whole.[10]

As with other settings, levels of acceptability to vaccination in Indonesia are associated with a fear of adverse effects, access to health services and cultural norms.[10] A survey conducted in 2002–2003 estimated the coverage of 3 doses of combined vaccine for DTP in rural areas of Indonesia to be 78%.[11] Similarly, WHO estimates of DTP3 coverage for Indonesia in 2012 were 83%, close to the herd immunity threshold of 85%[12] but below the 90% recommended by WHO.[13] In response to the diphtheria epidemic in East Java, a supplementary immunization campaign was implemented in November 2012 for all ≤18 years of age within districts of high incidence.

In March 2013, we conducted a cross-sectional survey in East Java with the primary objective of estimating seroprevalence of immunity to diphtheria in children 1–15 years of age in order to produce the first estimates of the seroprevalence of immunity to diphtheria in the province and to inform the development of future studies.

Two districts were chosen for the survey: Bangkalan on the island of Madura, an area of high disease incidence during the epidemic and where a recent catch-up vaccination campaign (November 2012) had been undertaken, and Kediri on the mainland of East Java where no laboratory confirmed diphtheria cases had been detected in 2012 and where the catch-up vaccination had not been implemented.

MATERIALS AND METHODS

Study Population

The survey took place in the districts of Bangkalan and Kediri in the province of East Java, Indonesia. The population eligible for sampling were those children registered with either a midwife (<5 years) or a school (5–15 years) within either of the 2 districts.

Survey Design

A complex sample design was used consisting of 2 strata (districts) with clustering within strata (10 villages per district). Villages were randomly selected with equal probability from a list of those within each district. Sampling was designed to ensure that 10 children were sampled for each single year of age between 1 and 15 years for each district (total of 150 children per district). Within villages, the number of children sampled for each year of age was proportional to the population size of the village. These clusters had a median size of 13 children (range: 13–41) for Bangkalan and a median size of 15 children (range: 5–25) for Kediri.

Sampling Frames

Participants were randomly selected in advance of fieldwork using sampling frames for each village. Sampling frames were made up of population registers obtained from midwifes and from schools. Sampling frames had a median coverage of 87% (range: 36–128%) of the population for each village. Sampling frames that exceeded >100% coverage did so because of the use of school registers where catchment areas included other villages. For each village, the required number of participants for each year of age was randomly selected from sampling frames with equal probability.

Sampling and Data Collection

Fieldwork took place over 4 days in March 2013. The parents of randomly selected children were notified in advance by local public health staff and asked to bring their child to the local health center on the day of sampling, where written consent was obtained. A serum sample and throat swab was taken from each child and the vaccination history of the child (for childhood and diphtheria-containing vaccines) obtained from the parent/guardian. Where recruitment of a randomly selected child was not possible, convenient replacements of the same age were made on the day of sampling using the contact network of the local public health team. For children where evidence of vaccination history was not provided by the parent/guardian (through a child health card), members of the study team later attempted to validate vaccination histories through records held at local health centers. Demographic data (age, sex, height and weight) and the number of household members (≤15 years, >15 years) were recorded on recruitment. Body mass index was calculated as weight (kg)/height (m^2) and standardized by single year of age and sex. Data collected was double entered and validated using EpiData.[14]

Examination of Throat Swabs

Throat swabs were placed in transport media and cultured onto selective media within 3 hours of collection to determine *C. diphtheriae* carriage status. Positive cultures were speciated and biotyped (API-Coryne, BioMèrieux, France), in accordance with World Health Organization guidelines[15] at the East Java public health microbiology laboratory. Cultures identified as *C. diphtheriae* were assessed for toxigenicity with a modified version of the Elek immunoprecipitation test.[16]

Serologic Testing

Sera were tested for reactivity to diphtheria toxin using an in vitro Vero cell challenge neutralization assay[17] at the East Java public health microbiology laboratory. Semiquantitative results were interpreted according to previously established criteria: no immunity [<0.01 international unit (IU)/mL), basic level of protection (0.01–0.09 IU/mL), full immunity (0.1–1.0 IU/mL) or long-term protection (>1 IU/mL).[18] Full or long-term levels of immunity were considered to be protective against infection.

Data Analysis

All data analysis was performed using Stata v11.2.[19] The sampling error calculation was specified as individuals (i), within clusters (villages, j) and strata (districts, k), where $n_{i,j,k}$ varied by village (proportional to the population size of that village, $N_{i,j,k}$), $n_{j,k} = 10$ and $n_k = 2$. Sampling weights were calculated for each participant as the reciprocal of the probability of selection: the product of the probability of selection for a village within each strata ($1/N_{i,k}$) and the probability of selection of a child within each cluster ($n_{i,j,k}/N_{i,j,k}$). Sampling weights were not adjusted for nonresponse and poststratification factors.

All weighted analyses, including multivariable models, incorporated the sampling error calculation. Predictors for immunity were assessed using logistic regression models built using a 3-stage model building strategy: (1) a main effects model produced through backwards selection using all predictor variables, (2) consideration of interaction terms through forward selection in order of decreasing statistical significance and (3) testing the assumption of linearity for continuous variables. A multivariable fractional polynomial model[20] of the final model was used to test the linearity assumption with suggested transformations tested for improved significance of coefficients by replacement of their untransformed version in the final model. The effect of being underweight or overweight was modeled as 2 separate variables, the number of standard deviations above or below the mean body mass index for each combination of sex and year of age. Household members were modeled as 2 groups based on the median number reported. Interactions considered were those between diphtheria-containing vaccines (DTP3, DT, and Td), between diphtheria-containing vaccines and age, between diphtheria-containing vaccines and district and between district and age. A subgroup sensitivity analysis was performed using randomly selected children only.

RESULTS

Sampling and Survey Participants

A total of 297 children were selected to participate in the study, although 7 children were excluded from analysis as either a blood sample was not obtained for serology (five children) or a questionnaire was not completed (2 children), leaving 290 participants for analysis.

For both districts combined, 68% (197/290) of children were randomly recruited, with a higher proportion of children conveniently selected in Bangkalan (41%, 59/143) than in Kediri (23%, 34/147). Because of the use of convenient sampling and the inaccuracy of ages listed in sampling frames, the actual number of children sampled per single year of age varied from 6 to 13 (see Table, Supplemental Digital Content 1, http://links.lww.com/INF/C221). Cluster sizes (villages) for each district were similar (Bangkalan:

mean = 14.3, range 3–38 and Kediri: mean = 14.7, range 5–25). Evidence of vaccine history was available for 62% (179/290) of children, with a slight reduction in this percentage in Bangkalan (56%, 80/143) compared with Kediri (67%, 99/147).

Vaccine Coverage

As little difference was found between weighted and crude estimates (results not shown), weighted estimates are presented throughout. Almost all children (98%) who received DTP1 and DTP2 also received DTP3 (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/C222), and as such vaccine coverage estimates for these 3 vaccines are almost identical. DTP3 coverage was significantly lower in Bangkalan than in Kediri for all 3 age groups (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/C222); in Kediri this coverage was well above 90% for all age groups, whereas in Bangkalan the overall coverage was <60%, but with evidence of significantly increasing DTP3 coverage with more recent birth cohorts [unadjusted odds ratio (OR_{UN}) = 2.00; 95% CI: 1.04–3.86; $P = 0.041$). Similar to DTP3, immunization with diphtheria-containing booster vaccines (DT and Td) was generally higher in Kediri than in Bangkalan, although this was only statistically significant with Td for children 1–5 years of age and with DT for children 11–15 years of age and for all ages (Table 2).

Coverage of nondiphtheria-containing childhood vaccines (BCG, HBV, polio and measles) reflected that of DTP3: close to complete coverage in Kediri (96–98%) with a statistically significant lower coverage in Bangkalan (57–69%; see Table, Supplemental Digital Content 1, http://links.lww.com/INF/C221). As with DTP3 coverage, coverage of the other childhood vaccines in Bangkalan suggests an increasing coverage for more recent birth cohorts, reaching statistical significance ($P < 0.05$) for HBV (OR_{UN} = 2.22; 95% CI: 1.09–4.57), polio vaccine (OR_{UN} = 2.21; 95% CI: 1.05–4.68) and measles vaccine (OR_{UN} = 1.97; 95% CI: 1.05–3.68), but not for BCG (OR_{UN} = 2.08; 95% CI: 0.99–4.40).

Seroprevalence

The percentage of children fully susceptible to diphtheria was higher in Bangkalan than in Kediri (Table 1), albeit not significantly, for all ages ($P = 0.287$) and for each age group (1–5: $P = 0.069$; 6–10: $P = 0.639$; and 11–15: $P = 0.190$). Although the OR for being fully susceptible to diphtheria increased significantly in Bangkalan with younger age groups (OR_{UN} = 3.87; 95% CI: 3.00–4.99), no such trend was evident in Kediri (OR_{UN} = 1.09; 95% CI: 0.39–3.04).

In Kediri, there were similar percentages of children with full or long-term immunity to diphtheria, whereas in Bangkalan, children had largely long-term immunity (Table 1). This is reflected in a significantly higher weighted seroprevalence of long-term immunity in Bangkalan than in Kediri (70%, 95% CI: 58–82 vs. 35%, 95% CI: 25–44; $P<0.001$) and a significantly higher weighted seroprevalence of full immunity in Kediri than in Bangkalan (37%, 95% CI: 25–48 vs. 13%, 95% CI: 5–22; $P = 0.004$; Table 1).

Despite significantly higher levels of DTP3 coverage, Kediri had an overall significantly lower seroprevalence of protection to diphtheria ($P = 0.039$; Table 2), largely because of the lower levels for older children (11–15 years: $P<0.001$). Seroprevalence levels for the 2 groups of younger children were not significantly different (1–5 years: $P = 0.297$; 6–10 years: $P = 0.957$). Analysis by age group showed a trend for increasing seroprevalence of immunity with age in Bangkalan but not in Kediri.

Predictors of Immunity to Diphtheria

Age and the interaction between age and district (ie, the change in OR for each 1 year increase in age for children living in Kediri) were the only statistically significant predictors of protection against diphtheria (Table 3). The significance of these terms remained after restricting the analysis to randomly selected children only (results not shown).

Given the differences in patterns of immunity levels between the 2 districts, a multivariable model was built to explore predictors of reaching long-term immunity rather than full immunity (Table 4). This model indicated a significantly higher OR for reaching long-term immunity if a child had received both the diphtheria-containing booster vaccines and a significantly reduced OR associated with having received DT in Kediri. Although both of these terms were no longer significant when restricting the analysis to randomly selected children only, this may be due, in part, to the reduced power of the subgroup analysis; point estimates of OR for both terms remained considerably above or below unity but with substantial loss of precision because of the reduced sample size (results not shown).

Carriage Status

Five throat swabs were positive for *C. diphtheriae* (4 of which were variant mitis and 1 variant gravis), 2 were toxigenic. All 5 positive throat swabs were from children living in Bangkalan, giving a weighed prevalence for *C. diphtheriae* carriage of 3% (95% CI: 0–7) and 1% (95% CI: 0–4) for carriage of toxigenic strains.

DISCUSSION

The recent outbreak of diphtheria in Indonesia was focused in the province of East Java. Within the province, there was a distinct clustering of cases; infection rates were clearly not uniform across the province. This survey has shown that there are significant differences in both vaccine coverage and the seroprevalence of immunity for children between districts. Moreover, different mechanisms appear to be determining a quantitatively different level of immunity in regions of high and low incidence. Despite high DTP3 coverage in the low incidence district surveyed, our results support the need for booster vaccines to achieve high protective levels of immunity. In the district of high incidence, in the absence of sufficient vaccine coverage, younger children are at risk of infection before the development of natural immunity. Children living in low

TABLE 1. Seroprevalence of Immunity to Diphtheria According to Level of Immunity and Age Group From a Survey of Children 1–15 Years of Age in 2 Districts of East Java, Indonesia, March 2012

	No. of Children by District and Level of Immunity (Weighted Percentage Seroprevalence)											
	Both Districts				Bangkalan				Kediri			
Age Group	Susceptible	Basic	Full	Long Term	Susceptible	Basic	Full	Long Term	Susceptible	Basic	Full	Long Term
1–5 yr	12 (12)	20 (24)	28 (31)	37 (33)	9 (20)	4 (9)	4 (8)	29 (63)	3 (7)	16 (34)	24 (46)	8 (14)
6–10 yr	8 (9)	9 (9)	18 (19)	63 (63)	5 (10)	4 (8)	7 (15)	34 (67)	3 (8)	5 (10)	11 (22)	29 (61)
11–15 yr	3 (4)	11 (14)	29 (33)	52 (49)	0 (0)	1 (2)	7 (17)	39 (81)	3 (6)	10 (22)	22 (44)	12 (28)
All ages	23 (8)	40 (16)	75 (27)	152 (49)	14 (10)	9 (7)	18 (13)	102 (70)	9 (7)	31 (22)	57 (37)	50 (35)

TABLE 2. Weighted Estimates of Seroprevalence of Immunity to Diphtheria According to Minimum Level of Immunity and Age Group From a Survey of Children 1–15 Years of Age in 2 Districts of East Java, Indonesia, March 2012

	Weighted Percentage Seroprevalence by District and Minimum Level of Immunity (95% CI)											
	Both Districts				Bangkalan				Kediri			
Age Group	Susceptible	≥Basic	≥Full	Long Term	Susceptible	≥Basic	≥Full	Long Term	Susceptible	≥Basic	≥Full	Long Term
1–5 yr	12 (6–18)	88 (82–94)	64 (51–76)	33 (21–45)	20 (12–28)	80 (72–88)	71 (74–90)	63 (47–79)	7 (0–15)	93 (85–100)	59 (41–78)	14 (5–22)
6–10 yr	9 (3–14)	91 (86–97)	82 (75–89)	63 (51–76)	10 (5–15)	90 (85–95)	82 (74–90)	67 (48–86)	8 (0–16)	92 (84–100)	82 (72–93)	60 (44–77)
11–15 yr	4 (0–8)	96 (92–100)	82 (75–89)	49 (35–63)	0	100	98 (93–100)	81 (68–94)	6 (0–13)	94 (87–100)	72 (61–83)	28 (12–45)
All ages	8 (5–11)	92 (89–94)	76 (70–82)	49 (39–58)	10 (6–14)	90 (86–94)	83 (76–90)	70 (58–82)	7 (3–11)	93 (89–97)	71 (63–80)	35 (25–44)

incidence areas with subprotective levels are potentially at risk of infection should exposure to the organism occur through incursion from areas of higher prevalence of carriage.

In the district with a high incidence of diphtheria, we found higher levels of antibody but in the absence of full immunization. It is likely that protection is arising through natural immunity because of repeat exposure to *C. diphtheriae*, probably through contact with skin infections, as occurred in the prevaccination era.[3] Our carriage data support the surveillance data: children living in the area of relative high incidence are more likely to be exposed to toxigenic *C. diphtheriae*. This is further supported by an increasing seroprevalence of immunity with age (both crudely and after adjustment) in the district with relatively high incidence.

In the absence of full immunization, a quantitatively greater level of immunity in the high incidence district is predicted to have been determined by the receipt of both diphtheria-containing boosters, and modified by a reduced likelihood if one of those boosters (DT) was received in the low incidence district. At the time of the survey the high incidence district had very recently been included in a supplementary immunization activity as a public health response to the outbreak—children had received a diphtheria booster just 5 months before sampling. In addition, the use of a second booster for older children in the low incidence district had recently been suspended, partially explaining the lower seroprevalence of immunity for the older age group in this district. Certainly, more recent diphtheria vaccination is a strong predictor of immunity in adults[7] and children,[21] where booster vaccines coincide with increasing antibody titre.[22] The combination of immunization (complete or partial) and natural challenge through exposure may also contribute to a quantitatively different level of response in areas where the reservoir of bacteria is relatively larger. We were unable to include in our analysis the time since immunization with specific vaccines, the inclusion of which would have helped to explore this further.

TABLE 3. Associations With Immunity to Diphtheria From a Survey of Children 1–15 Years of Age in 2 Districts of East Java, Indonesia, March 2012

Variable	Category	OR_{UN} (95% CI)	*P* value	OR_{AD} (95% CI)	*P* value
District	Kediri	0.50 (0.25–0.97)*	0.041*	1.46 (0.46–4.62)	0.495
Age	Increase of 1 yr	1.11 (1.06–1.17)*	<0.001*	1.33 (1.17–1.50)*	<0.0001*
Sex	Male	0.58 (0.28–1.21)	0.137	0.51 (0.24–1.07)	0.073
DTP3†	Received	0.95 (0.45–1.99)	0.875	2.00 (0.49–8.21)	0.316
DT	Received	1.56 (0.85–2.85)	0.143	1.77 (0.73–4.29)	0.189
Td	Received	1.41 (0.64–3.09)	0.374	1.11 (0.49–2.53)	0.791
BMI	Each SD below mean	1.68 (0.88–3.21)	0.109	–	–
	Each above mean	0.91 (0.56–1.48)	0.689	–	–
Household ≤15 yr	≥3	2.01 (0.93–4.32)	0.073	–	–
Household >15 yr	≥4	1.46 (0.64–3.31)	0.348	–	–
District × age	Kediri × 1 yr increase	–	–	0.81 (0.71–0.92)*	0.003*

*Significant at 5% level.

†Because of colinearity of prediction for DTP1, DTP2 and DTP3 coverage, only DTP3 was considered in the model.

× indicates interaction; BMI, body mass index; OR_{AD}, adjusted odds ratio; and SD, standard deviation.

Despite a larger proportion of children living in Bangkalan with protective levels of immunity to diphtheria, the number of cases remains much higher. Given vaccine coverage, and the lack of an association between immunization status and protective levels of antibody, the contrasting epidemiology between districts must equate to a higher effective contact rate within the high incidence district, reflecting the rate at which the susceptible population are exposed to the organism (carriers, cutaneous and infections). Given that natural immunity accumulates with age, the high incidence is likely linked to the subprotective immune levels in younger children (before the development of natural immunity) and contact with a reservoir of toxigenic *C. diphtheriae*. Although there were a small number of confirmed diphtheria cases in adults from Bangkalan in 2012, 79% (22/28) were in children ≤15 years (East Java Provincial Health Office, unpublished data), reflecting the immunity gaps in under-immunized children suggested by the vaccine coverage and seroprevalence data.

Given the high vaccine coverage, seroprevalence in Kediri would be expected to show a corresponding high proportion of children with protective antibody levels. However, our seroprevalence data suggest that the proportion of children protected against diphtheria in Kediri could be below that required for herd immunity. The low titres of antibody despite excellent coverage of DTP3 and diphtheria-containing boosters may require further investigation; the immunogenicity of the vaccine may have been compromised before administering and an audit of the stability of the cold chain and compliance with good vaccine delivery practices is necessary

TABLE 4. Associations With Reaching Higher Levels of Antibody* to Diphtheria From a Survey of Children 1–15 Years of Age in 2 Districts of East Java, Indonesia, March 2012

Variable	Category	OR_{UN} (95% CI)	*P* value	OR_{AD} (95% CI)	*P* value
District	Kediri	0.18 (0.07–0.45)†	0.001†	1.79 (0.12–25.80)	0.651
Age	Increase of 1 yr	1.02 (0.96–1.09)	0.503	1.03 (0.96–1.12)	0.369
Sex	Male	0.73 (0.43–1.22)	0.215	0.95 (0.49–1.82)	0.859
DTP3‡	Received	0.46 (0.20–1.07)	0.068	1.89 (0.47–7.55)	0.345
DT	Received	0.64 (0.30–1.36)	0.228	0.96 (0.32–2.82)	0.931
Td	Received	0.92 (0.31–2.75)	0.871	0.27 (0.70–1.05)	0.058
BMI	Each SD below mean	0.85 (0.48–1.52)	0.568	–	–
	Each SD above mean	1.06 (0.53–2.12)	0.853	–	–
Household ≤15 yr	≥3	1.12 (0.57–2.21)	0.728	–	–
Household >15 yr	≥4	1.06 (0.47–2.37)	0.892	–	–
Td × DT	Received both	–	–	6.22 (1.70–22.71)†	0.008†
DT × district	Received × Kediri	–	–	0.05 (0.01–0.72)†	0.030†

*Long-term immunity vs. full immunity.
‡Because of colinearity of prediction for DTP1, DTP2 and DTP3 coverage, only DTP3 was considered in the model.
†Significant at 5% level.
×indicates interaction; BMI, body mass index; OR_{AD}, adjusted odds ratio; and SD, standard deviation.

to explore this further. Certainly, geometric mean antibody titres for children with a validated history of receiving DTP3 plus both boosters are lower in Kediri (0.341, 95% CI: 0.235–0.495; $n = 91$) than in Bangkalan (1.515, 95% CI: 1.000–2.295, $n = 46$), although this could at least partially be explained by more recent receipt of boosters and immune priming through exposure to toxigenic *C. diphtheriae*.

The drivers for the current epidemiological situation in Kediri (no evidence of carriage and no endemic cases) may be extant: basic immunity may be sufficient to prevent transmission in the absence of high contact rates. Conversely, titers of antidiphtheria toxin antibodies may, indeed, be insufficient to prevent infections for a sufficient proportion of children; in which case, should contact rates with the organism increase through incursion of the organism, a resurgence of diphtheria could occur, similar to that seen on a larger scale in the former Soviet Union in the 1990s.[23] Further seroprevalence surveys across the province are needed before the full extent of this problem can be determined.

Despite the small size of this survey, both in terms of the sample size and the selection of just 2 districts, the findings of this study are felt to be transferable beyond the immediate study population, providing a reflection of how seroepidemiology is likely to vary between high and low areas of diphtheria incidence in East Java. The continued use of multiple booster vaccines and the maintenance of high DTP3 coverage are essential to ensure that young children are protected against diphtheria. Although DTP3 coverage (and that of other childhood vaccines) does encouragingly appear to be increasing with more recent birth cohorts in the high incidence district, effort should be made to ensure that the childhood vaccination program is fully promoted and that future birth cohorts achieve vaccine-acquired immunity before potential exposure to toxigenic *C. diphtheriae*.

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