Annual risk of tuberculous infection in the northern zone of India

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Objective To estimate the annual risk of infection with tubercle bacilli in the northern zone of India.

Methods A community-based cross-sectional tuberculin survey was conducted among children aged 1–9 years who lived in a sample of villages and urban blocks of six selected districts in a defined north zone of India. A two-stage cluster sampling method was used to select rural and urban clusters. A total of 48,624 children in 598 clusters were subjected to tuberculin testing with one tuberculin unit (1 TU) of PPD RT23 stabilized with Tween 80. The maximum transverse diameter of induration was measured about 72 hours after the test.

Findings Among the 48,624 test-read children, 22,064 (45.4%) had a bacille Calmette-Guérin (BCG) scar. On the basis of the frequency distribution of tuberculin reaction size among 25,816 children without a BCG scar, the prevalence of infection with tubercle bacilli was estimated as 10.3%. The annual risk of infection was computed as 1.9%. The proportion of infected children was significantly higher in urban than rural areas.

Conclusion The high rate of tuberculous infection in the north zone of India suggests the need for further intensification of tuberculosis control efforts on a sustained and long-term basis.

Keywords Tuberculosis, Pulmonary/epidemiology; Mycobacterium tuberculosis/pathogenicity; Tuberculin test; BCG vaccine; Periodicity; Epidemiologic factors; Cross-sectional studies; Cluster analysis; India (source: MeSH, NLM).

Mots clés Tuberculose pulmonaire/épidémiologie; Mycobacterium tuberculosis/pathogénicité; Réaction tuberculinique; Vaccin BCG; Périodicité; Facteurs épidémio-logiques; Étude section efficace; Sondage en grappes; Inde (source: MeSH, INSERM).

Palabras clave Tuberculosis pulmonar/epidemiología; Mycobacterium tuberculosis/patogenicidad; Test de tuberculina; Vacuna BCG; Periodicidad; Factores epidemiológicos; Estudios transversales; Análisis por conglomerados; India (fuente: DeCS, BIREME).

Introduction

The annual risk of infection is the preferred indicator of the epidemiological status of tuberculosis, especially in developing countries, because disease surveys are expensive and time consuming. The annual risk of infection is defined as the probability of acquiring new tuberculous infection or reinfection over a period of one year. It expresses the overall impact of various factors influencing the transmission of tubercle bacilli, specifically the load of infectious cases in the community and the efficiency of case finding and treatment programmes. The study of tuberculosis status in a large country such as India always has been a challenge. Although the importance of tuberculosis to public health has long been recognized, only in 1955–58 did a nationwide disease survey first reveal the magnitude of the problem (1). Most of the subsequent epidemiological studies to estimate the prevalence of disease or annual risk of infection have been confined to limited geographical areas of south India, close to two national tuberculosis institutes. Thus, information on the prevailing epidemiological status of tuberculosis has been largely unknown for most parts of the country.

The National Tuberculosis Program (NTP) has been implemented in India since 1962; however, its performance in terms of case finding and treatment has been below expectation (2,3). The Government of India therefore resolved to intensify efforts to control tuberculosis and to adopt the Direct Observation Treatment Strategy (DOTS) for the programme, which was renamed as the Revised National Tuberculosis Control Program (RNTCP). The RNTCP started to be introduced across the country in a phased manner in 1998, and it has shown promise, especially in terms of the cure rate for cases with sputum smears positive for tubercle bacilli (4). While efforts to control tuberculosis were being revitalized, it was decided to study the prevalent epidemiological situation of tuberculosis by conducting a nationwide tuberculin survey to estimate the annual risk of infection in different parts of the country. We stratified the country into four zones, each with about one quarter of the country’s population (Fig. 1). The survey was designed to obtain estimates of the average annual risk of tuberculous infection in each of the four zones. This paper presents the results from the north zone.
Methods

Study population and sampling

The study population comprised children aged 1–9 years without a scar from bacille Calmette–Guérin (BCG) vaccination. Older children were not included, as they were more likely to harbour infection with environmental mycobacteria that could interfere with interpretation of study results (5, 6). Children with a scar from BCG vaccination were excluded because BCG–induced tuberculin sensitivity could interfere with interpretation of the study results (6).

The states of Himachal Pradesh, Jammu and Kashmir, Punjab, Haryana, Uttar Pradesh, and Delhi made up the north zone. The sample size was estimated on the basis of an assumed minimum prevalence of infection of 8%, which was itself based on results obtained during earlier surveys in different parts of India (7–17). Other parameters considered for estimation of sample size are given in Table A (web version only, available from: http://www.who.int/bulletin/).

The sample size needed was estimated as 12,000 children without BCG scar, to be investigated in 600 clusters. For the purpose of the survey, a village was considered as a rural cluster and an urban census enumeration block as an urban cluster. The clusters in the zone were distributed among two strata — rural ($n = 453$) and urban ($n = 147$) — in proportion to the rural and urban population.

A two-stage sampling procedure was used to select clusters within a stratum. In the first stage, six districts were selected by systematic sampling: Rae Bareli, Hardoi, and Jaunpur (all from Uttar Pradesh — the most populous state...
Field procedures
Fieldwork was conducted by the National Tuberculosis Institute (NTI), Bangalore, with the support of district health authorities, from April 2000 to August 2001. Three visits were made to each cluster for planning, registration and testing, and reading. During the planning visit, important community leaders were apprised of the purpose of the survey. A sketch of the cluster, including hamlets and lanes, was made, and a lane in which fieldwork could be started was selected at random.

Children were registered on the day of testing, starting with those from the first house of the selected lane. Contiguous houses were visited in a clockwise direction until 85 children aged 1–9 years (completed one year and <10 completed years) were registered. An individual card was opened for each child, and identification particulars were noted. Tuberculin tests were performed at a temporary centre. Each child was injected intradermally on the volar aspect of the left forearm with 0.1 ml of tuberculin containing 1 TU of PPD RT23 stabilized with Tween 80 (WHO standard tuberculin test (18)). Tests were recorded as “satisfactory” when they raised a flat pale wheal with clearly visible pits and well demarcated borders, and “unsatisfactory” in case of leakage or subcutaneous injection. The presence or absence of a BCG scar was recorded after both of the child’s shoulders were palpated. The reader identified the margins of induration on careful palpation, and the maximum transverse diameter of induration was measured in millimetres. The presence of any vesicles, bullae, or necrosis also was recorded. The data were entered into the computer twice with FoxPro software and validated.

In addition, 493 cases of pulmonary tuberculosis were subjected to tuberculin testing within one week of being found positive for acid-fast bacilli on smear microscopy. This was intended to corroborate the criteria used to estimate prevalence of infection.

The tuberculin dilutions used in the survey were prepared by the BCG Laboratory, Guindy, Chennai, from the original seed lot of PPD RT23 stabilized with Tween 80 sent by the Statens Serum Institut, Copenhagen.

Statistical methods
Analysis was restricted to children without a BCG scar. The frequency distribution of tuberculin reaction sizes was plotted, and reactions equal to or greater than the identified cut-off point were considered attributable to “infection with tubercle bacilli” (19). Since the number of test-read children without a BCG scar varied from cluster to cluster, the proportion of children infected in each cluster was calculated first. After weights equal to the inverse of the initial probability of a cluster being selected were assigned, the proportions were pooled to estimate the proportion of infected children in the rural and urban areas of each district.

The proportions of infected children in the rural and urban strata of the entire zone were estimated further, by combining the estimates for the districts and by using the proportion of district population (rural/urban) in the zone as the weight. The zonal estimate of prevalence of infection was obtained similarly by pooling the rural and urban estimates. The formulae used for the above estimations are given in Table A (web version only, available from: http://www.who.int/bulletin/).

The annual risk of infection (ARI) was calculated from the estimated prevalence of infection (P) by using the following equation, where A = mean age of test-read children (20).

\[
ARI = 1 - \left(1 - P\right)^{1/A}
\]

The \(\chi^2\) test with continuity correction was used to test the significance of differences between proportions. The calculated values of the test criteria were compared with the tabular value at the 95% level to ascertain the significance of the test. The data were analysed with SPSS software.

Results
A total of 55 433 children were registered; 48 624 (87.7%) of these were test-read. Overall, 22 064 (45.4%) of the 48 624 test-read children had a BCG scar. In rural areas, 15 142

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cut-off pointa</th>
<th>Estimate</th>
<th>By mirror image technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (%)</td>
<td>11.8 (10.0–13.5)b</td>
<td>10.3 (8.4–12.2)</td>
<td>9.2 (7.3–11.0)</td>
</tr>
<tr>
<td>Annual risk of infection (%)</td>
<td>2.1 (1.8–2.5)</td>
<td>1.9 (1.5–2.2)</td>
<td>1.6 (1.3–2.0)</td>
</tr>
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a Cut-off point for reaction size after tuberculin test.
b Values in parentheses are 95% confidence intervals.
(40.5%) of the 37391 test-read children had a BCG scar and 6922 (61.6%) of the 11233 test-read children in urban areas had a BCG scar. This difference in the proportion of children with a BCG scar between rural and urban area was statistically significant ($P = 0.000$).

Data pertaining to 25816 children without a BCG scar were analysed after 22808 test-read children were excluded because of the presence of a BCG scar ($n = 22064$), the presence of a doubtful BCG scar ($n = 648$), and the absence of a BCG scar, but an unsatisfactory tuberculin test ($n = 96$).

The frequency distribution of tuberculin reaction size among children without a BCG scar was bimodal, with the mode of reactions attributable to infection with tubercle bacilli at 20 mm and an antimode apparent at 14 mm (Fig. 2). Similar distributions were obtained when tuberculin reaction sizes were plotted separately for rural and urban strata. All reactions $\geq 14$ mm therefore were considered to be attributable to infection with tubercle bacilli for the purpose of estimating the prevalence of infection.

On the basis of the above criterion, the prevalence of infection in the zone was estimated as 10.3% (95% confidence interval, 8.4–12.2). The annual risk of infection computed from the estimated prevalence was 1.9% (1.5–2.2).

Estimates of prevalence of infection and annual risk of infection in the zone were also made by varying the cut-off points by 2 mm on either side — that is to 12 mm and 16 mm. Finally, estimates made with the mirror image technique, with the mode considered to be at 20 mm, were found to be similar to estimates based on the cut-off point at 14 mm (Table 1).

Table 2 gives the proportion of infected children and computed annual risk of infection by stratum, sex, and age group, when 14 mm was used as the cut-off point. In rural areas, 2115 (9.1%) of 21816 children were infected compared with 675 (14.1%) out of 4000 in urban areas. The computed annual risks of infection were 1.6% and 2.6%, respectively ($P = 0.000$). No significant difference was seen between boys and girls in the proportion infected. In total, 525 (4.6%) out of 10196 children aged 1–4 years and 2265 (14.1%) out of 15620 children aged 5–9 years were infected. The annual risk of infection computed from the prevalence of infection estimates for these age groups were 1.5% and 2.0%, respectively. A small proportion (207, 0.8%) of the children presented with additional features, including vesicles and bullae at the test site.

Fig. 3 shows the frequency distribution of tuberculin reaction size among 493 test-read cases who were smear positive for pulmonary tuberculosis. The mode of reactions was at 20 mm, and 438 (88.8%) of cases elicited tuberculin reactions $\geq 14$ mm.
Discussion

The results of the survey provide useful information on the prevailing epidemiological status of tuberculosis in the north zone of India. They could be used as baseline data to evaluate the impact of disease control measures and epidemiological trends in the future.

The number of test-read children without a BCG scar was higher than the estimated sample size, because the proportion of children without a BCG scar was larger than expected and the drop-out rate was lower. The frequency distribution of tuberculin reactions among children without a BCG scar was bimodal, and the observed mode of reactions attributable to infection with tubercle bacilli was similar to the mode of reactions among smear-positive cases.

The annual risk of infection in the north zone of India was estimated as 1.9%. A higher rate of transmission of infection was observed in urban areas, which implied that such areas have a worse status of disease than rural areas. Our results differed from the findings obtained during the nationwide disease survey from 1955–58, when the prevalence of pulmonary tuberculosis was similar in urban and rural areas (1). The living conditions in urban areas have deteriorated in the intervening period, with about one quarter of the population now living in slums (22). Moreover, the delivery of anti-tuberculosis services has been more unsatisfactory in urban areas than in rural areas, with a multiplicity of services, most of which do not adopt standardized methods of case finding and treatment (22).

We cannot comment on any change from the previous epidemiological status of tuberculosis, because no previous data on risk of infection have been available from any part of north India. The relatively lower risk of infection in those aged 1–4 years compared with those aged 5–9 years might be attributable to a decline in the rates of transmission of infection, in addition to differences in the risk of exposure. Age dependency in the risk of infection has been reported from some other countries (23, 24). We also cannot comment on the possible contribution of the HIV epidemic, which is still at a relatively low level in the states of the north zone compared with the other zones. Sentinel surveillance data in these states in 2001 showed that the prevalence of HIV infection was <1% among antenatal women and 0.3–4.6% among those suffering from other sexually transmitted diseases (25).

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The estimated annual risk of infection was higher than most recent estimates from many other developing countries: Algeria, Egypt, the Republic of Korea, Kenya, and the United Republic of Tanzania (26–30). A higher rate of 2.3% was observed only in the Philippines (31). Annual risks of infection in most developed countries are <0.1% (32).

Most of the above-mentioned countries used 2 TU doses of PPD RT23 stabilized with Tween 80 dilutions prepared at Statens Serum Institut (except the Republic of Korea, where 1 TU of the same product was used). A series of comparative studies conducted earlier at the NTI suggested, however, that 1 TU dilution of PPD RT23 stabilized with Tween 80 prepared at the BCG Lab, Guindy, could be used for tuberculin surveys in India (33).

Most of the earlier tuberculin surveys in India were conducted in South India, with the exception of one conducted in a subdivision in Rajasthan and another in a tribal area of
Résultats

Parmi les 48 624 enfants dont le test a été lu, 22 064 (45,4 %) avaient une cicatrice de vaccin BCG (bacille Calmette-Guérin). D’après la distribution de fréquence du diamètre de la réaction tuberculinique chez les 25 816 enfants n’ayant pas de cicatrice de vaccin BCG, la prévalence de l’infection par le bacille tuberculeux a été estimée à 10,3 %. Le risque annuel d’infection calculé était de 1,9 %. La proportion d’enfants infectés était significativement plus élevée dans les zones urbaines que dans les zones rurales.

Conclusion

Le taux élevé d’infection tuberculeuse dans la partie nord de l’Inde indique la nécessité d’intensifier encore davantage les efforts de lutte contre la tuberculose, de façon soutenue et prolongée.

Conflicts of interest: None declared.

Résumé

Risque annuel d’infection tuberculeuse dans le nord de l’Inde

Objectif Estimer le risque annuel d’infection par le bacille tuberculeux dans la partie nord de l’Inde.

Méthodes Une enquête tuberculinique transversale a été réalisée au niveau communautaire sur des enfants de 1 à 9 ans résidant dans un échantillon de villages et de quartiers urbains de six districts choisis dans une zone définie du nord de l’Inde. Les grappes rurales et urbaines ont été sélectionnées selon une méthode de sondage à deux niveaux. Au total, 48 624 enfants appartenant à 598 grappes ont été soumis à un test tuberculinique avec une unité tuberculinique de PPD RT23 stabilisé par le fixwien 80. Le plus grand diamètre de l’induration a été mesuré 72 heures après le test.

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Conclusion Le taux élevé d’infection tuberculeuse dans la partie nord de l’Inde indique la nécessité d’intensifier encore davantage les efforts de lutte contre la tuberculose, de façon soutenue et prolongée.
Resumen
Riesgo anual de infección tuberculosa en el norte de la India

Objetivo Calcular el riesgo anual de infección por el bacilo de la tuberculosis en el norte de la India.

Métodos Se realizó una encuesta tuberculínica transversal de ámbito comunitario entre niños de 1–9 años que vivían en una muestra de aldeas y edificios urbanos de seis distritos seleccionados en una determinada zona del norte de la India. Se utilizó un método de muestreo por conglomerados en dos etapas para seleccionar conglomerados rurales y urbanos. Un total de 48 624 niños de 598 conglomerados fueron sometidos a la prueba tuberculínica con una unidad de tuberculina (1 UT) en forma de PPD RT23 estabilizado con Tween 80. Unas 72 horas después de la prueba se midió el diámetro transversal máximo de la induración.

Resultados Entre los 48 624 niños sometidos a la prueba, 22 064 (45,4%) presentaban una cicatriz de bacilo de Calmette-Guérin (BCG). Teniendo en cuenta la distribución de frecuencias del tamaño de la respuesta a la tuberculina entre 25 816 niños sin cicatriz de la BCG, se estimó que la prevalencia de la infección por el bacilo de la tuberculosis era del 10,3%. Se calculó así que el riesgo anual de infección era del 1,9%. La proporción de niños infectados fue significativamente mayor en las zonas urbanas que en las rurales.

Conclusión La alta tasa de infección tuberculosa detectada en el norte de la India muestra la necesidad de intensificar aún más los esfuerzos de control de la tuberculosis de forma sostenida y a largo plazo.

Dueños de animales, entre 1964 y 1986, en 488 comunidades de acceso difuso. Aunque las tasas de prevalencia fueron más altas en las comunidades de acceso difuso, la tasa media de prevalencia fue de 9% en ambas áreas. En cuanto a la tasa de prevalencia en niños, la tasa media fue de 13%, pero en las comunidades de acceso difuso fue de 18%.

Los resultados indican que la introducción de vacunas antitetánicas puede ser un factor important en la reducción de la tasa de infección tuberculosa en el norte de la India. Sin embargo, es necesario realizar más estudios para determinar la efectividad de estas vacunas en diferentes contextos y en diferentes grupos de edad.

El estudio también sugiere que la introducción de programas de control de la tuberculosis en áreas rurales es crucial para reducir la tasa de infección en estas áreas. Es importante que se continúen los esfuerzos para mejorar la cobertura y la efectividad de estos programas en áreas rurales.

Las conclusiones de este estudio son consistentes con los hallazgos de estudios previos que han demostrado que la introducción de programas de control de la tuberculosis en áreas rurales es crucial para reducir la tasa de infección en estas áreas. Es importante que se continúen los esfuerzos para mejorar la cobertura y la efectividad de estos programas en áreas rurales.
16. Chadha VK, Jagannatha PS, Shashidhar Savanur J. Annual risk of tubercu-
sis infection in Bangalore City. Indian Journal of Tuberculosis 2001;48:63-
71.

17. Tuberculosis Research Centre. Trends in the prevalence and incidence 
of tuberculosis in South India. International Journal of Tuberculosis and Lung 
Disease 2001;5:142-57.


for estimating the risk of tuberculosis infection from tuberculin test results 
Against Tuberculosis and Lung Disease 1989;64:7-12.

20. Cauthen GM, Pio A, ten Dam HG. Annual risk of tuberculous infection. 

21. Office of the Registrar General, India. Slum population in states/UTs. New 
http://www.censusindia.net/results/slum1.html

22. Upelkar MW, Rangan S. Private doctors and tuberculosis control in India. 

Bulletin of the International Union against Tuberculosis and Lung Disease 
1975;50:5-61.

24. Nagelkerke NJD, Heisterkamp S, Borgdorff MW, Broekmans J, Houwelingen 
H Van. Semi-parametric estimation of age-time specific infection incidence 

Control Organization; 2002. Available from: URL: 
http://www.naco.nic.in/indianscene/esthiv.htm

26. Lwanga SK, Lemeshow S. Sample size determination in health stu-

27. Hong YP, Kim SJ, Lew WJ, Lee EK, Han YC. The seventh nationwide tubercu-

of Tuberculosis and Lung Disease 1998;2:272-80.

29. El Biayy S, DeCoster EJM, Tolba FM, Van Maaren P, Wasley L, Van Cleeft M, 
et al. Trend in the annual risk of tuberculosis infection in Egypt, 

30. Tanzania Tuberculiu Survey Collaboration. Tuberculosis control in the era 

31. Tupasi TE, Radhakrishna S, Pasqual MI, Quelapio MID, Villa ML, Co VM, 
et al. BCG coverage and the annual risk of tuberculosis infection over a 
14-year period in the Philippines assessed from the nationwide prevalence 
surveys. International Journal of Tuberculosis and Lung Disease 

32. Murray CJL, Styblo K, Rouillon A. Tuberculosis in developing countries: bur-
den, intervention and cost. Bulletin of the International Union against 

33. Chadha VK, Jagannatha PS, Vaidyanathan P, Jagota P. PPD RT23 for tuber-
culin surveys in India. International Journal of Tuberculosis and Lung Disease 

34. TB India 2001, RNTCP Status Report. New Delhi: Central TB Division, 
Directorate General of Health Services, Ministry of Health and Family 

35. TB India 2002, RNTCP Status Report. New Delhi: Central TB Division, 
Directorate General of Health Services, Ministry of Health and Family 
Welfare; 2002.

36. RNTCP performance quarterly reports—First Quarter 2002. New Delhi: 
Central TB Division, Directorate General of Health Services, Ministry of 
dia.org

37. RNTCP performance quarterly reports—Second Quarter 2002. New Delhi: 
Central TB Division, Directorate General of Health Services, Ministry of 
dia.org

38. Report on performance of National Tuberculosis programme. DTP Quarterly 
reports; 24/4 January–December 2000. Bangalore: National Tuberculosis 

Institute; 2002.

40. Report on performance of National Tuberculosis programme. DTP Quarterly 
reports; 26/2 January–June 2002. Bangalore: National Tuberculosis 
Institute; 2002.

41. Styblo K. The relationship between the risk of tuberculous infection and the 
risk of developing infectious tuberculosis. Bulletin of the International 
Union Against Tuberculosis and Lung Disease 1985;60:117-99.

42. Liwanag SK, Lemeshev S. Sample size determination in health studies — 

43. Nagelkerke NJD, Borgdorff MW, Kalisvaart NA, Broekmans JF. The design of 
multi-stage tuberculosis surveys: some suggestions for sampling. 

44. Bennett S, Woods T, Winitha Liyanage M, Duana Smith LA. Simplified gene-
ral method for cluster-sample surveys of health in developing countries. 