

## Tuberculin survey in Bangladesh, 2007–2009: prevalence of tuberculous infection and implications for TB control

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### SUMMARY

**OBJECTIVES:** To assess the prevalence of tuberculous infection and the annual risk of tuberculous infection (ARTI) for 2007–2009 in Bangladesh, approximately 45 years after the first tuberculin survey in 1964–1966. **METHODS:** A tuberculin survey was conducted along with the National Tuberculosis Disease Prevalence Survey in 2007–2009. This was a multistaged community-based, cross-sectional survey, including 17 718 children aged 5–14 years. The prevalence of tuberculous infection was estimated using the mixture method and a cut-off point of  $\geq 8$  mm.

**RESULTS:** The prevalence of infection was 10.0% (interquartile range [IQR] 8.6–12.2) in children aged 5–9 years and 17.9% (IQR 15.4–20.2) in those aged 10–14 years

using the mixture analysis. Prevalence was 12.4% (95% confidence interval [CI] 11.7–13.1) in children aged 5–9 years and 22.6% (95% CI 21.6–23.4) in those aged 10–14 years using a cut-off point of  $\geq 8$  mm. The estimated ARTI was respectively 1.5% and 1.7% in 5–9 and 10–14 year olds using the mixture method and respectively 1.9% and 2.1% using the cut-off method.

**CONCLUSIONS:** The moderate reduction in the prevalence of infection and slow decline of the ARTI after two decades of DOTS implementation indicates considerable ongoing transmission.

**KEY WORDS:** community-based; tuberculous infection; annual risk; tuberculin skin test; Bangladesh

THE PREVALENCE of *Mycobacterium tuberculosis* infection is an indicator for tuberculosis (TB) transmission in a community and may be used to monitor the burden of disease.<sup>1</sup> Knowledge of the TB burden and its trends is imprecise in Bangladesh.<sup>2,3</sup> The prevalence of smear-positive cases was reported in two TB prevalence surveys at 318 per 100 000 population in 1964–1966 and 910/100 000 in 1987–1988.<sup>4,5</sup> The recently conducted 2007–2009 National Tuberculosis Prevalence Survey (NTPS) reported a markedly decreased prevalence of 79.4 smear-positive cases/100 000 in those aged  $\geq 15$  years.<sup>6</sup>

The causes of the decline in TB prevalence may be the result of successful TB control, socio-economic development<sup>7</sup> or concurrent development in other non-health sectors.<sup>8</sup> Cure rates in the National Tuberculosis Programme (NTP) increased from 75% in 1993–1995 to  $>90\%$  in 2011,<sup>9,10</sup> while the gross domestic

product per capita increased from US\$594 in 1994 to US\$1584 in 2010.<sup>11</sup>

As the NTP wished to examine whether the decline in TB prevalence was accompanied by a similar decline in the risk of *M. tuberculosis* infection, a national tuberculin survey was embedded in the latest NTPS. The only national tuberculin survey conducted in Bangladesh was in 1964–1966.<sup>4</sup> A reassessment of the survey data revealed some methodological problems, making the results for the 5–9 years age group questionable.<sup>12</sup> These included the probability of reduced sensitivity in children aged 5–9 years due to lower age and the low doses of antigen used (1 tuberculin unit [TU]), not reaching some of the remote areas, and the inability to read tested persons in some instances.<sup>4,12</sup>

In this article, we report the results of the 2007–2009 tuberculin survey. The results will provide an estimate of the annual risk of tuberculous infection

(ARTI) following countrywide implementation of the DOTS strategy in the 1990s.

## MATERIALS AND METHODS

### Setting

The survey was conducted by the Bangladesh NTP in collaboration with the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), with the support of the World Health Organization (WHO) and the KNCV Tuberculosis Foundation. As in the 1964–1966 survey, we included children aged 5–14 years. School attendance for children aged  $\geq 12$  years (secondary school) is considerably lower than for younger children in Bangladesh, with greater drop-out rates between primary and secondary schools among girls than among boys.<sup>13</sup> For this reason, we conducted a community-based survey to avoid the bias likely in a school-based survey.

### Design

The survey was carried out in children from 20 urban and 20 rural clusters. Stratified cluster sampling (by urban/rural) was performed, with sub-districts being selected with probability proportional to population size. A *mauza* or *muhallah* (smaller geographical units) from each sub-district was then selected by simple random sampling. In each cluster, inclusion of households started with random selection of an initial household after which other households were added consecutively following a pre-defined direction until the required sample size was achieved.<sup>14</sup> The selected households were visited several times to include as many eligible children as possible.

The sample size was calculated on the basis of an assumed decline in infection prevalence in children aged 5–9 years from 4.1% to 2.5% between 1964 and the current survey, and from 13.6% to 10% in those aged 10–14 years. With an anticipated participation rate of 90%, a design effect of 3, a significance level of  $P < 0.05$  and a power of 90%, the survey required a total study population of around 15 000. Given the operational context of the NTPS of which this tuberculin survey was a part, we anticipated that 500 children per cluster would be available from the same households, giving a total sample size of 20 000.

### Skin testing survey

All children aged 5–14 years who were resident in the selected households were eligible for inclusion. Only children whose legal guardians provided written informed consent were included. Children who were severely ill, mentally retarded, those with a history of convulsion or who had severe skin rashes were excluded.

Inclusion of the children started at the first household of the cluster and ended with the inclusion of all the children of the household in which the 500th

child lived. After enrolment, bacille Calmette-Guérin (BCG) status was checked and children were tested using intradermal administration of 0.1 ml of tuberculin containing 2 TU of purified protein derivative RT23 with Tween 80 (Statens Serum Institute, Copenhagen, Denmark) in the ventral aspect of the left forearm, regardless of BCG status.<sup>1,15</sup> Skin reactions were read after 72 h using a transparent ruler. Children who had been administered tuberculin but were not present for reading were traced and examined on the following day.

Parents were informed of the results and advised if any measures were necessary. All children with indurations of  $\geq 15$  mm were referred to the nearest diagnostic centre for clinical evaluation and management per NTP protocol.<sup>16</sup>

### Quality control of tuberculin testing

The design of the tuberculin survey followed the standard guidelines of the WHO/International Union Against Tuberculosis and Lung Disease for conducting tuberculin skin test (TST) surveys in high-prevalence countries.<sup>1,15</sup> Tuberculin testers received training by two international tuberculin reference nurses. Several refresher and on-the-job training courses were carried out to maintain adequate standards. A random sample of the children was read a second time by another tuberculin reader blinded to the initial results. If serious discordances were identified by the double reading, additional training in the reading of tuberculin results was provided.

The survey protocol was approved by the research review and ethical review committees of the icddr,b. The protocol, along with the NTPS, was approved by the Ministry of Health and Family Welfare of the Government of the Peoples' Republic of Bangladesh.

### Statistical analysis

This protocol followed recent recommendations to include all children,<sup>15,17</sup> irrespective of BCG status, for analysis. An initial frequency distribution was drawn to identify digit preference or any other distribution pattern. Data were smoothed by applying a moving average of five.

We used a mixture analysis for the study. We also explored the prevalence of tuberculous infection with a fixed cut-off point indicated by the graphic distribution obtained from the initial mixture analysis. The mixture method disentangled the overall distribution of the TST reaction sizes used in the cut-off method into separate distributions due to *M. tuberculosis*, non-specific causes (other mycobacterium, BCG) and non-infected individuals.<sup>18</sup> This allowed for a more precise estimate of the prevalence of infection by *M. tuberculosis*. The method is not new and had been successfully used in the analysis of previous TST surveys. It is a Bayesian Markov Chain Monte Carlo simulation approach which calculates a posterior

distribution of the prevalence of tuberculous infection with its associated interquartile ranges (IQRs). The cut-off method provided a single prevalence estimate with associated confidence intervals (CIs).

We assessed the fit of three parametric models (normal, log-normal and Weibull) to describe *M. tuberculosis* infection, and two models (log-normal and Weibull) to describe distribution among non-infected children. A model fit for each of the six possible combination models was assessed by examining maximum log-likelihood values, percentage of predictive failures and analysis of the graphical distributions of reactions. From the graphs produced by the distribution of indurations, a fixed cut-off point was chosen to estimate the prevalence of tuberculous infection using the conventional cut-off method. ARTI was estimated according to the following formula:  $ARTI = 1 - (1 - \text{prevalence})^{(1/\text{average age})}$ .<sup>19</sup>

In secondary analyses, we explored the relationship between tuberculin indurations and the socioeconomic position (SEP) of the household, and between tuberculin indurations and exposure to an adult with TB in the same household. SEP was assessed by validated asset items for each of the households included in the latest NTPS. A principal component analysis generated household scores and these were categorised in tertiles. Children belonging to a particular household were attributed the assets of that household. Details of the method used is described elsewhere.<sup>20,21</sup>

Data analysis was performed using the statistical package Stata, v. 10.0 (Stata Corp, College Station, TX, USA), and software 'R', version 2.4 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

Of 22 309 children included in the survey, 20 285 were eligible for the study. Among the children eligible, 2103 (10.4%) were absent and 364 (1.8%) refused or were excluded. A total of 17 718 (87.3%) children were included as study participants. There were no marked differences between those analysed and those who were not with respect to sex, age group, setting, SEP or exposure to TB in the household (data not shown). All study participants were tested with tuberculin, and almost all of them (99.3%) were read for reaction sizes. BCG scars were present in >85% of children, irrespective of age (Table 1).

The percentage of non-reactors (indurations = 0) was 76.6% in those aged 5–9 years, and 63.1% in those aged 10–14 years. More than 50% of the reactors in the 5–9 years age group and 46% in the 10–14 years age group had indurations of between 1 and 8 mm (Figure 1).

There were few predictive failures in any of the combinations in the mixture analysis; combinations did not differ much in their maximum log-likelihood

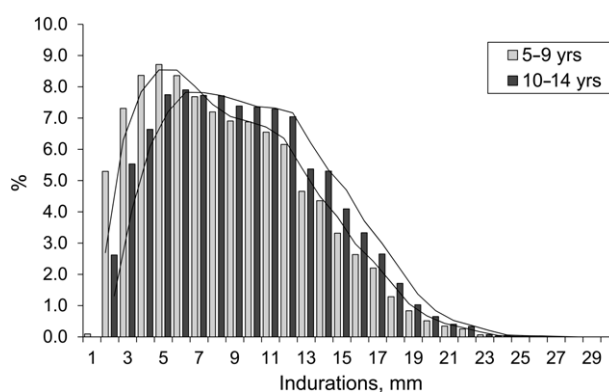
**Table 1** Participation and test status of children, 2007–2009 tuberculin survey, Bangladesh

Study population	Children aged 5–9 years <i>n</i>	Children aged 10–14 years <i>n</i>
Children in census	11 782	10 527
Eligible children	10 651	9 634
Absent	1 025	1 078
Refused	70	64
Excluded	128	102
Children tested	9 428	8 290
Children read, among those tested, <i>n</i> (%)	9 357 (99.2)	8 228 (99.3)

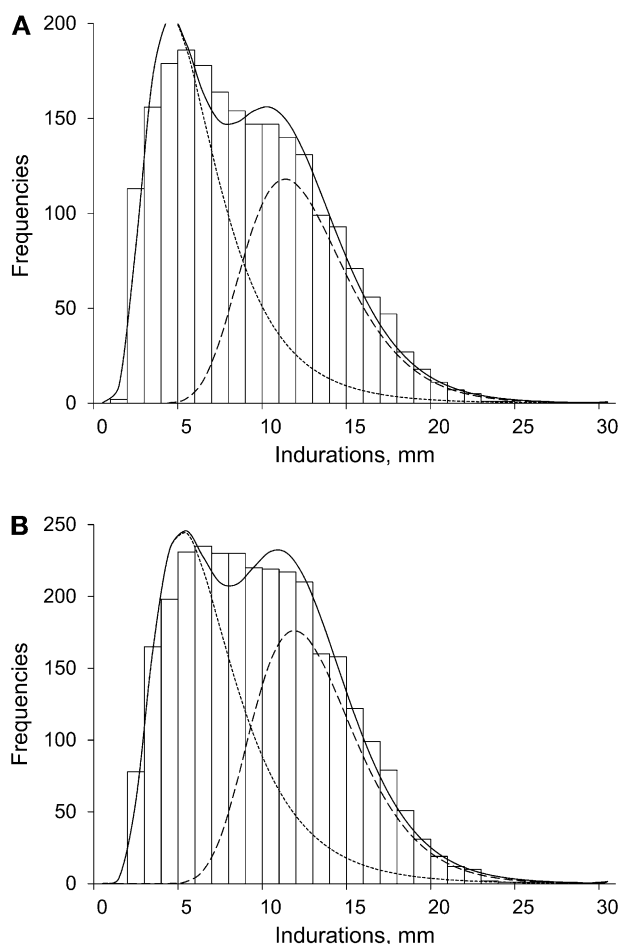
estimates. Among all combinations, the ln/ln graph showed a modest bimodal distribution with an anti-mode at 8 mm (Figure 2). This cut-off point was used for analysis based on the conventional fixed cut-off point approach.

Applying the mixture method, the prevalence of tuberculous infection was respectively 10.0% (IQR 8.6–12.2) and 17.9% (IQR 15.4–20.2) in those aged 5–9 years and 10–14 years. Using a cut-off point of  $\geq 8$  mm, the prevalence of tuberculous infection was respectively 12.4% (95%CI 11.7–13.1) and 22.6% (95%CI 21.6–23.4) in those aged 5–9 years and 10–14 years. The estimated ARTI for 5–9 years was 1.5%, while for the 10–14 years age group it was 1.7% using the mixture method. This was respectively 1.9% and 2.1% for both age groups using the fixed cut-off point at  $\geq 8$  mm (Table 2).

The 2007–2009 survey identified only 33 new smear-positive pulmonary TB cases.<sup>6</sup> Nineteen children were from households where a TB case was diagnosed during the survey and were considered exposed to a TB case. Among the children with exposure to a TB case, 9 (47.4%) had indurations of  $\geq 8$  mm compared to 2934 (16.7%) children without exposure



**Figure 1** Frequency distribution of reaction sizes in children aged 5–9 and 10–14 years, Bangladesh, 2007–2009 tuberculin survey. Proportion of children with indurations, %. Distribution after smoothing on five averages. All reactions of '0' mm have been omitted for clarity. All reactions of '0' were 76.6% in children aged 5–9 years and 63.1% in those aged 10–14 years.



**Figure 2** Histograms of frequencies of induration reactions to tuberculin in **A**) children aged 5–9 years and **B**) those aged 10–14 years in the 2007–2009 survey using the mixture analysis. In both histograms, the underlying distribution from non-specific cross-reactions is indicated by the tall dotted line (.....) between 0 and 10, the underlying distribution from *M. tuberculosis* infection by the dashed line (---) peaked after 5, and the mixture distribution by the solid line (—).

to a TB case (odds ratio [OR] 4.5, 95%CI 1.7–11.9; Table 3).

Among the children belonging to the lower SEP tertiles, 15.8% had indurations of  $\geq 8$  mm compared to 17.2% in both the middle and upper SEP

**Table 2** Prevalence of infection and ARTI, 2007–2009 tuberculin survey, Bangladesh

	Children aged 5–9 years		Children aged 10–14 years	
	Prevalence of infection	ARTI %	Prevalence of infection	ARTI %
Mixture method, % (IQR)	10.0 (8.6–12.2)	1.5	17.9 (15.4–20.2)	1.7
Cut-off at $\geq 8$ mm, % (95%CI)	12.4 (11.7–13.1)	1.9	22.6 (21.6–23.4)	2.1

ARTI = annual risk of tuberculous infection; IQR = interquartile range; CI = confidence interval.

**Table 3** Potential risk factors for infection: within-household exposure status and indurations, asset status and indurations

	Indurations		All n (%)
	<8 mm n (%)	$\geq 8$ mm n (%)	
Exposed*	10 (52.6)	9 (47.4) <sup>†</sup>	19 (0.1)
Not exposed	14 596 (83.3)	2 934 (16.7)	17 530 (99.9)
All	14 606 (83.2)	2 943 (16.8)	17 549 (100)
Asset status <sup>‡</sup>			
Lower	4 552 (84.2)	851 (15.8)	5 403 (30.8)
Middle	4 842 (82.8)	1 006 (17.2)	5 848 (33.3)
Upper	5 212 (82.8)	1 086 (17.2)	6 298 (35.9)
All	14 606 (83.2)	2 943 (16.8)	17 549 (100)

\* Children from a household where a smear-positive TB case was detected during the 2007–2009 survey.

<sup>†</sup>  $P < 0.000$ .

<sup>‡</sup>  $P = 0.055$ .

tertiles. The prevalence of tuberculous infection was not significantly associated with any of the SEP tertiles (Table 3).

## DISCUSSION

This tuberculin survey was conducted nearly 45 years after the first survey carried out in 1964–1966. There is therefore only limited information available to put this estimate into context. The 1964–1966 survey provided valid data only for the 10–14 years age group using the cut-off method with an 8-mm threshold. The prevalence of tuberculous infection was 34.4%.<sup>4,12</sup> The new prevalence of tuberculous infection in the same age group using the same methodology was 22.6%, showing an average decline of nearly 1% between the two surveys. This indicates that despite the efforts of the NTP, there has been little improvement in curbing the risk of tuberculous infection in the community since 1964.

This is in sharp contrast with the reported decline in the prevalence of smear-positive TB in the community, from 318/100 000 in 1964–1966 to only 79.4/100 000 in 2007–2009,<sup>6</sup> an annual decline of approximately 3.2%. A lower prevalence of infectious TB patients reduces TB transmission within the community, which should be reflected in a decline in the prevalence of tuberculous infection. Ascertaining the reasons why this has not been observed in Bangladesh is challenging.

We have shown earlier that the NTP does not have adequate coverage among the poorer population groups of Bangladesh.<sup>21</sup> Higher TB prevalence among the poor, combined with delays in care seeking and treatment,<sup>22</sup> may create a favourable environment for transmission. Our results, however, do not show any association between the prevalence of tuberculous infection and SEP, although they indicate a clear relationship between induration size and TB exposure. This would suggest that TB transmission in our study

was not influenced by SEP. However, our methods of data capture (for either SEP or prevalence of infection) might not be strong enough to establish such an association.

SEP distribution in the survey population was based on an asset score, which provided a relative classification of SEP rather than an absolute measure. Although the distribution of assets-based SEP was homogeneous in relation to the underlying absolute SEP, the method was insufficiently sensitive to detect a relationship between the SEP and the prevalence of tuberculous infection. Data on the relationship between tuberculous infection and SEP were not unanimous, despite the obvious link given the higher prevalence of TB in this setting. In South Africa, Mahomed et al. reported that low income and low education levels (factors strongly associated with SEP) increased the risk of latent tuberculous infection.<sup>23</sup> In contrast, Boccia et al. showed that in Zambia the risk of tuberculous infection was associated with a higher SEP.<sup>24</sup>

The ability of a tuberculin survey to measure the prevalence of tuberculous infection might be questionable in itself, given the well-known limitations in design and analysis.<sup>19</sup> We used a strict quality control strategy comprising training, monitoring and re-training to obtain high-quality data. Analyses were performed after smoothing of the data, and the mixture method was used instead of the crude cut-off approach. This strategy minimised the methodological problems seen in tuberculin surveys as much as possible. Problems with TST measurements and analysis can probably be overcome by using interferon-gamma release assays (IGRAs) as an alternative to TST, not only for their high specificity but also because the results are not affected by BCG vaccination status,<sup>25,26</sup> although IGRAs are costly and technically challenging in low-resource settings.<sup>17</sup>

The limited decline in ARTI or prevalence of tuberculous infection in Bangladesh in this study should be interpreted with caution, as not only were the two tuberculin surveys conducted 45 years apart, they differed methodologically with respect to sampling and the use of reagents for testing (1 TU instead of 2 TU);<sup>4</sup> also, considerable societal and programmatic changes have occurred in the intervening period. Despite this, the current study provides the first estimate of the prevalence of tuberculous infection after the nationwide implementation of the DOTS strategy. As such, it can serve as an important starting point for further studies. Despite the limitations of tuberculin surveys, multiple surveys with identical methodology in an appropriate time frame have been shown to provide information on trends.<sup>18,27,28</sup> Assessing the association between SEP and the risk of tuberculous infection should be conducted in specifically designed studies to ensure maximum power and attribution. Such studies will be of benefit to the Bangladesh NTP in designing interventions for effective TB control.

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## R É S U M É

**OBJECTIFS :** Evaluer la prévalence de l'infection tuberculeuse ainsi que le risque annuel d'infection tuberculeuse (ARTI) en Bangladesh pour la période 2007–2009, environ 45 ans après la première enquête tuberculique en 1964–1966.

**MÉTHODES :** En 2007–2009, on a mené une enquête tuberculique parallèlement avec une enquête de prévalence nationale sur la maladie tuberculeuse. Il s'agissait d'une enquête transversale à étapes multiples et basée sur la collectivité, comportant 17718 enfants âgés de 5 à 14 ans. On a estimé la prévalence de l'infection tuberculeuse au moyen d'une méthode de mixage et une limite de positivité de  $\geq 8$  mm.

**RÉSULTATS :** Selon l'analyse de mixage, la prévalence de

l'infection a été de 10,0% (IQR 8,6–12,2) chez les enfants âgés de 5 à 9 ans et 17,9% (IQR 15,4–20,2) chez ceux âgés de 10 à 14 ans. L'utilisation d'une limite de positivité  $\geq 8$  mm a conduit à des taux de 12,4% (IC95% 11,7–13,1) chez ceux âgés de 5 à 9 ans et 22,6% (IC95% 21,6–23,4) chez ceux âgés de 10 à 14 ans. L'ARTI estimé est de 1,5% pour les enfants âgés de 5 à 9 ans et de 1,7% pour ceux âgés de 10 à 14 ans par la méthode de mixage, et de 1,9% et 2,1% respectivement par la méthode de limite de positivité.

**CONCLUSIONS :** La réduction modérée de la prévalence de l'infection ainsi que le lent déclin de l'ARTI après deux décennies de mise en œuvre du DOTS indiquent la persistance d'une transmission considérable de la maladie.

## R E S U M E N

**OBJETIVOS:** Evaluar la prevalencia y el riesgo anual de infección tuberculosa (ARTI) entre el 2007 y el 2009, cerca de 45 años después de la primera encuesta tuberculínica realizada entre 1964 y 1966 en Bangladesh.

**MÉTODOS:** Se llevó a cabo una encuesta tuberculínica al mismo tiempo que una encuesta nacional de prevalencia de la enfermedad tuberculosa entre el 2007 y el 2009. El estudio consistió en una encuesta poblacional transversal con un muestreo en varias etapas que incluyó a 17718 niños entre los 5 y los 14 años de edad. Se calculó la prevalencia de infección tuberculosa mediante un método de mezcla y con un umbral discriminatorio de  $\geq 8$  mm de induración.

**RESULTADOS:** La prevalencia de infección fue 10,0% (IQR 8,6–12,2) en el grupo de 5 a 9 años de edad y

17,9% en el grupo de 10 a 14 años (IQR 15,4–20,2) según el análisis de mezcla. Cuando se aplicó el umbral de  $\geq 8$  mm de induración, la prevalencia fue 12,4% en el grupo de 5 a 9 años (IC95% 11,7–13,1) y 22,6% (IC95% 21,6–23,4) en el grupo de 10 a 14 años. El ARTI según el método de mezcla se calculó en 1,5% en el grupo de 5 a 9 años y en 1,7% en el grupo de 10 a 14 años y por el método del umbral discriminatorio fue 1,9% y 2,1%, respectivamente.

**CONCLUSIÓN:** La discreta disminución de prevalencia de la infección tuberculosa y la lenta disminución del ARTI después de dos decenios de ejecución de la estrategia DOTS indica una considerable transmisión activa de la enfermedad.