Tracing recent community *Mycobacterium tuberculosis* transmission using tuberculin skin testing in the under-5s in rural Malawi

Authors: Palwasha Y Khan,[1,2] Sebastian Mboma,[2] Dominic Mulawa,[2] Judith R Glynn,[1,2] Amelia C Crampin[1,2]
Affiliations: [1]London School of Hygiene and Tropical Medicine; [2]Karonga Prevention Study
Adddress: Karonga Prevention Study, PO BOX 46, Chilumba, Karonga District, Malawi
E-mail: palwasha.khan@lshtm.ac.uk

Background

Despite significant progress through the DOTS strategy in the 1990s and the STOP TB strategy in 2006,[1] tuberculosis (TB) control, especially in sub-Saharan Africa, remains immensely challenging in the face of the HIV epidemic.[2,3] A key factor sustaining the TB epidemic is our inability to interrupt the on-going transmission cycle of *Mycobacterium tuberculosis (Mtb).*[4] Even if current control measures are successful, future control is in jeopardy unless we can dramatically reduce the large reservoir of latent infections in high HIV/TB burden settings.[5] In order to attempt to meet the STOP TB Partnership target of TB elimination[i] by 2050,[6] a detailed understanding of *Mtb* transmission dynamics in HIV-prevalent settings is urgently required to effectively and efficiently implement interventions to reduce transmission and disease.[7-12]

The contribution of different non-household sources to *Mtb* transmission is unknown. Household contacts of a tuberculosis case are at high risk for *Mtb* infection and disease,[13] yet most *Mtb* transmission occurs outside the household in endemic areas.[14-19] In Karonga, Malawi, the setting for this study, the proportion attributable to household contact was < 20% whether estimated from molecular epidemiology,[16] case-control studies,[17] or skin-testing household contacts.[20]

The potentially long latent period of infection with Mtb and the inability to distinguish between recent and historic infection complicates studies examining transmission. However, *Mtb* infection in young children indicates recent transmission and acts as a sentinel of the presence of infectious adult tuberculosis.[21,22], as well as young children at risk of active disease. Identifying infection in pre-school children may pinpoint areas where control measures are failing. Ascertainment of infection relies on the tuberculin skin test (TST), one of the oldest diagnostic tests still in use,[23] which measures the host immune response to a extract?? of antigens from killed tubercule bacilli. [21] Despite concerns about lack of specificity due to cross-reactivity with non-tuberculous mycobacteria and BCG,[24] in endemic settings most positive indurations (≥10mm) can be attributed to *Mtb* infection,[25-27] and using interferon gamma release assays (IGRA) alone provides few advantages.[28,29] Young children spend more time near their home and close family than do adults, and are less likely to be already infected with *Mtb*, thus increasing the chance of being able to pinpoint the source of new infections.

In this study we aimed to identify locations and sources of recent transmission in a rural HIV-prevalent community using *Mtb* infection in a cohort of children aged under-5 years.

[i] TB elimination defined as less than one TB case per one million population per year

Methods

Study design

A population-wide tuberculin skin test (TST) survey in children under 5 years of age is currently underway within a demographic surveillance site in Karonga District, Malawi. Two TST are undertaken one year apart to identify incident *Mtb* infection. Adult (aged >15 years) household, neighbourhood and regular visitor contacts of children identified with incident *Mtb* infection are screened for active TB and their households using enhanced sputum collection. Children with presumptive incident infection on the basis of TST are asked to provide a blood sample for Quantiferon testing.

Study dates

The study started in January 2012 (after an initial pilot study) and the first round was completed in December 2012. Round 2 of the TST survey started in January 2013 and is in progress.

Study setting

This study is part of on-going research at the Karonga demographic surveillance site (population 36,000) in Karonga District, northern Malawi.[30] Karonga district has an adult HIV prevalence of approximately 10%,[31] and annual incidence of new smear-positive TB of approximately 75 per 100,000 adults per year (unpublished data). Bacille Calmette-Guérin (BCG) vaccination has been given on first health system contact as part of the Expanded Programme on Immunisation in the district since 1990.[32]

We have detailed data on familial relationships, socio-economic factors, household structure, HIV status of adults, vaccination history of children and clinical and socio-demographic data on all diagnosed TB patients resident within the demographic surveillance site.

Participants

All children resident within the demographic surveillance site aged less than 5 years, whose parents or guardians are able to give written informed consent were eligible to take part in round one of tuberculin skin test cohort study.

Definitions

- Positive TST defined as induration of ≥ 10mm in a child of any age
- Presumed incident *Mtb* infection defined as any infant aged ≤ 1year of age with induration ≥ 10mm, **or** any child with an increase in induration ≥ 6mm from <10mm to >10mm from round 1 to round 2. .
  Does quantiferon not come into this definition of incident infection

Study procedures and data collection

2 international units (IU) of Tuberculin purified protein derivative RT 23 (Statens Serum Institut, Copenhagen, Denmark) were injected into the volar surface of the forearm, and indurations were measured 48 hours later by experienced field staff. To ensure validity of TST reading and to reduce intraobserver and interobserver variability, induration measurement by each member of the field team was tested at regular intervals during the course of the study against the same reference reader (field supervisor). Any field workers with marked deviation from the standard reference reading were retrained.

Need to mention this higher upA QuantiFERON®-TB Gold In-tube test (QFT-GIT; Cellestis, Carnegie, VIC, Australia), was also carried out in children with evidence of incident *Mtb*

infection as per study case definition. 3-4ml of venous blood was collected in a syringe and immediately transferred into each of the 3 tubes (nil, mitogen, antigen) and immediately shaken. All samples were returned to the laboratory the same day for incubation.

The movement and contacts of each child identified with incident *Mtb* infection were recorded through structured guardian interviews. Information on duration of time spent in different households within the last year, history of TB within the family and close contacts within the last year and number of times the child has been exposed to crowded gathering places, such as at funerals, churches, healthcare facilities, markets and travel on minibuses within the last year, were ascertained using a standardised questionnaire.

Children identified with incident *Mtb* infection were evaluated for TB-related symptoms. Once active disease was excluded children with induration ≥15mm or children with a TST≥10mm with a positive IGRA were commenced on a 6-month course of isoniazid preventative therapy (5mg/kg once daily). Evidence of BCG scar and scar size, height, weight and mid-upper arm circumference were also recorded. HIV status was not determined. Field workers documented TST readings for all children with a TST≥10mm, irrespective of whether they met the criteria for case definition, in the child's health passport.

Adult contacts (household, regular visitors to household and members of households regularly visited by infant) identified by the questionnaire and neighbourhood (how defined) contacts within the demographic surveillance site were screened for tuberculosis using enhanced sputum collection techniques.[33]

Analyses

Descriptive categorical data is displayed as numbers and percentages. Histograms of induration size were evaluated for evidence of digit-preference and bimodal distributions.

Prevalence of *Mtb* infection was calculated as the proportion of all children with a positive TST reading divided by the total number of children with an administered and read skintest. Two methods were examined to define prevalence of infection: the fixed cut-off method, using 10mm cut-off (definition of positive TST in this study) and 15mm cut-off, and the fixed mirror method.[34]

Annual risk of infection was calculated using the formula:[21]

$$R_{b+a/2} \approx 1\text{-}\ (1 - P_{b+a})^{1/a}$$

where $R_{b+a/2}$ = annual risk of infection at the midpoint in calendar time between the year the cohort was born and the year of the TST survey; P_{b+a} = proportion of children with "positive" TST depending on method used to estimate prevalence of *Mtb* infection at the time of the survey; a = mean age of the study group at the time when the survey was conducted.

Spatial analysis was undertaken using Geographical Information Systems (GIS) techniques. ArcGIS® | ArcMap version 10 (ESRI, Redlands, CA) was to used for mapping all skin test readings of children and smear-positive TB cases diagnosed from January 2011 to December 2012. GIS was used to extract data pertaining to distance of child identified with incident *Mtb* infection from nearest diagnosed TB case.

Ethical approval

The study was approved by the Malawi National Health Sciences Research Committee and the London School of Hygiene and Tropical Medicine Ethics committee. Written informed consent was obtained from the parent or guardian of all the children who participated in the study and from the adults that were screened for TB as part of the study.

Results

Participants

5824 children aged < 5 years were eligible to participate in the study, of whom 4987 (85.6%) had a skin test placed and read during the initial round. Of these 4987 children, 737 (14.8%) were infants aged ≤ 1 year, in whom a single test was informative.

Descriptive data

Figure 1 and 2 illustrate percentage distribution of induration size for all children and for under-ones respectively.

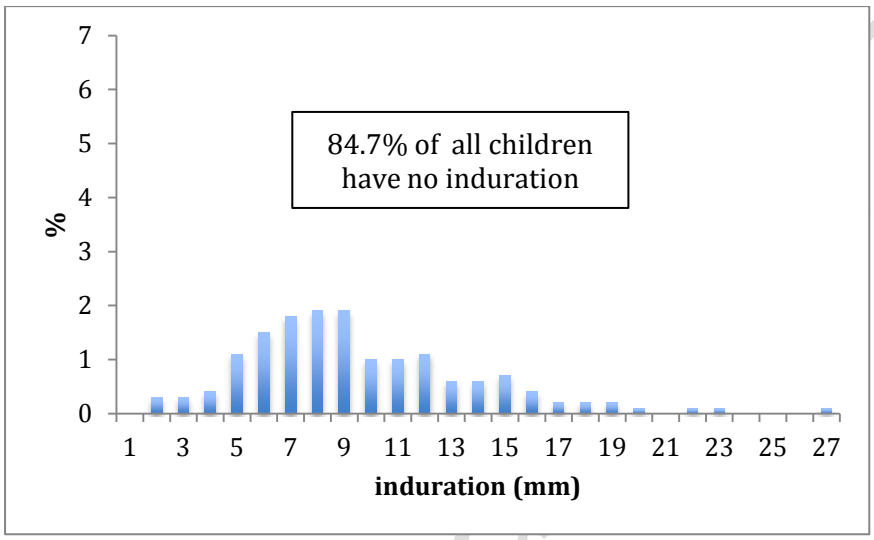

Figure 1. Histogram showing percentage distribution of induration size for all skin test performed and read (n=4987) in children aged 3-60 months in round 1 of the study

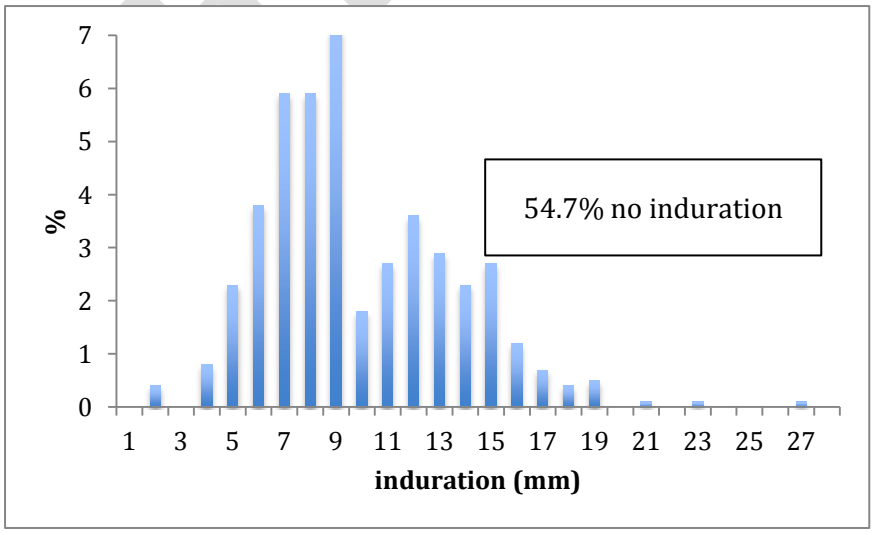

Figure 2. Histogram showing percentage distribution of induration size in under-ones (n=737)

Outcome data

TST induration (mm)	All children N	(%)	Age <1yr		1-2yrs		2-3yrs		3-4yrs		4-5yrs	
0	4225	(84.7)	404	(54.8)	902	(84.7)	969	(91.2)	1009	(92.9)	941	(90.7)
1-4	45	(0.9)	9	(1.2)	11	(1.0)	9	(0.8)	7	(0.6)	9	(0.9)
5-9	405	(8.1)	183	(24.8)	86	(8.1)	53	(5.0)	40	(3.7)	43	(4.2)
10-14	216	(4.4)	97	(13.2)	49	(4.6)	23	(2.2)	18	(1.7)	29	(2.8)
≥15	96	(1.9)	44	(6.0)	17	(1.6)	8	(0.8)	12	(1.1)	15	(1.4)

Table 1. Distribution of induration size among 4987 children aged 3-60 months

Table 2 shows the annual risk of infection (ARI) using different methods to estimate prevalence of *Mtb* infection demonstrating considerable variation in estimates of prevalence and ARI if you do put it in, should add >>=10mm alone

Method used	Prevalence of infection <12 mths n/N (%)		Prevalence of infection 12-60 mths n/N (%)		**Annual risk of infection (%) <12 mths** mean age=0.63yr	**Annual risk of infection (%) 12-60 mths** mean age=2.99yr
TST≥10mm & IGRA+	19/737	(2.6)	-		4.2	-
15mm*	74/737	(10.0)	78/4250	(1.8)	15.6	0.6
Fixed mirror	25/737	(3.4)	42/4250	(1.0)	5.4	0.3

*with adjustment for false negatives

Table 2. Annual risk of infection (using different methods to estimate prevalence of infection) in the under ones

Main results

Initial round of tuberculin skin testing identified possible "incident infection" in 141/737 (19%) of the under-ones (in whom a single test is informative) using a 10mm cut-off.

Of these 141 infants (how many had IGRA post and how many positive by other criteriaa) what proportion had a smear positive adult (or any TB patient) in their house since birth ? b) what proportion had a TB patient since birth resident in their contact network/neighbours (however we define) /

guardians of 133 (94%) agreed to an interview and a QFT-GIT assay. 19/133 (14%) had a positive QFT-GIT assay. Table 3 shows how QFT-GIT relates to induration size in this group of infants aged under one year.

		Induration size (mm)		
		N (%)		Total
		10-14	≥15	
QFT-GIT	Positive	14 (15.6)	5 (11.6)	19 (14.3)
	Negative	67 (74.4)	36 (83.8)	103 (77.4)
	Indeterminate	9 (10.0)	1 (2.3)	10 (7.5)
	Suspicious	0 (0)	1 (2.3)	1 (0.8)

Table 3. QFT-GIT assay result by induration size among under-ones

1114 contacts have been screened so far (relating to 55 incident infections). We have identified one minimally symptomatic smear-positive female, resident within 100 metres of 5 infants with incident infection. Of the 141 infants, 10 (7%) reside within 200 metres of a known smear-positive case (diagnosed within their lifetime).

Smear positive in 2011-2012 (ArcGIS map)

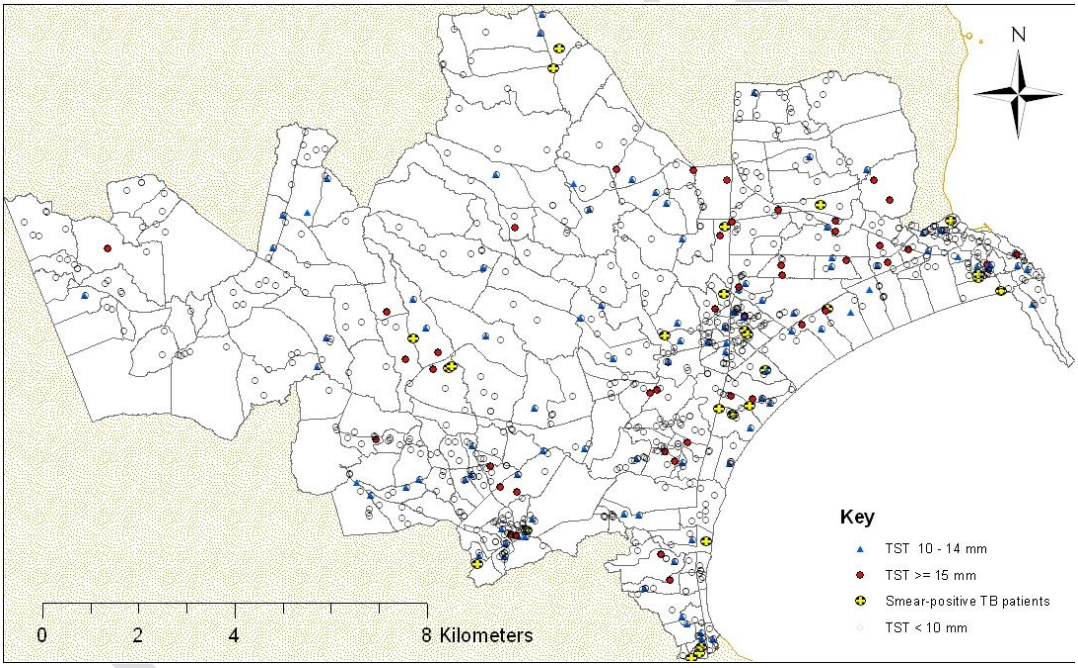

Figure 3. Map of the demographic surveillance site showing geographical position of all skin tests undertaken in under-ones in round 1 in relation to all smear-positive TB cases diagnosed from January 2011 to December 2012.

Conclusion

- *Key results- summarised with reference to study objective*
- *Limitations: taking into account sources of potential bias and imprecision, discuss both magnitude and direction of any potential bias*
- *Interpretation: cautious overall interpretation of the results considering study objectives, limitations, mulitiplicity of analyses, results from other studies, and other relevant evidence*
- *Generalisability: external validity of the study results*

Identifying recent transmission in a setting with a well-implemented tuberculosis control programme may pinpoint transmission "hot spots" and estimate the contribution of known and unknown "transmitters" at a community level.

Evidence in the under-ones suggests that even in this age group the majority of infection does not arise from diagnosed or undiagnosed neighbourhood transmitters but from more casual contact.

Fundingyes, including your own funding

References

1. WHO. Global tuberculosis control: WHO report 2011. http://www.who.int/tb/publications/global_report/2011/gtbr11_main.pdf. Geneva: World Health Organisation; 2011.

2. Harries AD, Zachariah R, Corbett EL, et al. The HIV-associated tuberculosis epidemic--when will we act? *Lancet.* May 29 2010;375(9729):1906-1919.

3. Lawn SD, Harries AD, Williams BG, et al. Antiretroviral therapy and the control of HIV-associated tuberculosis. Will ART do it? *Int J Tuberc Lung Dis.* May 2011;15(5):571-581.

4. Murray EJ, Marais BJ, Mans G, et al. A multidisciplinary method to map potential tuberculosis transmission 'hot spots' in high-burden communities. *Int J Tuberc Lung Dis.* Jun 2009;13(6):767-774.

5. Sandgren A, Cuevas LE, Dara M, et al. Childhood tuberculosis: progress requires advocacy strategy now. *Eur Respir J.* Feb 23 2012.

6. Glaziou P, Falzon D, Floyd K, Raviglione M. Global epidemiology of tuberculosis. *Seminars in respiratory and critical care medicine.* Feb 2013;34(1):3-16.

7. Bekker LG, Wood R. The changing natural history of tuberculosis and HIV coinfection in an urban area of hyperendemicity. *Clin Infect Dis.* May 15 2010;50 Suppl 3:S208-214.

8. Fine PE, Small PM. Exogenous reinfection in tuberculosis. *N Engl J Med.* Oct 14 1999;341(16):1226-1227.

9. Marais BJ, Obihara CC, Warren RM, Schaaf HS, Gie RP, Donald PR. The burden of childhood tuberculosis: a public health perspective. *Int J Tuberc Lung Dis.* Dec 2005;9(12):1305-1313.

10. Chamie G, Luetkemeyer A, Charlebois E, Havlir DV. Tuberculosis as part of the natural history of HIV infection in developing countries. *Clin Infect Dis.* May 15 2010;50 Suppl 3:S245-254.

11. Rylance J, Pai M, Lienhardt C, Garner P. Priorities for tuberculosis research: a systematic review. *Lancet Infect Dis.* Dec 2010;10(12):886-892.

12. Godfrey-Faussett P. Measuring TB transmission and its impact at community level: what is missing? HIV/TB research meeting in conjuction with the 19th Conference on Retroviruses and Opportunistic Infections; 2012; Seattle, USA: .

13. Morrison J, Pai M, Hopewell PC. Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income and middle-income countries: a systematic review and meta-analysis. *Lancet Infect Dis.* Jun 2008;8(6):359-368.

14. Schaaf HS, Michaelis IA, Richardson M, et al. Adult-to-child transmission of tuberculosis: household or community contact? *Int J Tuberc Lung Dis.* May 2003;7(5):426-431.

15. Verver S, Warren RM, Munch Z, et al. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *Lancet.* Jan 17 2004;363(9404):212-214.

16. Crampin AC, Glynn JR, Traore H, et al. Tuberculosis transmission attributable to close contacts and HIV status, Malawi. *Emerg Infect Dis.* May 2006;12(5):729-735.

17. Crampin AC, Floyd S, Ngwira BM, et al. Assessment and evaluation of contact as a risk factor for tuberculosis in rural Africa. *Int J Tuberc Lung Dis.* Jun 2008;12(6):612-618.

18. Buu TN, van Soolingen D, Huyen MN, et al. Tuberculosis acquired outside of households, rural Vietnam. *Emerg Infect Dis.* Sep 2010;16(9):1466-1468.

19. Brooks-Pollock E, Becerra MC, Goldstein E, Cohen T, Murray MB. Epidemiologic inference from the distribution of tuberculosis cases in households in Lima, Peru. *J Infect Dis.* Jun 1 2011;203(11):1582-1589.

20. Ho T. *The risk of household transmission of Mycobacterium tuberculosis infection to children under 11 years in rural Malawi*. London: Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine; 2012.

21. Rieder HL. *Epidemiological basis of tuberculosis control.* Paris: International Union Against Tuberculosis and Lung Disease; 1999.

22. Middelkoop K, Bekker LG, Morrow C, Zwane E, Wood R. Childhood tuberculosis infection and disease: a spatial and temporal transmission analysis in a South African township. *S Afr Med J.* Oct 2009;99(10):738-743.

23. Tissot F, Zanetti G, Francioli P, Zellweger JP, Zysset F. Influence of bacille Calmette-Guerin vaccination on size of tuberculin skin test reaction: to what size? *Clin Infect Dis.* Jan 15 2005;40(2):211-217.

24. Rieder HL. Methodological issues in the estimation of the tuberculosis problem from tuberculin surveys. *Tuber Lung Dis.* Apr 1995;76(2):114-121.

25. Tuberculin skin test survey in a pediatric population with high BCG vaccination coverage -- Botswana, 1996. *MMWR Morb Mortal Wkly Rep.* Sep 12 1997;46(36):846-851.

26. Lockman S, Tappero JW, Kenyon TA, Rumisha D, Huebner RE, Binkin NJ. Tuberculin reactivity in a pediatric population with high BCG vaccination coverage. *Int J Tuberc Lung Dis.* Jan 1999;3(1):23-30.

27. Araujo Z, de Waard JH, de Larrea CF, Borges R, Convit J. The effect of Bacille Calmette-Guerin vaccine on tuberculin reactivity in indigenous children from communities with high prevalence of tuberculosis. *Vaccine.* Oct 16 2008;26(44):5575-5581.

28. Dheda K. IGRAs: Utility in high and low burden settings [Stop TB Partnership New Diagnostics Working Group]. Paper presented at: 42nd Union World Conference on Lung Health2011; Lille, France.

29. Rutherford ME, Nataprawira M, Yulita I, et al. QuantiFERON(R)-TB Gold In-Tube assay vs. tuberculin skin test in Indonesian children living with a tuberculosis case. *Int J Tuberc Lung Dis.* Feb 8 2012.

30. Crampin AC, Dube A, Mboma S, et al. Profile: the Karonga Health and Demographic Surveillance System. *Int J Epidemiol.* Jun 2012;41(3):676-685.

31. Mboma SM, Houben RM, Glynn JR, et al. Control of (Multi)Drug Resistance and Tuberculosis Incidence over 23 Years in the Context of a Well-Supported Tuberculosis Programme in Rural Malawi. *PLoS One.* 2013;8(3):e58192.

32. Crampin AC, Glynn JR, Fine PE. What has Karonga taught us? Tuberculosis studied over three decades. *Int J Tuberc Lung Dis.* Feb 2009;13(2):153-164.

33. Heartland National TB Center. Collecting a Quality Sputum. 2012.

34. 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.