Prevalence and determinants of QuantiFERON-diagnosed tuberculosis infection in 9,810 Mongolian schoolchildren

Davaasambuu Ganmaa[1,2†]
Polyna Khudyakov[1]
Uyanga Buyanjargal[3]
Badamtsetseg Jargalsaikhan[2]
Delgerekh Baigal[2]
Oyunsuren Munkhjargal[2]
Narankhuu Yansan[2]
Sunjidmaa Bolormaa[2]
Enkhsaikhan Lkhagvasuren[2,4]
Christopher T Sempos[3]
Sabri Bromage[2]
Zhenqiang Wu[6]
Batbayar Ochirbat[2]
Batbaatar Gunchin[2,4]
Adrian R Martineau[5†]

1. Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA

2. Mongolian Health Initiative, Royal Plaza, Bayanzurkh District, Ulaanbaatar, Mongolia

3. Office of Dietary Supplements, National Institutes of Health, Bethesda, MD 20892, USA

4. Mongolian National Health Sciences University, Ulaanbaatar Mongolia

5. Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AB, UK

6. School of Population Health, The University of Auckland, Auckland 1142, New Zealand

† To whom correspondence should be addressed at The Department of Nutrition, Harvard T.H. Chan School of Public Health, Building 2, Room 211, 655 Huntington Ave, Boston, Massachusetts 02115, USA; gdavaasa@hsph.harvard.edu or at
The Centre for Primary Care and Public Health, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 58 Turner St, London E1 2AB, UK; a.martineau@qmul.ac.uk

Summary

This large community-based study of risk factors for QuantiFERON-positivity in Mongolian schoolchildren reports that household exposure to an index case of pulmonary TB, vitamin D deficiency, passive smoking and increasing age are risk factors for *Mycobacterium tuberculosis* infection in childhood.

Abstract

Background: There is controversy regarding the potential influence of vitamin D deficiency, exposure to environmental tobacco smoke, BCG vaccination, season and body habitus on susceptibility to *Mycobacterium tuberculosis* (MTB) infection.

Methods: We conducted a cross-sectional analysis to identify determinants of a positive QuantiFERON®-TB Gold (QFT) assay result in children aged 6-13 years attending 18 schools in Ulaanbaatar, Mongolia. Data relating to potential risk factors for MTB infection were collected by questionnaire, physical examination and determination of serum 25-hydroxyvitamin D (25[OH]D) concentrations. Risk ratios were calculated using generalized estimating equations with adjustment for potential confounders, and population attributable fractions (PAFs) were calculated for modifiable risk factors identified.

Results: 946/9,810 (9.6%) participants had a positive QFT result. QFT-positivity was independently associated with household exposure to pulmonary TB (adjusted risk ratio [aRR] 4.75, 95% CI 4.13-5.46, P<0.001; PAF 13.1%, 95% CI 11.1%-15.0%), vitamin D deficiency (aRR 1.23, 95% CI 1.08-1.40, P=0.002; PAF 5.7%, 1.9%-9.3%), exposure to environmental tobacco smoke (one indoor smoker, aRR 1.19, 95% CI 1.04-1.35; two or more indoor smokers, aRR 1.30, 95% CI 1.02-1.64; P for trend, 0.006; PAF 7.2%, 95% CI 2.2%-12.0%) and increasing age (aRR per additional year 1.14, 95% CI 1.10-1.19, P<0.001). No statistically significant independent association was seen for presence of a BCG scar, season of sampling or body mass index.

Conclusions: Our findings underline the importance of contact tracing in TB-exposed households as a strategy to identify MTB-infected children. Vitamin D deficiency and exposure to environmental tobacco smoke may be modifiable risk factors for MTB infection.

Keywords: Latent tuberculosis infection; environmental tobacco smoke; BCG vaccine; Vitamin D Standardization Program (VDSP).

INTRODUCTION

Mycobacterium tuberculosis (MTB) infection in children necessarily arises from recent transmission: this group therefore represents a sentinel population for infectious tuberculosis. Population-based cross-sectional studies to estimate the prevalence and determinants of MTB infection in children living in high-incidence settings can inform tuberculosis control programs by allowing estimates of on-going transmission and identifying risk factors for infection that are potentially amenable to intervention.

Exposure to an infectious index case and increasing age are well recognized risk factors for MTB infection in children that have been demonstrated in numerous settings [1]. The evidence for other potential risk factors is less consistent however. Specifically, some studies have reported associations with lack of BCG vaccination [2, 3], exposure to environmental tobacco smoke [4], vitamin D deficiency [5], winter and spring season [6] and lower body mass index [7] while others have found no such associations [8-10]. Existing studies in the literature are variously limited by lack of power, low participation rates, use of the tuberculin skin test to diagnose MTB infection (which may yield false positive results in BCG-vaccinated individuals), restriction to household contacts, and insufficiently detailed information on potential confounders, all of which may underlie the heterogeneity of results seen when their results are meta-analyzed [11-13]. Additional studies addressing these limitations are therefore needed to clarify whether or not these factors influence risk of MTB infection.

An opportunity to undertake such a study recently arose in the context of screening for a community-based Phase 3 clinical trial with very broad eligibility criteria that enrolled primary schoolchildren living in Ulaanbaatar, Mongolia [14], where BCG is administered at birth only. A total of 9,814 children underwent screening for MTB infection using the QuantiFERON TB Gold test. Comprehensive data relating to potential susceptibility factors were collected, and multivariable analyses were performed to identify those that were independently associated with increased risk of MTB infection. Population attributable fractions (PAF) were then calculated for modifiable risk factors identified.

METHODS AND MATERIALS

Study design, setting and ethical approval

We conducted a cross-sectional analysis of baseline data from children attending eighteen public schools located in six districts of Ulaanbaatar, Mongolia (Bayanzurkh, Songino-Kharkhan, Bayangol, Khan-Uul, Chingeltei and Sukhbaatar) who were being screened for participation in a randomized controlled trial of vitamin D supplementation for the prevention of MTB infection.[14] Mongolia is an East Asian country situated between China and Russia with a population of approximately 3.1 million people, of whom 1.2 million (39%) reside in the capital city, Ulaanbaatar. School attendance is mandatory for children aged 6-16 years. Incidence of active tuberculosis in Mongolia is estimated at 428 cases per 100,000 population per annum [15] and prevalence of HIV infection is very low at 0.02% [16]. The study was approved by Institutional Review Boards at the Mongolian Ministry of Health, the Mongolian National University and the Harvard T. H. Chan School of Public Health, USA (IRB reference number 14-0513).

Participants

Eligibility criteria were as for the clinical trial for which children were being screened.[14] Inclusion criteria were age 6-13 years at screening; provision of written informed assent to participate by the child; and provision of written informed consent for the child to participate from his/her parent/guardian. Exclusion criteria were known HIV sero-positivity, primary hyperparathyroidism, sarcoidosis or previous active or latent tuberculosis; taking cytotoxic therapy or other immunosuppressant medication, enzyme-inducing anticonvulsant therapy, cardiac glycoside, any preparation containing 1-alpha-hydroxylated vitamin D or vitamin D supplementation of >10 micrograms/day; planning to move away from Ulaanbaatar within 4 years of enrollment; and presence of clinical signs of rickets, assessed by school doctors who checked for leg bowing, knock knees, pectus carinatum and thickened wrists and ankles.

Data Collection and Measurements

Fieldworkers collected information from each child's parent for the following variables using an electronic questionnaire on the RedCAP database: age, sex, highest education level attained by either

parent, type of residence, monthly household income, home ownership, number of people per room, indoor tobacco smoking in the household, active smoking by the child themselves, presence of an index case of pulmonary TB living in the household during the child's lifetime, and the average amount of time the child spends outdoors per day. Height was measured to the nearest 0.1 cm using a portable stadiometer (SECA, Hamburg, Germany). Weight was measured to the nearest 0.1 kg using a Digital Floor Scale (SECA). Body mass index (BMI) was calculated using the formula BMI = weight (kg) / (height $[m]^2$). Percent body fat was estimated using a body composition analyzer (SC-331S, Tanita, Tokyo, Japan). School doctors ascertained the BCG status of participating children by clinical examination for a vaccination scar. One ml of venous blood was drawn into nil, TB antigen and mitogen QuantiFERON-TB Gold High Altitude tubes (Qiagen, Hilden, Germany), which were processed as described below. Children with positive QuantiFERON-TB Gold results were referred to the Mongolia National Centre for Communicable Disease for clinical and radiographic screening for active TB. QuantiFERON-positive children in whom active TB was excluded were not preventively treated for latent tuberculosis infection, in line with WHO recommendations [17].

The QuantiFERON-TB Gold assay was performed according to manufacturer's instructions at the Global Laboratory, Ulaanbaatar, Mongolia, which participates in the QuantiFERON Quality Assurance Program of the Royal College of Pathologists of Australasia. Serum 25(OH)D concentrations were determined using an enzyme linked fluorescent assay (VIDAS 25OH Vitamin D total, Biomerieux, Marcy-l'Étoile, France). Total CV was 7.9%, mean bias was 7.7% and the limit of quantitation (LOQ) was 8.1 ng/ml. Non-zero 25(OH)D values were standardized using a set of 40 DEQAS serum samples as previously described by the Vitamin D Standardization Program (VDSP).[18] Values below the LOQ were classified as <8.1 ng/ml. Season-adjusted (deseasonalized) values were then calculated for each participant from their individual standardized 25(OH)D concentration and date of blood sample collection,

using a sinusoidal model with values derived from standardized values for all participants [19].

Statistical analysis

Data were analyzed using SAS software (version 9.4; SAS Institute, Cary, NC USA) and STATA (version 15; StataCorp. 2017, College Station, TX USA). Annual risk of tuberculosis infection (R) was estimated using the formula $R = 1-(1-\text{Prevalence})^{1/(\text{mean age})}$ [20]. The following factors were investigated

as risk factors for QuantiFERON-positivity, and handled as independent variables in the analysis: sex, age, parental education, type of residence, monthly household income, home ownership, number of people per room, month of sampling, number of people smoking cigarettes in the home, active smoking by the child, presence of BCG scar, body mass index, % body fat, household exposure to an index case of pulmonary tuberculosis, time spent outdoors and vitamin D deficiency, defined as serum 25(OH)D concentration <10 ng/ml; this threshold was pre-specified, based on findings of a previous study that reported susceptibility to *M. tuberculosis* infection to be increased below this cut-off [5]. Risk ratios for the association between these independent variables and the dependent variable of MTB infection (categorized as QuantiFERON-positive vs. –negative) were estimated using generalized estimating equations (GEE) with the binary distribution, log link function and exchangeable working correlation structure [21]. When the log-binomial model failed to converge, Log-Poisson models, which provide consistent but not fully efficient estimates of the risk ratio and its confidence intervals, were used [22]. We conducted two multivariable analyses to identify factors that were independently associated with risk of MTB infection: one adjusted for age and sex only, and the other additionally adjusted for all covariates that were associated with QuantiFERON-positivity with P<0.20 in the age- and sex-adjusted analysis. PAFs and their 95% confidence intervals were calculated for modifiable risk factors for MTB infection using STATA as previously described [23]. Participants with indeterminate QuantiFERON results (n=4) were excluded from analyses of risk factors for MTB infection.

RESULTS

A total of 11,475 children were invited to participate in the study from July 2015 to January 2017, of whom 1,065 (9.3%) declined and 596 (5.2%) were ineligible; reasons for ineligibility are presented in Supplementary Table 1. Socio-demographic characteristics of the remaining 9,814 children who participated in the study are presented in Table 1. Males and females were equally represented, mean age was 9.4 years and mean household income was US$ 840 per month. The BCG strains in use over the period of participants' birth were Japan BCG (2001-2003), Intervax Toronto (2003-2006) and SI India (2007-2009). Two thousand three hundred and sixty-five (24.1%) participants lived in a centrally heated house or apartment, 3,774 (38.5%) lived in a house or apartment without central heating and 3,675 (37.4%) lived in a ger (traditional Mongolian yurt). Three thousand five hundred and sixty-two (36.3%) participants lived in a household where at least one person smoked tobacco indoors, and 374 (3.8%) participants had a history of household exposure to a case of pulmonary TB. Deseasonalized 25(OH)D concentrations were available for 9,760/9,814 (99.4%) participants; they ranged from undetectable to 41.9 ng/ml, with a mean value of 12.1 ng/ml and standard deviation of 4.1 ng/ml. Two thousand four hundred and thirty-two participants (24.9%) were vitamin D deficient (25[OH]D <10 ng/ml).

Of the 9,814 children who underwent QuantiFERON testing, 8,864 (90.3%) had a negative result, 946 (9.6%) had a positive result, and 4 (0.04%) had an indeterminate result and were excluded from analyses. Based on a mean participant age of 9.4 years, the annual risk of tuberculosis infection (R) estimated using the formula $R = 1 - (1 - \text{Prevalence})^{1/(\text{mean age})}$ was 1.1% [20]. Nine hundred and thirty-eight of the 946 QuantiFERON-positive children (99.2%) were screened for active TB; of these, 129 (13.8%) were diagnosed with active TB.

Table 2 presents results of univariable and multivariable analyses evaluating potential determinants of QuantiFERON positivity in the 9,810 participants who had a positive or negative result. The following factors were found to associate with increased risk of QFT-positivity after adjustment for age, sex, parental education, type of residence, monthly household income, home ownership, number of people per room, month of sampling, exposure to household environmental tobacco smoke, child active smoking status, household PTB contact and deseasonalized serum 25(OH)D concentration: household

exposure to pulmonary TB (adjusted risk ratio [aRR] 4.75, 95% CI 4.13 to 5.46), vitamin D deficiency (aRR 1.23, 95% CI 1.08 to 1.40), number of people smoking indoors (aRR for one indoor smoker 1.19, 95% CI 1.04 to 1.35, aRR for two or more indoor smokers 1.30, 95% CI 1.02 to 1.64; P for trend =0.006) and increasing age (aRR per additional year 1.14, 95% CI 1.10 to 1.19). Population attributable risk fractions for modifiable risk factors for MTB infection were 13.1% for household TB contact (95% CI 11.1% to 15.0%), 5.7% for vitamin D deficiency (95% CI 1.9% to 9.3%) and 7.2% for passive smoking (95% CI 2.2% to 12.0%). No independent associations were seen for sex, socio-economic indices, presence of a BCG scar, body mass index, % body fat or season of sampling. A sensitivity analysis excluding children diagnosed with active TB yielded similar results (Supplementary Table 2).

In a sub-set of 373 children with a history of household exposure to an index case of pulmonary TB, risk of QFT-positivity was independently associated with the total number of index cases to whom the child had been exposed (aRR per additional index case 1.72, 95% CI 1.33 to 2.23, P<0.001) and increasing age (aRR per additional year 1.08, 95% CI 1.01 to 1.16, P=0.04), but not with any other potential risk factor investigated (Table 3).

DISCUSSION

We present results of the largest and most comprehensive study investigating risk factors for MTB infection in children conducted to date, and the first such investigation to be done in Mongolia. In a representative population of schoolchildren aged 6-13 years living in the capital city, Ulaanbaatar, we found that household contact with a case of pulmonary TB, vitamin D deficiency, household exposure to environmental tobacco smoke and increasing age were independent risk factors for infection. We found no association between risk of MTB infection and gender, socioeconomic factors, presence of BCG scar, season or body mass index.

Our study has several positive findings. The observation that MTB infection risk associates with household contact and increasing age is consistent with results from community-based studies and household contact studies in the literature [1]. The former finding emphasizes the importance of efforts to protect children from MTB infection in the home, and highlights the potential for a policy of household contact tracing with provision of preventive therapy to reduce the population-level burden of TB in low- and middle-income countries [17, 24, 25]. Demonstration of an independent association between household exposure to environmental tobacco smoke and increased risk of MTB infection suggests that this association is not explained by confounding due to socio-economic factors, as has previously been suggested [12]. The case for a causal interpretation is further supported by our demonstration of a dose-response relationship between increasing number of people per household smoking indoors and increasing risk of QuantiFERON-positivity, and by results of mechanistic studies showing that tobacco smoke attenuates innate immune responses to MTB both *in vitro* and *in vivo* [26-29]. Vitamin D metabolites have also been shown to support innate immune responses to MTB *in vitro* [30, 31], and our finding of an independent association between vitamin D deficiency and QuantiFERON-positivity supports the case for conducting clinical trials of vitamin D supplementation to prevent acquisition of MTB infection, two of which are currently in progress [14, 32].

With regard to the magnitude of protective associations observed, we found that 13.1% of the risk of acquiring MTB infection was attributable to household TB contact. This figure is similar to that reported from a meta-analysis of ten studies which yielded an estimate of 14.1% (95% confidence interval, 11.6%–16.3%) for the PAF for household transmission [33]. This relatively low figure reflects the fact that tuberculosis disease affects less than 1% of households at any time, even in high incidence settings, making exposure opportunities between a person with tuberculosis and their social network outside the household more numerous [34]. Studies in South Africa have indicated that significant

transmission occurs in public transportation [35] and in schools [36]. Further study to investigate sites of TB transmission in community settings in Mongolia is needed. The relatively high PAF for passive smoking (7.0%) demonstrated in our study is also striking: it highlights the importance of tobacco control for TB prevention [37]. The PAF for vitamin D deficiency (5.7%) echoes results of a recent ecological analysis, indicating that 6.3% of global variation in tuberculosis incidence is attributable to variations in exposure to ultraviolet-B radiation [38], which is a key determinant of vitamin D status.

Our study also has some important null findings. In contrast to others, [2, 3] we did not find that presence of a BCG scar associated with protection against MTB infection. In considering the significance of this observation, it is important to note that absence of a BCG scar does not necessarily signify that BCG vaccine was not given, since a proportion of BCG-vaccinated children do not develop a scar. Coverage of BCG vaccination has been estimated to be as high as 98.6% in Mongolia [39]; thus, children without BCG scars in this study may have been vaccinated. The fact that we found no statistically significant association between active smoking and risk of MTB infection may be explained by a lack of statistical power to detect such an association, reflecting the rarity of this practice: just 49/9810 (0.5%) participants smoked cigarettes. Accordingly, the 95% confidence interval for the risk ratio for active smoking was very wide (0.10 to 1.57).

Our study has a number of strengths. Our use of the QuantiFERON test (as opposed to TST) to detect MTB infection allowed for MTB infection status to be evaluated without confounding by sensitization to BCG or environmental mycobacteria. The sample size was very large, reducing the potential for type 2 error, and we recorded detailed information on a wide range of potential determinants of infection risk, allowing for comprehensive adjustment for confounders. We employed an objective assessment of BCG status (presence vs absence of BCG scar, evaluated by school doctors) rather than a subjective assessment such as eliciting a history of BCG vaccination. The lab performing QuantiFERON tests participated in an External Quality Assurance scheme performed by an ISO 9001-accredited laboratory, and rates of indeterminate results were extremely low (0.04%).

Our study also has some limitations. As with any observational study, associations observed may be due to residual and/or unmeasured confounding. However, the associations that we report are all biologically plausible and independent, withstanding adjustment for a wide range of potential confounders; moreover, a dose-response relationship is seen for some risk factors (e.g. age, number of TB contacts, number of people per household smoking cigarettes indoors). All of these factors strengthen the case for causal inference. A second potential limitation is that the study was a cross-

sectional analysis of baseline data from clinical trial participants: if this approach had resulted in exclusion of significant numbers of children, it could have compromised generalizability of our findings. However, participation rates in our study were high (85.5%), comparing favorably with those of cross-sectional studies investigating prevalence of MTB infection in other settings [40]. A third limitation is that we did not test for HIV infection; however, the prevalence of HIV infection in Mongolia is very low at 0.02% [16].

In conclusion, this very large, cross-sectional analysis identifies household contact with an index case of pulmonary TB, vitamin D deficiency and passive smoking as potentially modifiable risk factors for QuantiFERON-diagnosed MTB infection among Mongolian schoolchildren.

Authors' Contributions: GD and ARM designed the study. GD, UB, BJ, DB, OM, NY, SB, EL, BO, SB and BG participated in implementation of the study, data management and data collection. BJ, DB and OM performed laboratory assays. CTS implemented standardization of 25(OH)D levels. ZW calculated deseasonalized 25-hydroxyvitamin D levels. PK performed data analysis, with input from GD and ARM. ARM, DG and PK wrote the article; all other authors critically reviewed it and approved the final version.

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References

1. Narasimhan P, Wood J, Macintyre CR, Mathai D. Risk factors for tuberculosis. Pulmonary medicine **2013**; 2013: 828939.

2. Soysal A, Millington KA, Bakir M, et al. Effect of BCG vaccination on risk of Mycobacterium tuberculosis infection in children with household tuberculosis contact: a prospective community-based study. Lancet **2005**; 366: 1443-51.

3. Roy RB, Sotgiu G, Altet-Gomez N, et al. Identifying Predictors of Interferon-gamma Release Assay Results in Pediatric Latent Tuberculosis: A Protective Role of Bacillus Calmette-Guerin?: A pTB-NET Collaborative Study. Am J Respir Crit Care Med **2012**; 186(4): 378-84.

4. du Preez K, Mandalakas AM, Kirchner HL, et al. Environmental tobacco smoke exposure increases Mycobacterium tuberculosis infection risk in children. Int J Tuberc Lung Dis **2011**; 15(11): 1490-6, i.

5. Gibney KB, MacGregor L, Leder K, et al. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clin Infect Dis **2008**; 46(3): 443-6.

6. Wingfield T, Schumacher SG, Sandhu G, et al. The seasonality of tuberculosis, sunlight, vitamin D, and household crowding. J Infect Dis **2014**; 210(5): 774-83.

7. Cegielski JP, Arab L, Cornoni-Huntley J. Nutritional risk factors for tuberculosis among adults in the United States, 1971-1992. Am J Epidemiol **2012**; 176(5): 409-22.

8. Adetifa IM, Ota MO, Jeffries DJ, et al. Commercial interferon gamma release assays compared to the tuberculin skin test for diagnosis of latent Mycobacterium tuberculosis infection in childhood contacts in the Gambia. Pediatr Infect Dis J **2010**; 29(5): 439-43.

9. Hocaoglu AB, Erge DO, Anal O, Makay B, Uzuner N, Karaman O. Characteristics of children with positive tuberculin skin test. Tuberk Toraks **2011**; 59(2): 158-63.

10. Martineau AR, Newton SM, Wilkinson KA, et al. Neutrophil-mediated innate immune resistance to mycobacteria. J Clin Invest **2007**; 117(7): 1988-94.

11. Roy A, Eisenhut M, Harris RJ, et al. Effect of BCG vaccination against Mycobacterium tuberculosis infection in children: systematic review and meta-analysis. Bmj **2014**; 349: g4643.

12. Patra J, Bhatia M, Suraweera W, et al. Exposure to second-hand smoke and the risk of tuberculosis in children and adults: a systematic review and meta-analysis of 18 observational studies. PLoS Med **2015**; 12(6): e1001835; discussion e.

13. Saag LA, LaValley MP, Hochberg NS, et al. Low body mass index and latent tuberculous infection: a systematic review and meta-analysis. Int J Tuberc Lung Dis **2018**; 22(4): 358-65.

14. Ganmaa D, Martineau AR. Trial of vitamin D supplementation to prevent acquisition of latent M. tuberculosis infection in Mongolian primary schoolchildren. Available at: https://clinicaltrials.gov/ct2/show/NCT02276755.

15. WHO. Tuberculosis country profile: Mongolia. Available at: https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2=MN&LAN=EN&outtype=html. Accessed 10/16/2018.

16. Jagdagsuren D, Hayashida T, Takano M, et al. The second molecular epidemiological study of HIV infection in Mongolia between 2010 and 2016. PLoS One **2017**; 12(12): e0189605.

17. World Health Organisation. Guidance for national tuberculosis programmes on the management of tuberculosis in children: second edition. Geneva: World Health Organisation, **2014**.

18. Sempos CT, Betz JM, Camara JE, et al. General Steps to Standardize the Laboratory Measurement of Serum Total 25-Hydroxyvitamin D. J AOAC Int **2017**; 100(5): 1230-3.

19. Sachs MC, Shoben A, Levin GP, et al. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr **2013**; 97(6): 1243-51.

20. Shanaube K, Sismanidis C, Ayles H, et al. Annual risk of tuberculous infection using different methods in communities with a high prevalence of TB and HIV in Zambia and South Africa. PLoS One **2009**; 4(11): e7749.

21. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol **2005**; 162(3): 199-200.

22. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol **2004**; 159(7): 702-6.

23. Brady A. Adjusted population attributable fractions from logistic regression. Stata Tech Bull **1998**; 42: 8-12.

24. Kasaie P, Andrews JR, Kelton WD, Dowdy DW. Timing of tuberculosis transmission and the impact of household contact tracing. An agent-based simulation model. Am J Respir Crit Care Med **2014**; 189(7): 845-52.

25. Egere U, Togun T, Sillah A, et al. Identifying children with tuberculosis among household contacts in The Gambia. Int J Tuberc Lung Dis **2017**; 21(1): 46-52.

26. Gaschler GJ, Zavitz CC, Bauer CM, et al. Cigarette smoke exposure attenuates cytokine production by mouse alveolar macrophages. Am J Respir Cell Mol Biol **2008**; 38(2): 218-26.

27. van Zyl-Smit RN, Binder A, Meldau R, et al. Cigarette smoke impairs cytokine responses and BCG containment in alveolar macrophages. Thorax **2014**; 69(4): 363-70.

28. Bai X, Stitzel JA, Bai A, et al. Nicotine Impairs Macrophage Control of Mycobacterium tuberculosis. Am J Respir Cell Mol Biol **2017**; 57(3): 324-33.

29. Shang S, Ordway D, Henao-Tamayo M, et al. Cigarette smoke increases susceptibility to tuberculosis--evidence from in vivo and in vitro models. J Infect Dis **2011**; 203(9): 1240-8.

30. Martineau AR, Wilkinson KA, Newton SM, et al. IFN-γ- and TNF-Independent Vitamin D-Inducible Human Suppression of Mycobacteria: The Role of Cathelicidin LL-37. J Immunol **2007**; 178(11): 7190-8.

31. Martineau AR. Old wine in new bottles: vitamin D in the treatment and prevention of tuberculosis. Proc Nutr Soc **2012**; 71(1): 84-9.

32. Martineau AR, Middelkoop K. Trial of vitamin D supplementation in Cape Town primary schoolchildren. Available at: https://clinicaltrials.gov/ct2/show/NCT02880982.

33. Martinez L, Shen Y, Mupere E, Kizza A, Hill PC, Whalen CC. Transmission of Mycobacterium Tuberculosis in Households and the Community: A Systematic Review and Meta-Analysis. Am J Epidemiol **2017**; 185(12): 1327-39.

34. Mathema B, Andrews JR, Cohen T, et al. Drivers of Tuberculosis Transmission. J Infect Dis **2017**; 216(suppl_6): S644-S53.

35. Andrews JR, Morrow C, Wood R. Modeling the role of public transportation in sustaining tuberculosis transmission in South Africa. Am J Epidemiol **2013**; 177(6): 556-61.

36. Andrews JR, Morrow C, Walensky RP, Wood R. Integrating social contact and environmental data in evaluating tuberculosis transmission in a South African township. J Infect Dis **2014**; 210(4): 597-603.

37. Schneider NK, Novotny TE. Addressing smoking cessation in tuberculosis control. Bulletin of the World Health Organization **2007**; 85(10): 820-1.

38. Boere TM, Visser DH, van Furth AM, Lips P, Cobelens FGJ. Solar ultraviolet B exposure and global variation in tuberculosis incidence: an ecological analysis. Eur Respir J **2017**; 49(6).

39. Pai M, Zwerling A. The BCG World Atlas. Available at: http://www.bcgatlas.org/. Accessed 10/16/2018.

40. Mahomed H, Hawkridge T, Verver S, et al. Predictive factors for latent tuberculosis infection among adolescents in a high-burden area in South Africa. Int J Tuberc Lung Dis **2011**; 15(3): 331-6.

Table 1. Characteristics of study participants (n=9,814)

Sex	Female, n (%)	4,868 (49.6)
	Male, n (%)	4,946 (50.4)
Mean age, years (s.d.)		9.4 (1.6)
Parental education[1]	University/polytechnic, n (%)	1,881 (19.2)
	Secondary school or lower, n (%)	7,933 (80.8)
Type of residence	Centrally heated, n (%)	2,365 (24.1)
	Not centrally heated, n (%)	3,774 (38.5)
	Ger (Yurt) , n (%)	3,675 (37.4)
Mean monthly household income, US dollars (s.d.)[2]		840 (580)
Home ownership	No, n (%)	2,114 (21.5)
	Yes, n (%)	7,700 (78.5)
Mean number of people / room (s.d.)		4.7 (1.3)
No. of people in household smoking indoors	0	6,252 (63.7)
	1	3141 (32.0)
	≥2	421 (4.3)
Child actively smoking	No, n (%)	9,765 (99.5)
	Yes, n (%)	49 (0.5)
Household PTB contact	No, n (%)	9,440 (96.2)
	Yes, n (%)	374 (3.8)
Deseasonalized serum 25(OH)D[2]	<10 ng/ml, n (%)	2432 (24.9)
	≥10 ng/ml, n (%)	7328 (75.1)

Abbreviation: PTB, pulmonary tuberculosis; QFT, QuantiFERON®-TB Gold; s.d., standard deviation; US, United States.
[1] Highest educational level attained by either parent
[2] Data missing for mean monthly household income (10), Serum 25(OH)D (54)
[3] Defined as the presence of at least one person other than the participating child smoking tobacco indoors

Table 2. Risk factors for QuantiFERON®-TB Gold-positivity, all participants with non-indeterminate result (n=9,810)

Risk factors		Proportion QFT-positive (%)	Univariable analysis		Adjusted for age and sex only		Adjusted for age, sex and other covariates	
			Risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P
Sex	Female	494/4867 (10.2%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Male	452/4943 (9.1%)	0.90 (0.80, 1.02)	0.09	0.89 (0.79, 1.01)	0.07	0.92 (0.82, 1.04)	0.18
Age, years		-	1.17 (1.13, 1.22)	<0.001	1.17 (1.13, 1.22)	<0.001	1.14 (1.10, 1.19)	<0.001
Parental education[2]	University / polytechnic	128/1881 (6.8%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Secondary school or lower	818/7929 (10.3%)	1.52 (1.27, 1.81)	<0.001	1.43 (1.20, 1.71)	<0.001	1.16 (0.95, 1.42)	0.14
Type of residence	Centrally heated	170/2365 (7.2%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Not centrally heated	377/3772 (10.0%)	1.39 (1.17, 1.65)	<0.001	1.36 (1.14, 1.62)	<0.001	1.10 (0.92, 1.33)	0.29
	Ger (Yurt)	399/3673 (10.9%)	1.51 (1.27, 1.79)	<0.001	1.45 (1.22, 1.72)	<0.001	1.09 (0.88, 1.35)	0.41
Monthly household income[3], 100 US dollars		-	0.97 (0.95, 0.99)	<0.001	0.97 (0.96, 0.99)	0.003	0.99 (0.98, 1.01)	0.49
Home ownership	No	223/2113 (10.6%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Yes	723/7697 (9.4%)	0.89 (0.77, 1.03)	0.11	0.90 (0.78, 1.03)	0.13	0.95 (0.83, 1.09)	0.48
Number of people/room[3]		-	1.06 (1.03, 1.10)	<0.001	1.05 (1.02, 1.09)	0.003	1.01 (0.97, 1.05)	0.63
Month of sampling	Jun-Nov	247/3259 (7.6%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Dec-Feb	381/3671 (10.4%)	1.37 (1.18, 1.60)	<0.001	1.31 (1.12, 1.53)	<0.001	1.19 (1.00, 1.41)	0.06
	Mar-May	318/2880 (11.0%)	1.46 (1.24, 1.71)	<0.001	1.45 (1.23, 1.69)	<0.001	1.31 (1.10, 1.56)	0.003
No. of people in household smoking indoors	0	534/6249 (8.5%)	1.00 (ref)	<0.001[4]	1.00 (ref)	<0.001[4]	1.00 (ref)	0.006[4]
	1	347/3140 (11.1%)	1.29 (1.14, 1.47)	<0.001	1.26 (1.11, 1.44)	<0.001	1.19 (1.04, 1.35)	0.009
	2 or more	65/421 (15.4%)	1.81 (1.42, 2.29)	<0.001	1.68 (1.33, 2.13)	<0.001	1.30 (1.02, 1.64)	0.03

Child actively smoking[3]	No	944/9761 (9.7%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Yes	2/49 (4.1%)	0.42 (0.11, 1.64)	0.21	0.40 (0.10, 1.54)	0.18	0.40 (0.10, 1.57)	0.19
BCG scar	Absent	195/1958 (10.0%)	1.00 (ref)	-	1.00 (ref)	-		
	Present	751/7852 (9.6%)	0.96 (0.83, 1.12)	0.60	0.98 (0.84, 1.14)	0.78		
Body mass index[3], kg/m^2		-	1.02 (0.99, 1.04)	0.17	1.00 (0.97, 1.02)	0.84	-	
Body fat[3], %		-	0.99 (0.98, 1.00)	0.24	1.00 (0.99, 1.01)	0.56	1.00 (ref)	-
Household PTB contact	No	788/9437 (8.4%)	1.00 (ref)	-	1.00 (ref)	-	4.75 (4.13, 5.46)	<0.001
	Yes	158/373 (42.4%)	5.07 (4.43, 5.81)	<0.001	4.96 (4.33, 5.68)	<0.001		
Time spent outdoors, hours / day[3]	<1	414/4120 (10.1%)	1.00 (ref)	0.17[4]	1.00 (ref)	0.13[4]	1.00 (ref)	0.56[4]
	1-2	290/3013 (9.6%)	0.96 (0.83, 1.10)	0.55	0.95 (0.83, 1.10)	0.49	0.96 (0.83, 1.10)	0.53
	>2	242/2677 (9.0%)	0.90 (0.77, 1.05)	0.17	0.89 (0.76, 1.03)	0.13	0.95 (0.80, 1.12)	0.52
Deseasonalized serum 25(OH)D[3], ng/ml	≥10	656/7325 (9.0%)	1.00 (ref)	-	1.00 (ref)	-		
	<10	285/2431 (11.7%)	1.32 (1.16, 1.50)	<0.001	1.20 (1.05, 1.37)	0.01	1.23 (1.08, 1.40)	0.002

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BCG, Bacille Calmette–Guérin vaccine; CI, confidence interval; PTB, pulmonary tuberculosis; QFT, QuantiFERON®-TB Gold; US, United States.

[1] Adjusted for age, sex and the following covariates with P<0.2 in age/sex adjusted analysis: parental education, type of residence, monthly household income, home ownership, number of people per room, month of sampling, exposure to household environmental tobacco smoke, child active smoking status, household PTB contact and serum 25(OH)D concentration; 9,745 participants with non-missing data for all covariates included in this multivariable analysis.

[2] Highest educational level attained by either parent

[3] Missing values (income, 10 missing; number per room, 1 missing; body mass index, 2 missing; body fat, 42 missing; 25(OH)D, 54 missing)

[4] P-value for trend

Table 3. Risk factors for QuantiFERON®-TB Gold-positivity, sub-set of household pulmonary tuberculosis contacts (n=373)

Risk factors		Proportion QFT-positive (%)	Univariable analysis		Adjusted for age and sex only		Adjusted for age, sex and other covariates[1]	
			Risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P
Sex	Female	79/185 (42.7%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Male	79/188 (42.0%)	0.98 (0.78, 1.25)	0.89	1.00 (0.79, 1.26)	0.98	1.01 (0.80, 1.28)	0.92
Age, years				0.02	1.09 (1.01, 1.17)	0.02	1.08 (1.01, 1.16)	0.04
Parental education[2]	University / polytechnic	20/52 (38.5%)	1.00 (ref)	-	1.00 (ref)	-	-	-
	Secondary school or lower	138/321 (43.0%)	1.12 (0.78, 1.61)	0.55	1.09 (0.76, 1.55)	0.66	-	-
Type of residence	Centrally heated	27/74 (36.5%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Not centrally heated	51/140 (36.4%)	1.00 (0.69, 1.45)	0.99	1.00 (0.70, 1.45)	0.98	0.94 (0.65, 1.37)	0.76
	Ger (Yurt)	80/159 (50.3%)	1.38 (0.98, 1.93)	0.06	1.40 (1.00, 1.94)	0.05	1.36 (0.91, 2.03)	0.14
Household income, 100 US dollars[3]		-	-	0.71	1.00 (0.97, 1.02)	0.85	-	-
Home ownership	No	45/103 (43.7%)	1.00 (ref)	-	1.00 (ref)	-	-	-
	Yes	113/270 (41.9%)	0.96 (0.74, 1.24)	0.75	0.95 (0.74, 1.24)	0.72	-	-
Number of people/room[3]		-	-	0.10	1.05 (0.99, 1.11)	0.09	0.99 (0.92, 1.07)	0.85
Month of sampling	Jun-Nov	40/115 (34.8%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Dec-Feb	67/147 (45.6%)	1.31 (0.96, 1.78)	0.08	1.28 (0.94, 1.74)	0.11	1.23 (0.90, 1.67)	0.19
	Mar-May	51/111 (45.9%)	1.32 (0.96, 1.82)	0.09	1.33 (0.96, 1.83)	0.08	1.22 (0.87, 1.70)	0.25
No. of people in household smoking indoors	0	79/207 (38.2%)	1.00 (ref)	0.09[5]	1.00 (ref)	0.20[5]	1.00 (ref)	0.17[5]
	1	60/126 (47.6%)	1.25 (0.97, 1.61)	0.09	1.23 (0.96, 1.59)	0.10	1.16 (0.90, 1.50)	0.24
	2 or more	19/40 (47.5%)	1.24 (0.86, 1.80)	0.25	1.19 (0.82, 1.73)	0.35	1.19 (0.83, 1.73)	0.35
BCG scar	Absent	24/66 (36.4%)	1.00 (ref)	-	1.00 (ref)	-	-	-
	Present	134/307 (43.6%)	1.20 (0.85, 1.69)	0.30	1.19 (0.85, 1.68)	0.31	-	-
Body mass index, kg/m^2		-	1.01 (0.97, 1.05)	0.73	0.99 (0.95, 1.04)	0.78	-	-
Body fat, %[3]		-	0.99 (0.96, 1.01)	0.32	0.99 (0.96, 1.01)	0.34	-	-
Number of PTB index cases		-	1.62 (1.32, 2.00)	<0.001	1.58 (1.27, 1.96)	<0.001	1.72 (1.33, 2.23)	<0.001
Deseasonalized serum 25(OH)D, ng/ml[3]	≥10	113/280 (40.4%)	1.00 (ref)	-	1.00 (ref)	-		-
	<10	44/91 (48.4%)	1.20 (0.93, 1.55)	0.17	1.14 (0.87, 1.48)	0.35		

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BCG, Bacille Calmette–Guérin vaccine; CI, confidence interval; PTB, pulmonary tuberculosis; QFT, QuantiFERON®-TB Gold; US, United State

[1] Adjusted for age, sex and the following covariates with P<0.2 in age/sex adjusted analysis: type of residence, number of people per room, month of sampling, household environmental tobacco smoke, number of PTB index cases and serum 25(OH)D; 370 participants with non-missing data for all covariates were included in this multivariable analysis.

[2] Highest educational level attained by either parent

[3] Missing values (income, 1 missing; number per room, 1 missing; body fat, 2 missing; 25(OH)D, 2 missing)

[4] Defined as the presence of at least one person other than the participating child smoking indoors
[5] P-value for trend