

1 **Incidence of tuberculosis among young children in rural Mozambique**

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23 **Running title:** Tuberculosis in Mozambique

24 **Abbreviated title:** Incidence of Childhood TB in Rural Mozambique

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33 **Sources of support:** This work was supported by the European & Developing Countries
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35 Clinical Trials Partnership [IP_07_32080_003].
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38 **Keywords:** tuberculosis; pediatrics; epidemiology; incidence; Mozambique
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Background. Tuberculosis contributes significantly to child morbidity and mortality. This study aimed to estimate the minimum community based incidence rate of TB among children <3 years of age in Southern Mozambique.

Methods. Between October 2011 and October 2012, in the Manhiça District Health and Demographic Surveillance System (HDSS) we enrolled prospectively all presumptive TB cases younger than under 3 years through passive and active case finding. Participants included all children who were either symptomatic or were close contacts of a notified adult smear positive pulmonary TB. Children were clinically evaluated at baseline and follow-up visits. Investigation for TB disease included chest radiography, HIV and tuberculin skin testing as well as gastric aspirate and induced sputum sampling, which were processed for smear, culture, and mycobacterial molecular identification.

Results. During the study period, 13.764 children <3yr contributed to a total of 9.575 person years. Out of the 789 presumptive TB cases enrolled, 13 had TB culture confirmation and 32 were probable TB cases. The minimum community-based incidence rate of TB (confirmed plus probable cases) was 470/100.000 person-years (95% CI: 343 to 629/100.000). HIV co- infection was present in 44% of the TB cases.

Conclusion. These data highlight the huge burden of pediatric TB. This study provides one of the first prospective population-based incidence data of childhood tuberculosis and adds valuable information to the global effort of producing better estimates, a critical step to inform public health policy.

Introduction

1
2 Tuberculosis (TB) is an under-recognized but potentially important cause of morbidity
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4 and mortality in children in TB endemic settings^{1,2}. Infants and young children
5
6 (<3years) and those with immunodeficiency caused by HIV or severe malnutrition are
7
8 at highest risk of developing TB disease following infection³. Delay of diagnosis and
9
10 treatment in these children increases the risk of rapid disease progression and
11
12 mortality⁴. TB diagnosis is particularly challenging in this population, given the lack of
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14 specific symptoms, the difficulty in obtaining samples for microbiological examination,
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16 and the often pauci-bacillary disease. The diagnostic yield of samples is often <20%
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18 under TB program conditions^{5,6}. These diagnostic difficulties result in delayed and
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20 under-diagnosis of the disease, contributing to the hidden burden of TB in children.
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31 Child TB is receiving more attention³ as the World Health Organization (WHO) post-
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33 2015 TB strategy seeks to engage the wider health sector including the child health-
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35 care sector⁷. The WHO Global Tuberculosis Report 2014 estimates that 550 000
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37 children developed tuberculosis during 2013, representing 6% of the global TB
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39 burden⁸. However, several factors suggest that the true burden of disease may be
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41 higher as these estimates assume an equal ratio of notified cases in children and
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43 adults (whereas under-reporting in children is very common⁹), and estimated deaths
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45 only include those in HIV negative children³. As a setting's total TB burden increases,
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47 there tends to be a rise in the proportion of TB cases attributable to children¹⁰. Thus, in
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49 high TB burden settings, children may represent up to 10-20% of TB cases, with
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51 increased TB incidence in <5years (yr) and >15yr^{4,8,11}.
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1 Mozambique is one of the high TB burden countries listed by the WHO but has a very
2 low reported case-detection rate of 37%⁸. Improved reliable estimates are required
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4 to quantify the hidden burden of disease and measure future progress towards the
5 control of TB in the country, especially for vulnerable populations such as children
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8,12,13. We therefore aimed to determine the minimum community-based incidence rate (IR) of childhood TB.

Materials and Methods

Setting

The study was conducted in the Manhiça District (rural southern Mozambique), where the Manhiça Health Research Center (Centro de Investigação em Saúde de Manhiça, CISM) runs a Health and Demographic Surveillance System (HDSS) including the Manhiça District Hospital (MDH) and other peripheral health posts in the area. The HDSS links demographic and clinical data and covers a population of around 92,000 inhabitants, of which approximately 11% are <3yr¹⁴. A full description of the site can be found elsewhere¹⁴. In 2011, the <5yr mortality rate was 70/1000 live births. Severe malnutrition is common with an estimated IR of 35/1000 person-years among children from 1-2yr¹⁵.

TB treatment is offered free of charge at the health units and children are routinely vaccinated at birth with Bacille Calmette-Guérin (BCG), with estimated coverage ranging from 86-90%^{16,17}. The 2013 WHO TB incidence estimates for the country is 552/100.000 population⁸. The HIV prevalence in the district is among the highest in the

1 world, reaching 39.9% in the community among individuals aged 18-47 yr and 29.4%
2 for women attending the antenatal clinic¹⁸.
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7 **Study Design and participants**

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10 A prospective study was designed to recruit participants through passive and active
11 case finding in the community, MDH and peripheral health centers during a 1yr period
12 (2011-2012). Participants included all children from the HDSS who were <3yr at the
13 time of enrolment and had either TB symptoms or were close contacts of a notified
14 adult smear positive pulmonary TB case (PTB). Relapse or recurrent cases were
15 excluded.
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28 **Clinical Procedures**

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30 Presumptive TB cases were identified through two strategies: A) Passive case detection
31 of children presenting to the health facility with ≥ 1 symptoms compatible with TB (see
32 **Table 1** for a complete list of symptoms). Those not recruited at the time of the visit to
33 the clinic were later identified through the clinical data collected at the health unit by
34 the HDSS. B) Active case finding consisted of linking the adult smear positive PTB cases
35 registered at the district National TB Program (NTP) in the previous 24 months to the
36 HDSS database in order to identify all household contacts <3yr. At enrolment,
37 demographic and clinical information was collected through interviewing of parents
38 and physical examination. Participants had a chest radiography (CXR) performed,
39 followed by HIV antibody testing and tuberculin skin testing (TST). CXR were
40 performed with a digital X-ray machine and included posteroanterior and lateral
41 projections. For clinical purposes, an initial reading was performed on site by the
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1 clinician. Subsequently, all CXR were reviewed and re-assessed by an experienced
2 pediatric radiologist (JR) who was blinded to the clinical information. TST was
3 performed with intradermal injection of 2 units (Serum Staten's Institute, Denmark)
4 and reading at 48-96h, according to the study protocol. For symptomatic cases, in the
5 same day 2 ambulatory samples were obtained in a negative pressure facility available
6 at the MDH: one gastric aspirate (GA) and one induced sputum (IS) with
7 nasopharyngeal suction, following WHO recommendations¹⁹. Asymptomatic cases with
8 abnormal CXR did not undergo sampling but were re-evaluated at further visits. For
9 suspected extrapulmonary TB (EPTB) appropriate samples were obtained.
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26 All case management was performed by the NTP according to established national
27 clinical guidelines. Those patients with clinical or microbiological diagnosis of TB were
28 started on TB treatment at the NTP with the standard 3 or 4 first-line regimens
29 according to WHO category. Other symptomatic patients were referred for specific
30 treatment and follow-up including antibiotics or nutritional supplementation if
31 indicated. Presumptive cases had a follow-up visit within the next six months
32 regardless of initial disease classification to assess resolution of symptoms without
33 anti-TB treatment and /or clinical response to alternative therapy (if any). If
34 persistently symptomatic, further evaluation and testing including CXR and samples
35 was performed to rule out TB. Contacts had a follow-up visit which included physical
36 examination and CXR, as well as GA and IS samples for those symptomatic or with an
37 abnormal CXR.
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59 **Laboratory Procedures**

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1 Samples were transported within 4 hours of collection and processed in the Biosafety
2 Level III TB laboratory at CISM. Following NaCl/NaOH digestion and concentration
3 through centrifugation, all samples were processed for acid fast bacilli smear testing
4 using LED Microscopy and Zielh Nielsen staining and inoculated into liquid culture
5 media (BACTEC MGIT 960® -automated) and solid media (Lowenstein Jensen). Positive
6 cultures were confirmed using Zielh Nielsen staining and rapid test as well as Xpert
7 MTB/RIF and identified through mycobacterial molecular identification (HAIN
8 GenoType® Mycobacterium CM/AS). First line drug sensitivity testing was performed
9 either on liquid culture or line probe assays. The laboratory is subject to an external
10 quality assurance program.
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28 **BOX: Study definitions**

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31 ■ Exposure to TB was defined as either documented (identified through active case
32 finding) or reported contact (household or regular contact during child lifetime).
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- 36 ■ Positive TST was defined as an induration >5mm for HIV or malnourished children
37 and >10mm for the rest of participants.
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41 ■ HIV infection was defined as: positive antibody test in children >18 months
42 (Determine, Abbott Laboratories and confirmed with Unigold, Trinity Biotech); or
43 positive HIV PCR in those <18 months; or a strong clinical suspicion with positive
44 antibody test in the absence of a PCR result.
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51 ■ CXR were classified as compatible if presented ≥ 1 of the following radiographic
52 abnormalities: airway compression, lymphadenopathy, opacification, nodular
53 picture, effusion, cavities, spondylitis or Ghon focus²⁰.
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- 1 ▪ Presumptive TB cases included all children <3 with compatible TB sign or
2 symptoms.
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- 4 ▪ Confirmed TB cases included those with compatible symptoms plus a positive
5 culture with *Mycobacterium Tuberculosis* . Probable TB cases were defined as
6 those with:(1) compatible symptoms unresolved at last clinical follow up visit (prior
7 to any TB treatment initiation) plus (2) compatible CXR (for children with ≥1CXR,
8 the latter was used given the likelihood of seeing resolving pneumonias) plus (3) at
9 least one of the following: TB exposure, positive TST or positive response to TB
10 treatment. EPTB cases followed the same definition except for the requirement of
11 having an abnormal CXR. The study TB case definition was adapted a standardized
12 clinical case definition of intrathoracic TB disease and included confirmed plus
13 probable cases²¹ (See Figure 2 for complete case definition).
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31 **Ethical approval**

32 The study protocol was approved by the Mozambican National Bioethics Committee
33 and the Hospital Clinic of Barcelona Ethics Review Committee.
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36 **Data Analysis and Statistical Considerations**

37 Clinical data was double entered in an electronic data capture system (OpenClinica™
38 www.openclinica.org) and checked for discrepancies. Statistical software for analysis
39 was Stata 13.0 (StataCorp. 2013. Stata: Release 13. Statistical Software. College
40 Station, TX: StataCorp LP). We calculated z-scores for weight-for-age, height-for-age
41 and weight-for-height using World Health Organization 2006 reference data²² .
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1 The minimum community-based IR was calculated as a density rate with the age-
2 specific yearly number of TB cases (according to the study case definition) among
3 study participants divided by the total age-specific population at risk during a period of
4 12 months (person-time at risk). Time at risk was individually measured using DSS data
5 taking into account demographic events (births, deaths, migrations) of all children
6 included in the study. The IR is considered to be minimum as the case detection
7 system cannot ensure that all TB cases are detected. For each IR 95% exact Poisson
8 confidence interval were calculated. Proportions were compared using the Pearson or
9 Fisher exact Chi-square test and odds ratio (OR) and 95% confidence intervals (CI) were
10 estimated using logistic regression. Variables at a significance level below 0.2 were
11 chosen and placed on stepwise backward multivariate logistic regression. Only factors
12 with a p-value on likelihood ratio tested were retained on the model.

33 Results

34 During the study period, 13.764 children <3yr contributed to a total of 9.575py in the
35 Manhiça DSS (**Figure 1**). A total of 789 presumptive TB cases were enrolled (42 and 747
36 identified through active and passive case finding respectively). Forty-five children
37 fulfilled the TB case definition -13 microbiologically confirmed plus 32 probable TB
38 (**Figure 2**). Thus, the minimum community-based IR was 470/100.000person-year
39 (95%CI 343/100.000 to 629/100.000) for confirmed plus probable cases and
40 135/100.000person year for confirmed cases (95%CI 72 to 232/100.000)(**Table 2**).

41 Baseline characteristics of presumptive TB cases are presented in **Table 1**. Fifty-four
42 percent were males, and the age distribution showed a predominance of children
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1 between the ages 12 to 24 months (51%). The most frequent clinical feature at
2 enrolment was severe malnutrition, which was the only symptom in 72% of cases.
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4 Nutritional assessment found that almost a quarter had severe undernutrition (weight
5 for age Z score <3). Of the 1347 total CXR performed during the study, 27% had only
6 one projection. Twenty-one percent of all presumptive TB cases had a CXR compatible
7 with TB. Thirty percent of presumptive cases had a second TST of which, 9% had a
8 positive TST. Among all presumptive TB cases, nine had a positive smear, although
9 none of the 9 had a positive MTBC culture (4 were NTM and 5 were culture negative).
10 Non-TB mycobacteria (NTM) were isolated in 27% of all cultures of presumptive cases.
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12 We found 7 EPTB cases - 4 lymph node and 3 disseminated- and no TB meningial
13 cases. A total of 104 children were diagnosed as HIV positive (13%).
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31 We identified 13 confirmed TB cases (7 in GA, 4 in IS and 2 both in GA and IS). The %
32 of confirmed cases among TB cases was highest for those <1y (40% vs 29 and 22%
33 among children with 1-2yr and 2-3yr respectively), and statistically significantly lower
34 for HIV-TB co-infected cases (10 vs 44%, p=0.02). Confirmed cases presented a higher
35 frequency of cough or fever as compared to probable cases. Furthermore, the
36 confirmed cases appeared to be more symptomatic at enrolment than did the
37 probable cases (53.8% vs 15.6% presenting with ≥ 1 TB symptom respectively,
38 p<0.001). Probable cases had a higher proportion of HIV-infection (p=0.01), positive
39 TST (p=0.001) or BCG scarring (p=0.08) as compared to confirmed cases.
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57 Multivariate logistic regression analysis for TB risk factors showed that HIV infection,
58 and number of previous outpatient consultations were predictors of TB disease when
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1 compared to unlikely TB cases. After adjusting for other variables, HIV infected
2 children were six times more likely to have TB disease than uninfected ones (OR 8.4,
3 95% CI 4 to 17) (**Table 3, available online**).
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10 Fifty-two patients were started on TB treatment based on clinical or microbiological
11 criteria, 67% fulfilled the study TB case definition (**Table 4**). A total of 97 children
12 initiated isoniazide preventive treatment (IPT) (71 based on exposure history, 21 on
13 TST results and 5 unspecified) and 5 were later diagnosed as TB cases while on IPT. Due
14 to drug supply shortages, isoniazide was not always available and 47 % of children with
15 criteria did not initiate IPT. The mortality rate for all presumptive cases at 12 months
16 after enrolment was 5.2% and increased with decreasing age (10.9, 5.7 and 0.8% of
17 children in the first, second and third year of life respectively, $p < 0.001$). Mortality was
18 also higher in TB cases as compared to non-TB (13% vs 5% respectively, $p = 0.02$) as it
19 was in HIV infected children as compared to HIV-uninfected (14.4% vs 3.8%, $p < 0.001$).
20 The case fatality rate was 9% (N=4/45 TB cases), all deaths taking place in the first 6
21 months after enrollment.
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43 **Discussion**

44 This study provides one of the first prospective population-based incidence estimates
45 of childhood tuberculosis in a high TB-HIV endemic setting and shows a consistently
46 high IR across all ages. These results underscore the hypothesis of a gross under-
47 detection and under-reporting of childhood TB in Mozambique and globally²³.
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1 Mozambique has almost half its population below the age of <15yr, and yet, pediatric
2 TB only accounted for 7% of all new cases notified in 2012, much lower than the
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4 expected 10-20% of the total burden of TB disease seen in high burden countries. In
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6 the Manhiça District, the notified IR for children <1yr and between 1-4 yr in 2011 was
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8 163 and 399/100.000 respectively (personal communication²⁴). This corresponds to
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10 half the TB IR reported in this study and may suggest under-detection and under-
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12 diagnosing. Furthermore, under-detection is common in the wider Mozambican
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14 context, where WHO estimates that only 37% of actual TB cases are detected⁸. While
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16 the latest WHO country incidence estimates are 552 per 100.000 population, data
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18 from Manhiça suggest that the burden of disease in Southern Mozambique might be
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20 much higher. In fact, while the national 2011 notified rate was 186/100.000
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22 population, same-year data from the Manhiça suggest the TB incidence rate of smear
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24 positive cases could be as high as 456 per 100,000 population among adults aged 18 to
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26 47 yr²⁵.

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29 Globally, several pediatric TB incidence estimates have been published recently, with
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31 results varying from less than 200 000 new cases in 2013²⁶ to 970 000 in 2010⁶. The
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33 large variation in the estimates highlights the challenges in estimating the burden of
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35 pediatric TB and the need for population-based data to inform predictive models.
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40 There are few studies reporting age-specific pediatric TB incidence in high burden
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42 countries. Most studies are based on hospital-based retrospective reviews of
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44 notification rates and, to our knowledge, none have reported community IR using DSS
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11,27-35. However, in areas where health seeking behavior strongly modifies the pattern

1 of attendance, community based studies that use active case detection rather than
2 notified TB rates are necessary to provide accurate estimates. Moreover, childhood
3 mortality and frequent migration are potential causes of disease underestimation if
4 DSS person-years are not available. Inconsistency in TB clinical definitions among
5 studies are a challenge for comparability and have been a limitation in obtaining data
6 for meta-analyses. The recently proposed definition, applied in this study, may pave
7 the road for future comparisons⁹. The IR we report is significantly higher than in other
8 high burden African countries such as Malawi (notified IR<1y of 78/100.000) or
9 Tanzania (theoretical IR<5y based on likelihood of disease progression of 134.5 to
10 308.5/100.000) and similar to data from Gabon (extrapolated IR<15y 366/100.000), or
11 neighboring South Africa (notified IR<5y 770/100.000)^{30 31 32}.

12 There are several limitations to this study, mostly leading to a possible
13 underestimation of the true TB incidence. First, only single-day samples were obtained
14 as most patients would not accept overnight admission, decreasing the chances of
15 microbiological confirmation. Second, for study purposes CXR were read by a single
16 blinded experienced pediatric radiologist rather than the two independent CXR readers
17 are often recommended to prevent bias, given the pivotal role of CXR in case
18 definition, and the poor inter and intra-observer agreement among reviewers³⁶. Third,
19 contact tracing could not be fully implemented mainly due to difficulties in patient
20 identification and poor recording.. Fourth, the % of EPTB cases was lower than the 20-
21 30% expected and reported by others^{11,30,31,37-39}. While BCG protection may have a
22 role, it is likely that some EPTB cases in this study were missed; the reason may be due
23 to a stronger focus on pulmonary TB in the study design, errors in classification

1 (disease localization, including disseminated TB, may be confounding in young children
2 in the absence of CT-scan), or lost cases due to the fact that severely ill children are
3 often transferred to the tertiary reference hospital in the capital for specific diagnostic
4 procedures. Finally, there is a risk of overestimating TB IR by either including TB
5 prevalent cases at enrollment or adding new incident cases during follow-up beyond
6 the one year enrollment period. Although this possibility can't be ruled out, we believe
7 the effect would be minimal and probably outweighed by the above-mentioned risk
8 of underestimation.
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23 In this study, HIV prevalence was high regardless of disease classification, reaching 56%
24 of probable cases. The fact that significantly fewer HIV infected children had TB
25 confirmation reflects the diagnostic difficulties in this group. Given the overlap
26 between symptoms from both TB and HIV, HIV infected cases of TB pose the greatest
27 ascertainment bias with the highest risk of over or under estimation. Even though IPT
28 is indicated in all HIV positive children, the implementation of IPT among African NTP
29 remains very poor⁴⁰, and in this study, the high proportion of missed opportunities for
30 chemoprophylaxis in HIV and or TB exposed should raise alert. There may also be
31 missed preventable child deaths in HIV-infected presumptive TB cases and possible TB
32 cases. It has been reported that many children who die of diseases such as
33 malnutrition or respiratory infections may have in fact, undiagnosed TB⁴¹. There is thus
34 a need for widespread recognition that TB control is crucial for childhood survival¹.
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1 This study highlights the huge burden of pediatric TB under detection in children under

2 3. These data add valuable information to the global effort of producing better

3 estimates of childhood TB burden, a critical step to inform public health policy.

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10 **Acknowledgement**

11 We thank the participants and their families. We are grateful to the National TB

12 Program for, health staff at the Manhiça District Hospital and peripheral health centers

13 for their contribution. We thank Dr Bassat and Dr Naniche(Barcelona Centre for

14 International Health Research) for reviewing the paper.

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Table 1. Baseline characteristics of presumptive TB cases (n=789), N (%)

Sex (male)	430 (54.5)
Age in months (Median [IQR])	19.8 (13.8-25.9)
Age group	
< 1 yr	146 (18.5)
1-2 yr	403 (51.1)
>2 yr	240 (30.4)
BCG scar (n=785)	686 (87.4)
> 1 hospitalization in previous year	206 (26.1)
≥ 10 consultations in previous year	131 (16.6)
TB contact (documented or reported)	87 (11)
Symptoms *	
Cough ≥2 weeks	156 (19.8)
Fever ≥2 weeks	50 (6.3)
Malnutrition (chronic or acute)	668 (84.7)
Wheeze or lower respiratory infection	43 (5.5)
Adenopathy	3 (0.4)
Number of presenting symptoms	
One symptom	608 (77.1)
Only malnutrition	565 (71.6)
Hospitalized at time of enrolment	101 (12.8)
Physical examination	
Stunting (n=775) †	404 (52.1)
Undernutrition (n=777) ‡	184 (23.7)
Wasting (n=775) §	92 (11.9)
Kwashiorkor (n= 780)	33 (4.2)
Febrile (N=780)	44 (5.6)
Crackles on chest examination	27 (3.4)
TST positive (n=787)	74 (9.4)
HIV positive	104 (13.2)
Radiological changes suggestive of TB (n= 766)	160 (20.9)

Footnote:

Abbreviations: IQR, interquartile range; BCG, Bacille Calmette Guerin; HIV, human immunodeficiency virus; TST, tuberculin skin test

* Compatible TB symptoms: cough for ≥ 14 days not responding to appropriate course of antibiotics; fever greater than 38°C ≥ 14 days . after common causes like malaria or pneumonia were excluded; malnutrition defined as under 60% weight for height, failure to gain weight for more than 2 months or any loss of weight and not responded to nutritional interventional; unexplained wheeze ≥ 14 days not responding to standard treatments; lower respiratory tract infection ≥ 14 days not responding to antibiotics after 72hours; TB exposure in the last 12 months; symptoms compatible with extrapulmonary TB (EPTB) such as painless enlarged lymph nodes with or without fistula formation ≥ 14 days, arthritis, gibbus, meningitis, effusion, or unexplained hematuria, dysuria or polaquiuria for ≥21 days.

† Stunting: Height for age Z score <2.

‡ Undernutrition: Weight for age Z score < 2.

§ Wasting: Weight for height <2.

Table 2: Community based incidence rate of confirmed and probable tuberculosis cases by age group**All tuberculosis cases**

	Cases	PY	Incidence Rate per 100000py	95% Confidence Interval	
Confirmed	13	9575,6	135,8	72,3	232,2
Probable	32	9575,6	334,2	228,6	471,8
Total	45	9575,6	470,0	342,8	628,8

Confirmed cases by age group

Age (years)	Cases	PY	Incidence Rate per 100000py	95% Confidence Interval	
< 1	4	3265,6	122,5	33,3	313,3
1-2	5	3199,4	156,3	68,9	407,8
2-+	4	3110,5	128,6	52,2	374,5
All Confirmed	13	9575,6	135,8	72,3	232,2

Probable cases by age group

Age (years)	Cases	PY	Incidence Rate per 100000py	95% Confidence Interval	
< 1	6	3265,6	183,7	67,4	399,9
1-2	12	3199,4	375,1	193,8	655,2
2-+	14	3110,5	450,1	246,1	755,2
All Probable	32	9575,6	334,2	228,6	471,8

All cases by age group

Age	Cases	PY	Incidence Rate per 100000py	95% Confidence Interval	
< 1	10	3265,6	306,2	146,9	563,2
1-2	17	3199,4	531,4	309,5	850,7
2-+	18	3110,5	578,7	343,0	914,6
All TB Cases	45	9575,6	470,0	342,8	628,8

Footnote:

Abbreviations: PY, person years

Table 3. TB risk factor analysis

	TB Unlikey (N=603)		TB Case (N=45)		Univariate		Adjusted OR (95% CI)	
					OR (95% CI)	p	OR (95% CI)	p
Sex								
Male	322	53,4%	20	44,4%	-			
Female	281	46,6%	25	55,6%	1.43 (0.78-2.64)	0.246	1.52 (0.79-2.92)	0.210
Age in months (Median [IQR])	19.8 (14.1 - 26.0)		19.5 (13.8 - 26.4)					
Age category, N (%)								
< 1	100	16,6%	10	22,2%	-			
1-2	318	52,7%	17	37,8%	0.53 (0.24 - 1.21)		0.51 (0.21-1.23)	0.133
2-+	185	30,7%	18	40,0%	0.95 (0.43 - 2.19)	0.153	0.94 (0.39-2.26)	0.890
BCG Scar								
Absent	74	12,3%	7	15,6%	-			
Present	527	87,7%	37	82,2%	0.74 (0.32 - 1.73)	0.488		
TB contact								
No	557	92,4%	32	71,1%	-			
Yes	46	7,6%	13	28,9%	4.92 (2.38 - 10.15)	< 0.001		
Nº consultations in previous year								
Median (IQR)	5 (3 - 8)		7 (2 - 11)		-	0.074		
< 10	505	83,7%	29	64,4%	-			
10 - +	98	16,3%	16	35,6%	2.84 (1.48 - 5.47)	0.001	2.72 (1.35-5.49)	0.005
Nº of hospitalizations in previous year								
None	446	74,0%	26	57,8%	-			
At least one	157	26,0%	19	42,2%	2.08 (1.11 - 3.87)	0.019		
Symptoms at enrollment								
Cough ≥2 weeks								
no	489	81,1%	36	80,0%	-			
yes	114	18,9%	9	20,0%	1.07 (0.50 - 2.29)	0.857		
Fever ≥2 weeks								
no	567	94,0%	40	88,9%	-			
yes	36	6,0%	5	11,1%	1.97 (0.73 - 5.30)	0.172		
Chronic or Acute Malnutrition malnutrition								
no	82	13,6%	12	26,7%	-			
yes	521	86,4%	33	73,3%	0.43 (0.21 - 0.88)	0.016	0.42 (0.19-0.89)	0.024
Physical Exam								
Stunting †								
no	281	46,6%	16	35,6%	-			
yes	312	51,7%	28	62,2%	1.58 (0.83 - 2.98)	0.158		
Undernutrition ‡								
no	460	76,3%	30	66,7%	-			
yes	134	22,2%	14	31,1%	1.60 (0.82 - 3.11)	0.161		
Wasting ¥								
no	531	88,1%	37	82,2%	-			
yes	62	10,3%	7	15,6%	1.62 (0.69 - 3.80)	0.262		

Crackles on chest examination								
no	584	97,0%	39	90,7%	-			
yes	18	3,0%	4	9,3%	3.32 (1.07 - 10.36)	0.052*		
Tuberculin skin test								
Postive	0	0,0%	23	51,1%	-		< 0.001 μ	
Negative	603	100,0%	21	46,7%	-			
HIV Reported								
Not positive	549	91,0%	25	55,6%	-			
Postive	54	9,0%	20	44,4%	8.13 (4.12 - 16.04)	< 0.001	8.42(4.26-16.62)	< 0.001

Footnote:

Abbreviations: IQR, interquartile range; BCG, Bacille Calmette Guerin; HIV, human immunodeficiency virus; TST, tuberculin skin test

† Stunting: Height for age Z score <2.

‡ Undernutrition: Weight for age Z score < 2.

¥ Wasting: Weight for height <2

μ This variable was not included in multivariate analysis as a positive TST is part of the TB definition

* Exact Chi-squared test

Table 4. Outcome by group classification, N (%)

	Presumptive (N=789)	Definite (N=13)	Probable (N=32)	Possible (N=96)	Unlikely (N=603)	MTB infection (N=45)
Sex (male)	430 (54.5%)	5 (38.5%)	15 (46.9%)	57 (59.4)	322 (53.4%)	31 (68.9%)
HIV positive	104(13.2%)	2 (15.4%)	18 (56.3%)	24 (25%)	54 (9%)	6 (13.3%)
IPT	64 (8.1%)	1 (7.7%)	4 (12.5%)	4 (4.2%)	24 (4%)	31 (68.9%)
TB treatment	52 (6.6%)	9 (69.2%)	26 (81.3%)	14 (14.6%)	1 (0.2%)	2 (4.4%)
Median time to diagnosis (days) N (IQR)	115 (35-224)	41 (35-115)	184 (55-224)	60 (35-235)	14 (14 - 14)	148 (89 - 207)
Mortality at 12 months	41 (5.2)	3 (23.1%)	1 (3.1%)	13 (13.5%)	23 (3.8%)	1 (2.2%)

Footnote:

Abbreviations: HIV, human immunodeficiency virus; IPT, isoniazide preventive treatment; IQR, interquartile range; TST, tuberculin skin test

Figure 1. Study Profile

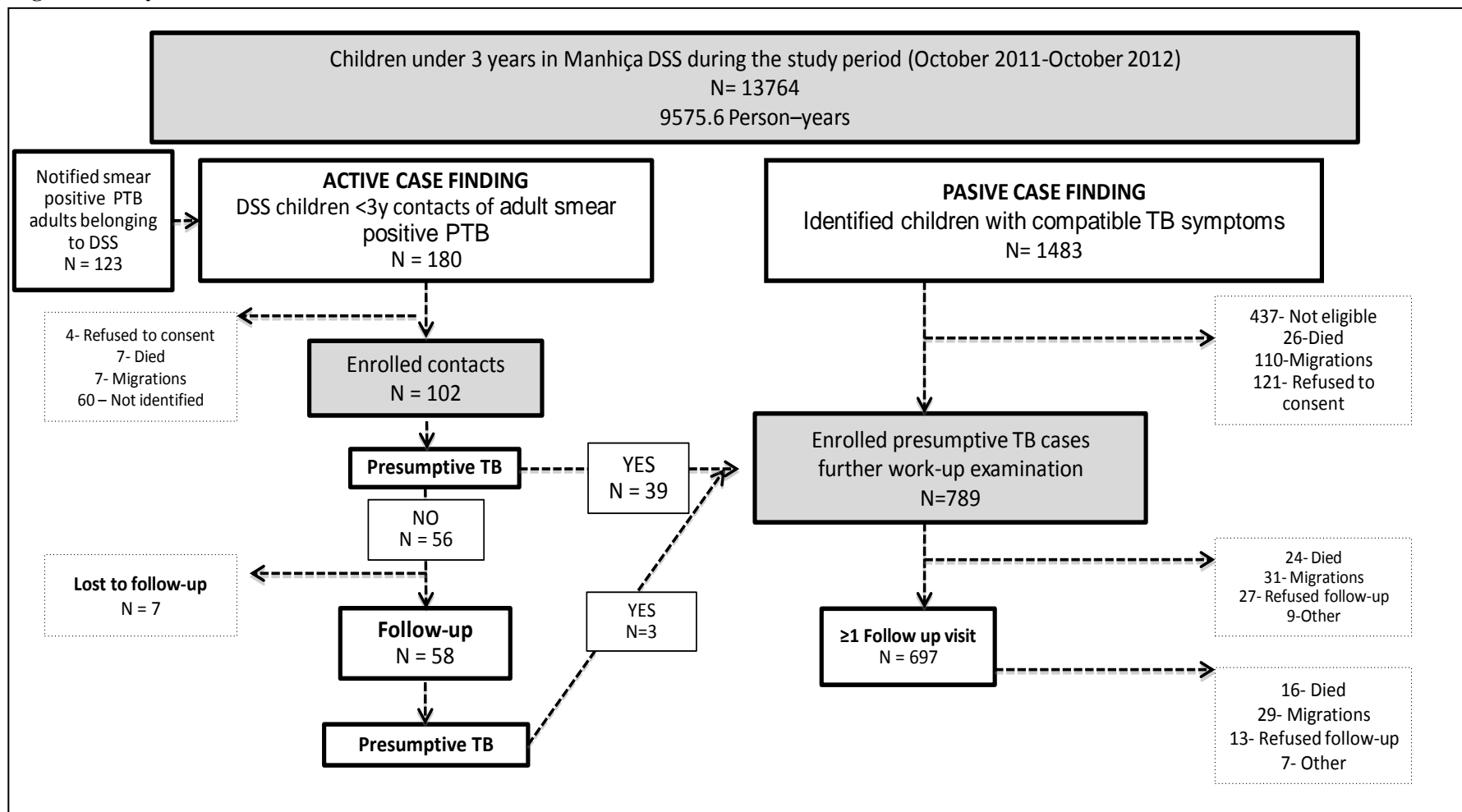


Figure 2. Algorithm for study case classification adapted from Graham et al (JID, 2012).

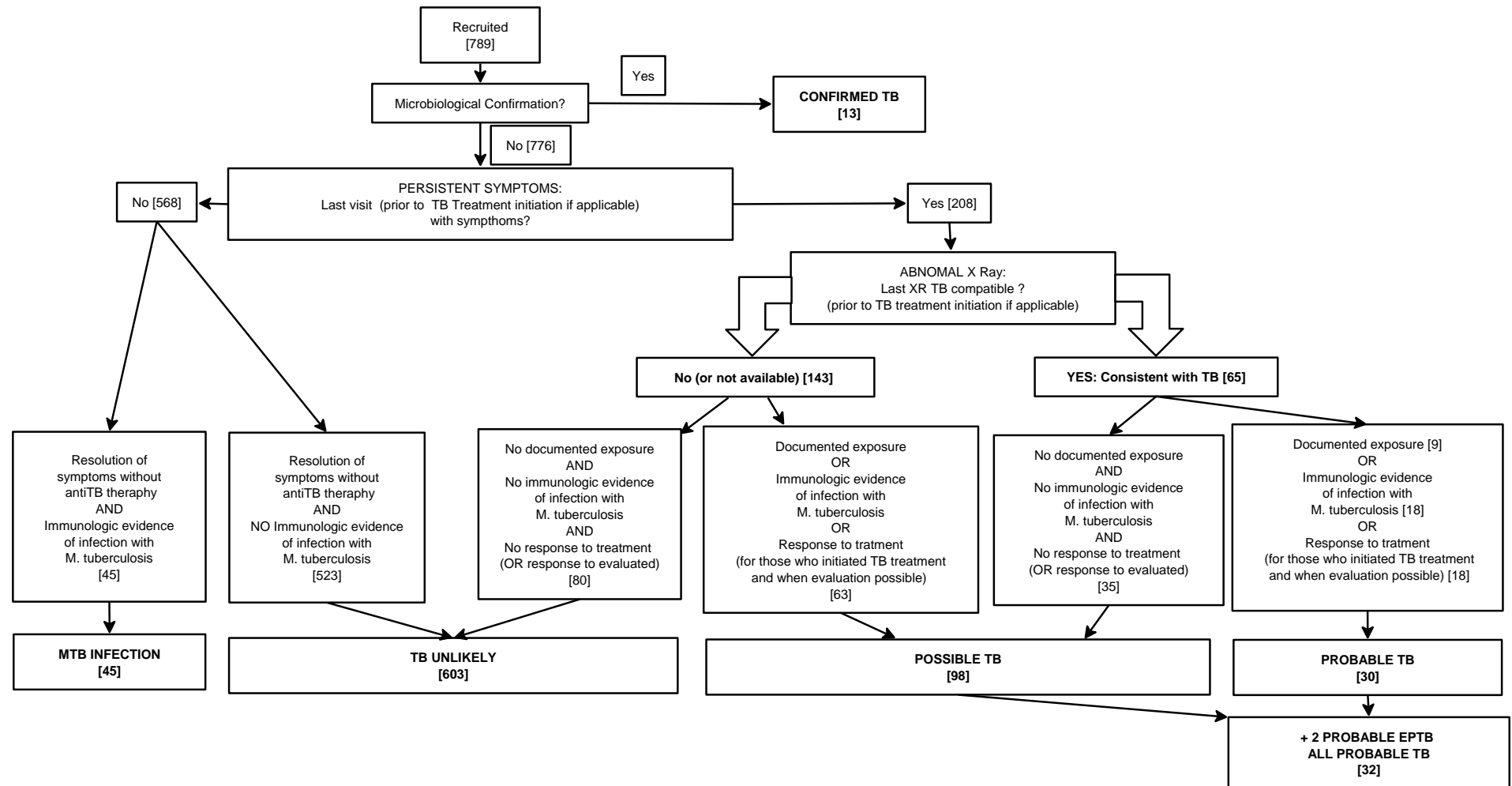


Figure 1. Study Profile

Flowchart showing the number of children under three in the study area and those enrolled in the study. A total of 1483 children were identified with at least one compatible TB symptom and 747 presumptive TB cases were enrolled in the study for further work-up. Among the 329 adult smear positive cases registered at the NTP between October 2010-October 2012, we identified 123 belonging to the study area and 180 contacts <3y, of whom 102 accepted to participate in the study yielding an additional 42 presumptive TB cases. Among the remaining 60 contacts, 7 were lost to follow-up and the rest had at least one follow up visit. Eighty-eight percent presumptive TB cases enrolled had at least one follow-up visit and 632/697 completed follow-up (had follow-up visits until alternative diagnosis was made or became asymptomatic). Abbreviations: DSS, Demographic Surveillance System; PTB, pulmonary tuberculosis.

Figure 2. Algorithm for study case classification adapted from Graham et al (JID, 2012)

Definitions. Confirmed TB: Compatible symptoms plus a positive culture with MTBC
Persistent symptoms: Compatible symptoms unresolved at last clinical follow up visit (prior to any TB treatment initiation). Consistent CXR: CXR read by blinded experienced pediatric radiologist with one or more radiographic abnormalities (airway compression, lymphadenopathy, opacification, nodular picture, effusion, cavities, spondylitis or Ghon focus). For children with more than one CXR, the latter was used given the likelihood of seeing resolving pneumonias. Positive response to TB treatment: determined by the clinician at first possible follow-up visit and defined as total/partial resolution of clinical features suggestive of TB present at baseline with no new clinical features suggestive of TB for those patients adherent to TB treatment. Resolution of symptoms without anti-TB treatment was evaluated by the clinical at first follow-up visit as total/partial resolution of

clinical symptoms suggestive of TB present at baseline and no new clinical features suggestive of TB in the absence of TB treatment.

Description: Of the initial 789 presumptive TB cases, 568 had symptoms resolved at follow-up visits, leaving 208 presumptive cases with persistent TB symptoms, of which 65 had an abnormal CXR at last visit. Probable cases include 30 children who fulfilled the definition plus an additional 2 extrapulmonary-cases. Contacts not enrolled as presumptive TB cases are not included in the algorithm.