



**Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau**

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Complete List of Authors:	Gomes, Victor; Bandim Health Project, ; Serum Statens Institute, Epidemiology Andersen, Andreas; Serum Satatens Institut, Epidemiology Lemvik, Grethe; Bandim Health Project, Wejse, Christian; Aarhus University Hospital, Department of Infectious Diseases Oliveira, Ines; Bandim Health Project, Vieira, Fina; Hospital de Pneumologia "Raoul Follereau, Joaquim, Luis; Hospital Nacional "Simao Mendes", Vieira, Cesaltina; Hospital de Pneumologia "Raoul Follereau, Aaby, Peter; Serum Satatens Institut, Epidemiology; Bandim Health Project, Gustafson, Per; Infectious Diseases Research Group, Lund University, Department of Clinical Sciences
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3 **Impact of isoniazid preventive therapy on mortality among children less than 5**  
4 **years old following exposure to tuberculosis at home in Guinea-Bissau**  
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8 Victor F. Gomes<sup>1</sup> MD, MSc, Andreas Andersen<sup>1</sup>, statistician, Grethe Lemvik<sup>1,2</sup> MD,  
9 Christian Wejse<sup>1,2</sup> MD, PhD, Ines Oliveira<sup>1</sup> MD, MSc, Fina J. Vieira<sup>3</sup> MD, Luis J. Carlos  
10 <sup>4</sup> MD, Cesaltina S. Vieira<sup>3</sup> MD, Peter Aaby<sup>1</sup> DSc, Per Gustafson<sup>5</sup> MD, PhD  
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16 1) Bandim Health Project, Indepth Network, Bissau, Guinea-Bissau,  
17 <http://www.bandim.org>, Statens Serum Institut, Copenhagen, Denmark  
18  
19 2) Department of Infectious Diseases, Aarhus University Hospital, Denmark  
20  
21 3) Hospital de Pneumologia “Raoul Follereau”, Bissau, Guinea-Bissau  
22  
23 4) Hospital Nacional Simao Mendes, Bissau, Guinea-Bissau  
24  
25 5) Infectious Diseases Research Group, Department of Clinical Sciences, Malmö,  
26 Lund University, Sweden  
27  
28  
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39  
40 Correspondence to:

41  
42 Victor Francisco Gomes

43 PROJECTO DE SAÚDE DE BANDIM

44 APARTADO 861, 1004 BISSAU CODEX, GUINÉ-BISSAU

45  
46 FAX No: +245 320 16 72

47  
48 Phone No: +245 320 14 89/3204460; Mobile: +245 6658334

49  
50 Email: [victorfranciscogomes@yahoo.co.uk](mailto:victorfranciscogomes@yahoo.co.uk)  
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## ABSTRACT

**Background and objective:** In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

**Setting:** This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

**Participants:** All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT programme.

**Main outcome measures (end points):** The all cause mortality rate ratio (MRR) between exposed children and unexposed community control children.

**Results:** A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT programme. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1). The mortality in IPT children compared with community controls in the 2005-2008 period differed significantly from the mortality of exposed but untreated children in the 1996-1998 cohort (test of interaction,  $p=0.01$ ).

**Conclusion:** The mortality among children who received IPT was significantly lower than the previously observed in the 1996-1998 period. The excess mortality was

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3 completely removed in the cohort of children receiving IPT in the 2005-2008 period.

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5 Children exposed who received IPT had a lower mortality than children exposed who did  
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8 not receive IPT.  
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### 10 11 12 **Article summary**

13 This article focuses on:

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17 • impact of IPT on mortality among children exposed to an adult with intrathoracic  
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19 TB at home and
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22 • Mortality in children exposed to TB who were enrolled on IPT compared to those  
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24 exposed but not receiving IPT in a previous study in the same setting.  
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### 28 29 **Strengths and limitations**

30  
31 Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills  
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33 were taken. Given the low mortality in the cohort it was not possible to test to what extent  
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35 adherence mattered for a beneficial effect of IPT.  
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39 Mortality in the study area declined dramatically between the two study periods, and the  
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41 study therefore had much less power than originally expected. Nonetheless, results were  
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43 so marked that it was still possible to show the hypothesized inversion of the mortality  
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45 rate ratios between TB-exposed children and community controls between the pre-IPT  
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47 period and the IPT period.  
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53 In an intervention study in an area with a very mobile population as in Bissau, it is not  
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55 possible to enrol all eligible children. There are always some children travelling or absent  
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3 at inclusion visits. This obviously opens for the possibility of selection biases as to who  
4 participated in the study.  
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8 Finally, due to the current WHO recommendation it was decided not to conduct a  
9 randomised study, hence there are a number of theoretical biases.  
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## Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the tuberculosis epidemic<sup>1:2</sup>. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe tuberculosis-related morbidity and mortality<sup>2</sup>. Of the estimated 8.3 million new tuberculosis cases diagnosed in 2000, almost 900,000 were children<sup>3</sup>, and the proportion of children in the high-burden countries is estimated to be higher<sup>4:5</sup>.

Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home<sup>6</sup> and reliable medical information on causes of death is therefore lacking<sup>7</sup>. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries<sup>7:8</sup>. Necropsy studies conducted in Africa have shown that tuberculosis rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas<sup>9</sup>.

An intervention known to contribute to the reduction of morbidity and mortality due to tuberculosis is isoniazid preventive therapy (IPT). Isoniazid (INH) was recommended for tuberculosis chemoprophylaxis during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70.000 people of all ages<sup>10</sup>. After all this time, INH continues to be the drug of choice<sup>11</sup>, and WHO recommends that all TB contacts under the age of 5 should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane

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3 review concluded that there was not enough evidence for general recommendation of IPT  
4 for HIV infected children<sup>12</sup>. The use of INH in low income countries is, however, limited  
5 by several circumstances: difficulties of ruling out active tuberculosis in children, mainly  
6 those infected with HIV, before initiation of prophylaxis<sup>13</sup>, liver toxicity<sup>14-16</sup>, and poor  
7 adherence<sup>17</sup>. These circumstances may limit the widespread use of IPT in the resource-  
8 constrained settings, where provision of TB care often falls short of internationally  
9 recommended standards<sup>13</sup>. Isoniazid chemoprophylaxis has been shown to be effective in  
10 recent skin test converters and recent contacts of identified cases of active  
11 tuberculosis<sup>17;18</sup>. In one study of tuberculosis preventive therapy with isoniazid in HIV-  
12 positive subjects, a 20% reduction of mortality was found in those with positive  
13 tuberculin skin test (TST)<sup>18</sup>. The effect of such prophylaxis in children, however, is not  
14 well established.

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34 The present study aimed to assess the impact of IPT on mortality in children less than 5  
35 years of age exposed to intrathoracic TB at home in an urban area of Bissau. We  
36 compared the mortality of children on IPT with the mortality of community control  
37 children who had not been exposed to TB at home. In a previous study in the same  
38 community, we found that exposure to TB at home was associated with a 66% excess  
39 mortality among children under five years of age<sup>19</sup>. Hence, the purpose of the present  
40 study was to assess whether this differential excess mortality could be removed.  
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## Materials and methods

### *Setting*

The study was conducted as a prospective cohort study from September 2005 to October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years<sup>20</sup>.

Due to difficulties in obtaining specific causes of deaths in our setting the all cause mortality was used as the main outcome measurement of the effect of IPT.

The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

### *Houses and household contacts*

- Houses in the study area are one-storey, rectangular constructions, usually with six to eight rooms and are inhabited by 2 to 4 households (families). The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the



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3 same space in the house, eating from the same pot and recognizing one person as  
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5 the head of the household.  
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## 10 **Recruitment of participants and patients**

### 11 *Identification of adult TB index cases*

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13 Since 1996 a TB surveillance system, implemented in collaboration with the national TB  
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15 hospital (“Hospital Raoul Follereau”), has identified adult ( $\geq 15$  years) intrathoracic TB  
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17 cases using passive and active case finding<sup>20</sup>. The procedures for identification and  
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19 diagnosis of the cases within this TB surveillance system have previously been  
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21 described<sup>20</sup>.  
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### 29 *Enrolment in the IPT cohort*

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31 Children less than 5 years of age living in the house when the adult TB case started  
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33 treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the  
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35 later of the following two dates: 3 month before treatment or date of registration. The  
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37 children were followed until 5 years of age contributing follow-up time until the date of  
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39 the last follow-up information. Children lost to follow-up were censored.  
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44 Prior to the initiation of IPT, the children were investigated for active TB in a clinical  
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46 examination for signs and symptoms using the Keith Edwards score<sup>21</sup>. If the investigation  
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48 suggested active TB the children were submitted to a careful and thorough assessment of  
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50 all evidence from history, clinical examination and relevant investigations, e.g. laboratory  
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52 examination, including HIV testing, and chest x-ray. Broad spectrum antibiotics were  
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54 administered for 10-15 days. Children who failed to improve clinically and radiologically  
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3 after 2 weeks of broad spectrum antibiotics, and without other explanatory disease were  
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5 given a full TB treatment regimen according to the national protocol. Children who  
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7 developed signs and symptoms suggestive of active TB while on IPT were evaluated and  
8  
9 treated in a similar way.  
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12 Children with active TB, those who did not give consent, who were absent from first,  
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14 second or third visit or at enrolment consultation were excluded from the IPT cohort.  
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17 There were several steps in the enrolment procedure as depicted in Figure 1. Once an  
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19 adult TB case from the study area was identified a project assistant went to the patient's  
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21 house to update the census for the families living in the house, and socio-economic and  
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23 demographic information was noted on the questionnaire. Following the census-update, a  
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25 field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house  
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27 was visited by the nurse who read the TST and referred potential TB cases for further  
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29 clinical examination. Children less than 5 years of age without active TB were eligible  
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31 for enrolment in the IPT cohort and were invited to attend the enrolment visit at the local  
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33 health centre. Eligible children who did not show up at inclusion were traced again. If not  
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35 found they were considered absent, but still followed up using basic census information.  
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37 Due to limited time frame, logistic reasons and limited funding they were not included  
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39 later.  
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43 For children enrolled in the IPT programme, INH tablets were administered at 5  
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45 mg/kg/day together with Pyridoxine (Vitamin B6) tablets. The Vitamin B6 dosage was  
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47 25 mg for children receiving <100 mg of INH and 50 mg for children receiving >100 mg  
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49 of INH<sup>22</sup>. The medicine was provided at the house every two weeks by a field assistant.  
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51 Study children were visited after 1, 4, 7 and 9 months of INH treatment. The follow-up  
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3 visits at 1 and 7 months were performed by the research clinician at the local health  
4 centre and at 4 and 9 months by a field assistant at the child's home. Evaluation at  
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6 follow-up visits included questions about side effects and a physical assessment of signs  
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8 and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The cohort and study  
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10 routines are described in detail elsewhere<sup>23</sup>. The initially intended IPT enrolment period  
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12 was September 2005 to October 2007 with 9 months of follow-up to June 2008.  
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14 However, children continued to be enrolled on IPT until the end of the study period in  
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16 June 2008.  
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#### 24 *Pre-IPT cohort*

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26 As previously described; children less than 5 years of age living in the same house as an  
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28 adult index TB case at the time of initiation of treatment during the period May 1996 to  
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30 July 1998 were retrieved from the BHP register<sup>19</sup>. To assess the impact of TB exposure at  
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32 home in the absence of IPT, their mortality was compared with the mortality of children  
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34 living in the study area who had not been exposed to TB at home, during the same period.  
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#### 41 *Effect-size calculation*

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43 For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses  
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45 during a 4½ year period. An average of 3 children < 5 years of age per house and a mean  
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47 follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children  
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49 with approximately 4725 child years of observation. We anticipated the TB surveillance  
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51 system to identify an increased number of TB patients during the IPT period. An  
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53 estimated 300 index cases per year during a 2½ year period would give 750 index cases.  
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3 An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25  
4 years (half of the 2½ year study period) would yield 2250 children with approximately  
5 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort  
6 we initially expected to be able to detect a 27% mortality reduction in the IPT cohort.  
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12 Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau  
13 we limited follow-up for the pre-IPT cohort to the period from February 1996 to June  
14 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT  
15 cohort lowering the number of identified children. In addition, <5-mortality dropped  
16 considerably more than we had anticipated.  
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#### 24 25 26 27 *Ethical approval*

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29 The parents or caregivers were informed about the study in writing (Portuguese) and  
30 verbally in the common language, Creol, before the child was enrolled in the IPT study.  
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32 Informed consent was obtained from all the parents or caregivers before enrolment. The  
33 study protocol was approved by the Guinea-Bissau National Research Coordination and  
34 Ethics Committee.  
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#### 43 44 *Statistical analysis*

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46 Data regarding adult TB cases were obtained from the general TB identification system  
47 in the study area while demographic information was taken from the basic surveillance  
48 system of the BHP. Statistical analyses were conducted in STATA version 10.  
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3 Similar to the analysis of the impact of TB exposure in the absence of IPT, the average  
4 delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months.  
5 Hence, children registered in the same house as an adult index TB case 0-3 months before  
6 treatment were considered exposed. The effect of exposure on mortality was evaluated by  
7 rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in  
8 Guinea-Bissau is very high. To be exposed a child had to be born and registered at the  
9 time of exposure. Consequently only few children were exposed before 3 months of age.  
10 Therefore we have chosen to commence the analyses at 3 months of age.  
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24 IPT treatment was initiated in September 2005. To allow exposure time before IPT,  
25 follow-up started in July 2005. IPT enrolment for the present study ended in October  
26 2007 with treatment ending in June 2008. Thus, the study period for the present study is  
27 July 2005 to June 2008. Exposed children counted as unexposed controls until the time of  
28 exposure. Exposed children never receiving IPT counted as exposed without IPT from  
29 the start of exposure to the end of follow-up. Children subsequently enrolled on IPT  
30 counted as exposed without IPT until the start of IPT and then as exposed with IPT to the  
31 end of follow-up. However, enrolment into the IPT program continued after the  
32 enrolment period of the present study. Children enrolled on IPT after October 2007 were  
33 also counted as exposed on IPT even though they did not finish the treatment within the  
34 study period. Censoring the children enrolled on IPT after October 2007 at the time of  
35 IPT had little impact on the results. Some children who were present when the TB team  
36 visited the TB case house (figure 1) were enrolled on IPT even though they were not born  
37 or registered in the house before the TB case initiated treatment. According to the  
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3 epidemiological definitions these children were not exposed and they have counted as  
4 unexposed with IPT. A separate analysis was conducted excluding these subjects.  
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8 An adjusted analysis was conducted including possible confounders related to child  
9 mortality: gender, ethnicity, district, socio-economic status, schooling of the mother,  
10 child crowding (<5 years) and crowding among older individuals (>5 years). A score for  
11 socio-economic status was calculated adding house indicators (yes=1; no/missing=0):  
12 corrugated iron roof, electricity, television and in-door toilet. A separate “Missing”  
13 category was constructed when information was missing on all four variables. Crowding  
14 was defined as the number of individuals in the house of the TB case on January 1, 2007,  
15 the mid-point of the examined period. Crowding was included in the analysis as a linear  
16 predictor.  
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32 It was further examined whether differential mortality not related to TB exposure may  
33 have existed in the exposed houses. Mortality was compared between children living in  
34 the house of the adult TB case three years before TB exposure began and children living  
35 in the remaining houses. As for the main study<sup>19</sup>, the comparison was made over a 3-year  
36 period from July 2002 to June 2005. The period was chosen not to overlap the study  
37 period.  
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**Results:***Index TB cases and included children*

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from “case houses with no inclusion” and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Follow-up ended before 3 months of age for 14 of these children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

*TB among exposed children*

One child was on TB treatment at the time of inclusion. Active TB was diagnosed in 2 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical

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3 and chest X-ray findings. One of these children tested HIV-positive and was enrolled in  
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5 an antiretroviral (ARV) programme.  
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### 10 *TB exposure and mortality*

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12 Two children died during IPT. For a 6-month old boy, hospital records stated the cause of  
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14 death as severe malaria and anaemia. The mother of a 2-year old girl, who died two  
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16 months after the initiation of the IPT program, reported that the child had had diarrhoea,  
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18 cough and fever prior to death. Antibiotics and other medications had been prescribed.  
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20 The research clinician had requested a chest X-ray, but the result was never received. No  
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22 further IPT children died during the follow-up period.  
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30 In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of  
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32 observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT  
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34 contributed with 1023 PYO and the controls contributed with 30713 PYO.  
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37 Though not statistically significant, the exposed children receiving IPT had a lower  
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39 mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-  
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41 1.2) (Table 1). This estimate changed little when controlled for background factors  
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43 (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and  
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45 some exposed children did not receive IPT (Figure 1). In this group of TB-exposed  
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47 children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7)  
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49 (Table 1).  
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3 There were 68 children on IPT who were not formally exposed to TB because the child  
4 was born or registered after exposure occurred in the house. Excluding these from the  
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8 IPT group we observed one death from 642 person years of observation giving a MRR of  
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10 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of  
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12 whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).  
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#### 15 16 17 *Comparison of mortality among TB-exposed children in the absence and presence of IPT*

18 We previously found that the excess mortality after exposure to TB only started 6 months  
19 after exposure<sup>19</sup>. We made a similar analysis for the period 2005-2008, table 3 shows the  
20 comparison of exposed children without IPT and unexposed children, stratified by time  
21 since exposure and age at exposure.  
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28 Restricted to the period after 6 months, the mortality rate relative to community controls  
29 in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children  
30 without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-  
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32 1.1).  
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38 It was furthermore examined whether the effect in the exposed house was caused by  
39 lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in  
40 the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among  
41 children in the houses which later had TB cases was the same as the mortality in the  
42 control houses, the MRR being 1.04 (0.7-1.5) (Table 4).  
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53 The purpose of the present study was to assess whether the excess mortality associated  
54 with TB exposure at home could be removed by implementing an IPT programme. In a  
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3 previous study in the same community, exposure to TB was associated with an MRR of  
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5 1.66 (1.2-2.3) among children under five years of age<sup>19</sup>. In the present study both the  
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7 MRR of 0.30 among children who received IPT and the overall MRR of 0.71 for all  
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9 exposed children in the 2005-2008 period were significantly lower than the previously  
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11 observed MRR of 1.66 (respective tests of interaction,  $p=0.01$  and  $p=0.004$ ).  
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15 It should be noted that the general child mortality declined markedly between the two  
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17 periods studied (Table 5); among community controls mortality declined by more than  
18  
19 50%. Given that excess mortality associated with TB exposure was 66% in the period  
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21 from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total  
22  
23 48/1000. If the impact of TB exposure had been similar during the period from 2005-  
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25 2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as  
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27 the unexposed children in the community, with an additional 19/1000 due to TB  
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29 exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).  
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## 36 Discussion

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38 In the present study we have shown a considerable impact of the IPT programme on  
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40 mortality among children less than 5 years of age exposed to adult TB. Children exposed  
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42 to TB in 1996-1998, when IPT was not available in the area, suffered a 66% excess  
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44 mortality compared with unexposed children. This excess mortality was completely  
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46 removed in the cohort of children receiving IPT from 2005-2008. It should be  
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48 emphasized that comparing data from different time-periods is not straight-forward, as  
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50 the conditions have changed in many ways that cannot be completely deduced, and the  
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52 results can be biased. In our situation, the child mortality has dropped markedly between  
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3 these two time-periods. However, the excess mortality from the 1996-1998 cohort has  
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5 changed to a trend of lower mortality in 2005-2008, which cannot solely be tribute to the  
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7 drop in child mortality, and our data suggest that this is partly due to the introduction of  
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9 IPT.  
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### 12 13 14 15 **Unexpected observations**

16  
17 Mortality in the study area declined dramatically between the two study periods and  
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19 mortality declined more than expected among both exposed children receiving IPT and  
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21 exposed children not receiving IPT. Based on the experience from the 1996-1998 period,  
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23 TB exposure at home in the absence of IPT should have been associated with a 19/1000  
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25 person-years excess mortality.  
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29 Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa.  
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31 However, despite the recent HIV epidemic, mortality rates have decreased drastically  
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33 over the last decade following the same pattern observed in the other sub-Saharan  
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35 African countries<sup>24-26</sup>. The reasons for the mortality decline are not fully understood but  
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37 systematic annual vitamin A campaigns and the marked decrease in malaria incidence are  
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39 likely to have contributed.  
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43 The strong reduction in mortality among IPT-treated children could possibly be linked to  
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45 increased attention to these children including easier access to other forms of treatment  
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47 and more attention from the parents. However, this would be unlikely to explain why  
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49 mortality also declined more than expected among the children who did not receive IPT.  
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51 This may suggest that all TB-associated mortality is not directly due to clinical TB  
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53 disease, but may be due to interactions with other infections. If the incidence or severity  
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3 of these other infections goes down, as has happened in Bissau, the mortality associated  
4 with TB exposure would also decline.  
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### 10 **Interpretation and consistency with previous studies**

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12 Children exposed to TB at home had excess mortality from around 6 months after  
13 exposure in the present study as well as in the previous study from 1996-1998. In both  
14 studies, we showed that the TB houses 3 years earlier had exactly the same mortality as  
15 community controls. It therefore seems unlikely that general social conditions in houses  
16 with TB cases explain the higher mortality of TB-exposed children. Furthermore,  
17 children enrolled on IPT, compared with community controls, had significantly lower  
18 mortality ratio than the TB exposed children not receiving IPT, compared with  
19 community controls, in both study periods. Hence, our results suggest that use of  
20 isoniazid plays an important role in decreasing mortality in children exposed to  
21 tuberculosis.  
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36 Studies conducted in South Africa and Zambia have reported isoniazid to be highly  
37 effective in reducing the mortality and incidence of tuberculosis in HIV-infected children  
38 and adults living in an area with a high prevalence of tuberculosis<sup>14;27;28</sup>. Our findings  
39 support those studies, but also observations made by Dr. Lincoln in the early 1950s  
40 showing that isoniazid chemotherapy reduced the case fatality from primary tuberculosis  
41 among children<sup>29</sup>.  
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### 53 **Implications and conclusions**

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3 In the period 1996-1998 an excess mortality of 66% was found in children in TB  
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5 households compared to controls. This excess mortality was completely removed in the  
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7 cohort of children receiving IPT from 2005-2008, and the all-cause mortality in children  
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9 from TB households was lower than the controls, though not reaching statistical  
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11 significance. When comparing the mortality between the exposed children to the controls  
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13 across the two different time periods, a significant difference in mortality was found.  
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15 More, in the 2005-2008 cohort, there was a trend that exposed children receiving IPT had  
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17 lower all-cause mortality than exposed children not receiving IPT.  
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22 Our study clearly shows that children less than 5 years of age exposed to TB at home  
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24 have a high mortality that can be prevented with IPT. All our data indicate that IPT  
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26 should be part of the standard TB program and would have a large impact on child  
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28 mortality in low-income countries.  
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#### 43 **Author's contributions**

44 PG, PA and VG designed the study. VG supervised and run data collection, AA run  
45  
46 statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data  
47  
48 handling, CW and PG carried out adult TB study, GL contributed in writing the article.  
49  
50 VG drafted the article and all authors contributed to the final version.  
51  
52

53 **Conflict of interest:** There are no competing or conflicting interests.  
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**Table 1: The effect of exposure on mortality according to age**

Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR <sup>#1</sup> IPT/Unexposed	MRR <sup>#2</sup> IPT/Unexposed	MRR <sup>#1</sup> No IPT/Unexposed	MRR <sup>#2</sup> No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

\* Include unexposed children on IPT

<sup>#1</sup> Mortality Rate Ratio from a model with age as underlying time

<sup>#2</sup> Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

**Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008**

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

**Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.**

Months since exposure	Age at exposure (Months)			Total
	0-11	12-35	36-59	
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

**Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005**

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

**Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home**

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	2005-2008
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)



Figure 1: Illustration of the inclusion process

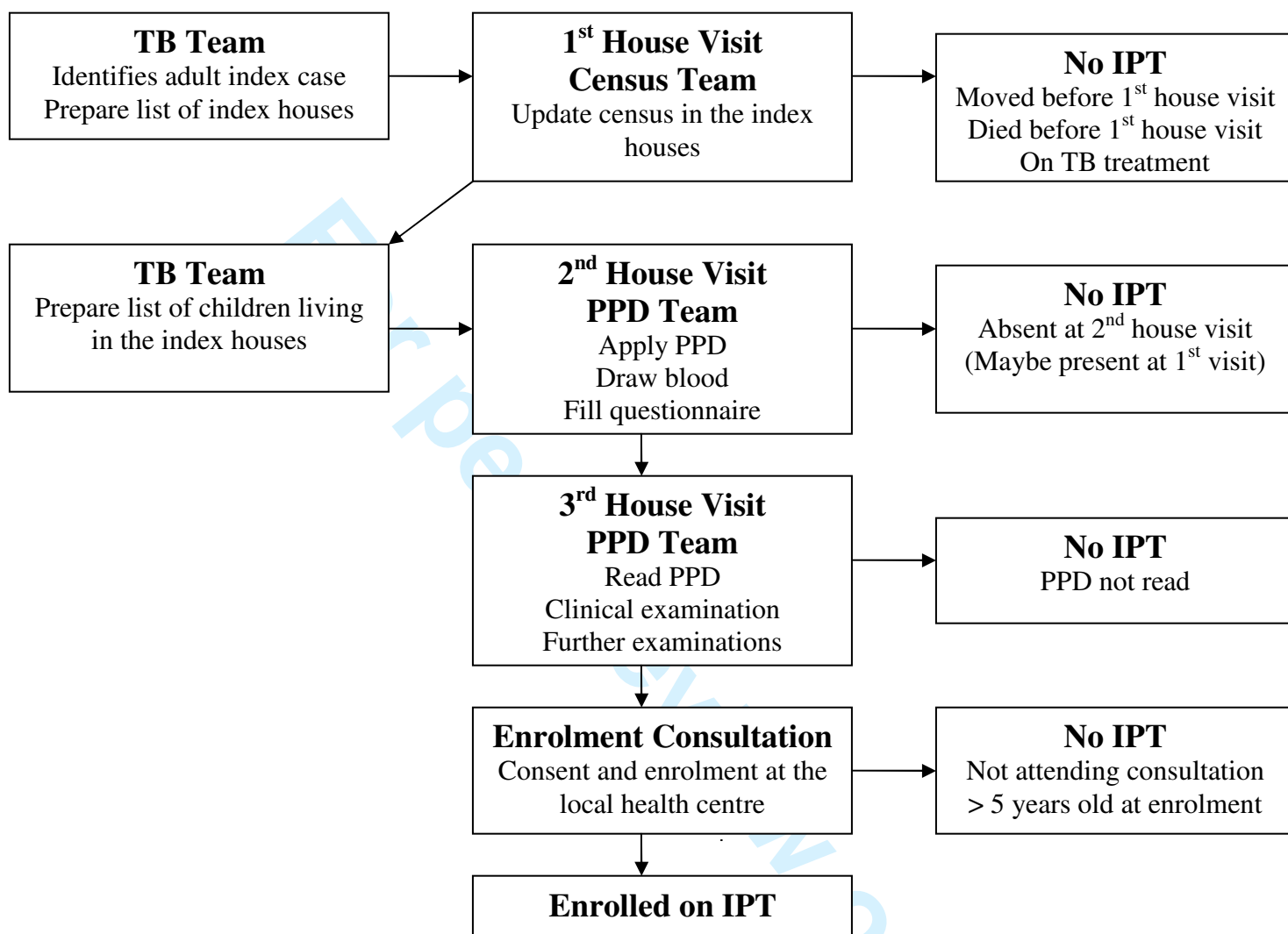
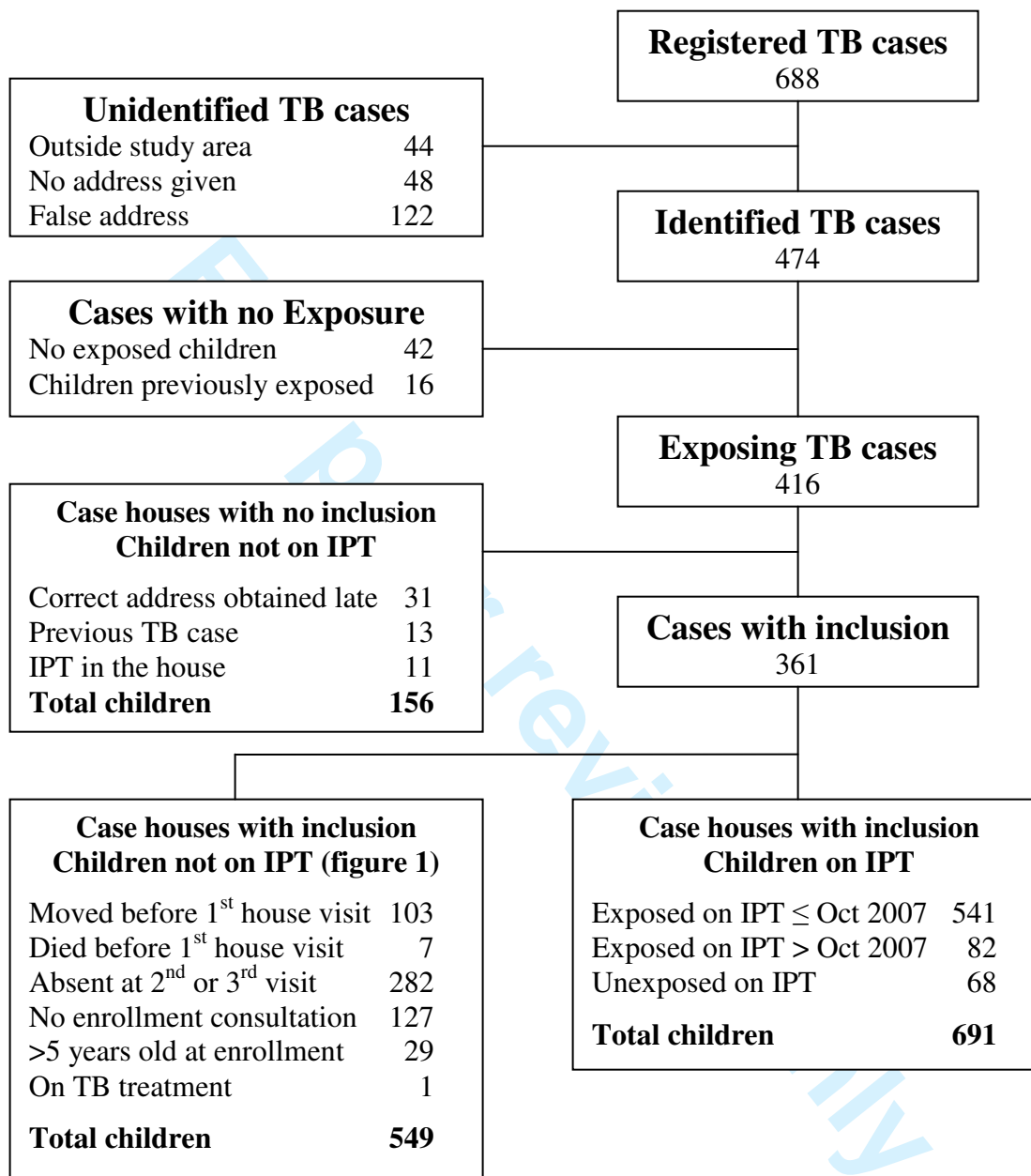


Figure 2: Flow chart of inclusion



**Figure 3: Person years of observation (PYO)**

<b>Children not on IPT</b>		<b>Children on IPT</b>	
<b>Exposed</b>		<b>Exposed</b>	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months of age at death/censoring		<b>Unexposed</b>	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64

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## Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancanha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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**Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau**

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3 **Impact of isoniazid preventive therapy on mortality among children less than 5**  
4 **years old following exposure to tuberculosis at home in Guinea-Bissau**  
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8 Victor F. Gomes<sup>1</sup> MD, MSc, Andreas Andersen<sup>1</sup>, statistician, Grethe Lemvik<sup>1,2</sup> MD,  
9 Christian Wejse<sup>1,2</sup> MD, PhD, Ines Oliveira<sup>1</sup> MD, MSc, Fina J. Vieira<sup>3</sup> MD, Luis J. Carlos  
10 <sup>4</sup> MD, Cesaltina S. Vieira<sup>3</sup> MD, Peter Aaby<sup>1</sup> DSc, Per Gustafson<sup>5</sup> MD, PhD  
11  
12  
13

- 14  
15  
16 1) Bandim Health Project, Indepth Network, Bissau, Guinea-Bissau,  
17 <http://www.bandim.org>, Statens Serum Institut, Copenhagen, Denmark  
18  
19 2) Department of Infectious Diseases, Aarhus University Hospital, Denmark  
20  
21 3) Hospital de Pneumologia “Raoul Follereau”, Bissau, Guinea-Bissau  
22  
23 4) Hospital Nacional Simao Mendes, Bissau, Guinea-Bissau  
24  
25 5) Infectious Diseases Research Group, Department of Clinical Sciences, Malmö,  
26 Lund University, Sweden  
27  
28  
29  
30  
31

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39  
40 Correspondence to:

41  
42 Victor Francisco Gomes

43 PROJECTO DE SAÚDE DE BANDIM

44 APARTADO 861, 1004 BISSAU CODEX, GUINÉ-BISSAU

45  
46 FAX No: +245 320 16 72

47  
48 Phone No: +245 320 14 89/3204460; Mobile: +245 6658334

49  
50 Email: [victorfranciscogomes@yahoo.co.uk](mailto:victorfranciscogomes@yahoo.co.uk)  
51  
52  
53  
54  
55  
56  
57  
58  
59  
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## ABSTRACT

**Background and objective:** In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

**Setting:** This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

**Participants:** All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT programme.

**Main outcome measures (end points):** The all-cause mortality rate ratio (MRR) between exposed children on IPT and unexposed community control children.

**Results:** A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT programme. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1). The relative mortality in IPT children compared with community controls in 2005-2008 differed significantly from the relative mortality of exposed untreated children compared to the community controls in the 1996-1998 (test of interaction,  $p=0.01$ ).

**Conclusion:** In 2005-2008, exposed children on IPT had 70% lower mortality than the community control children, though not significantly. Relative to the community control

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3 children, the mortality among TB-exposed children on IPT in 2005-2008 was  
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5 significantly lower than the mortality among TB-exposed children not on IPT in 1996-  
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### 10 11 12 **Article summary**

13 This article focuses on:

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17 • impact of IPT on mortality among children exposed to an adult with intrathoracic  
18 TB at home and  
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22 • Mortality in children exposed to TB who were enrolled on IPT compared to those  
23 exposed but not receiving IPT in a previous study in the same setting.  
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### 28 29 30 **Strengths and limitations**

31 Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills  
32 were taken. Given the low mortality in the cohort it was not possible to test to what extent  
33 adherence mattered for a beneficial effect of IPT.  
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36 Mortality in the study area declined dramatically between the two study periods, and the  
37 study therefore had much less power than originally expected. Nonetheless, results were  
38 so marked that it was still possible to show the hypothesized inversion of the mortality  
39 rate ratios between TB-exposed children and community controls between the pre-IPT  
40 period and the IPT period.  
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53 In an intervention study in an area with a very mobile population as in Bissau, it is not  
54 possible to enrol all eligible children. There are always some children travelling or absent  
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3 at inclusion visits. This obviously opens for the possibility of selection biases as to who  
4 participated in the study. Due to the current World Health Organisation (WHO)  
5 recommendation it was decided not to conduct a randomised study, hence, there are a  
6 number of theoretical biases. Furthermore, the previous study was conducted 10 years  
7 earlier than the present study and many things changed in the meantime. Another  
8 limitation was that the children were not HIV tested, it might bias the results if there were  
9 more HIV infected children in the IPT group compared to the no IPT group. However,  
10 we would then expect higher mortality in the no IPT group compared to the community  
11 controls.  
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## Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the TB epidemic<sup>1,2</sup>. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe TB-related morbidity and mortality<sup>2</sup>. Of the estimated 8.3 million new TB cases diagnosed in 2000, almost 900,000 were children<sup>3</sup>, and the proportion of children in the high-burden countries is estimated to be higher<sup>4,5</sup>.

Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home<sup>6</sup> and reliable medical information on causes of death is therefore lacking<sup>7</sup>. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries<sup>7,8</sup>. Necropsy studies conducted in Africa have shown that TB rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas<sup>9</sup>.

An intervention known to contribute to the reduction of morbidity and mortality due to TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive therapy during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70,000 people of all ages<sup>10</sup>. After all this time, isoniazid continues to be the drug of choice<sup>11</sup>, and WHO recommends that all TB contacts under the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane review concluded

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3 that there was not enough evidence for general recommendation of IPT for HIV infected  
4 children<sup>12</sup>. The use of isoniazid in low income countries is, however, limited by several  
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6 circumstances: difficulties of ruling out TB disease in children, mainly those infected  
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8 with HIV, before initiation of IPT<sup>13</sup>, liver toxicity<sup>14-16</sup>, and poor adherence<sup>17</sup>. These  
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10 circumstances may limit the widespread use of IPT in the resource-constrained settings,  
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12 where provision of TB care often falls short of internationally recommended standards<sup>13</sup>.  
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14 IPT has been shown to be effective in recent tuberculin skin test (TST) converters and  
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16 recent contacts of identified cases of TB disease<sup>17;18</sup>. In one study of IPT in HIV-positive  
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18 subjects, a 20% reduction of mortality was found in those with positive TST<sup>18</sup>. The effect  
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20 of such preventive therapy in children, however, is not well established.  
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29 The present study examined the impact of IPT on mortality in children less than 5 years  
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31 of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous  
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33 study in the same community, we found that exposure to TB at home was associated with  
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35 66% excess mortality compared to community control children not exposed to TB at  
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37 home<sup>19</sup>. The aim of the present study was to compare mortality between exposed children  
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39 on IPT and community control children, and to compare this relative mortality to the  
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41 previously observed excess mortality.  
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## Materials and methods

### *Setting*

The study was conducted as a prospective cohort study from 1 September 2005 to 31 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site, located in Bissau, the capital city of Guinea-Bissau. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years<sup>20</sup>.

Due to difficulties in obtaining specific causes of deaths in our setting the all cause mortality was used as the main outcome measurement of the effect of IPT.

The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

### *Houses and household contacts*

Houses in the study area are one-storey, rectangular constructions, usually with 6-8 rooms and are inhabited by 2 to 4 households (families), which can be extended families or not. The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the same space in the house, eating from the same pot.

## Recruitment of participants and patients

### *Identification of adult TB index cases*

Since May 1996 a TB surveillance system, implemented in collaboration with the national TB hospital (“Hospital Raoul Follereau”), has identified adult ( $\geq 15$  years) intrathoracic TB cases using passive and active case finding<sup>20</sup>. As previously described in more detail<sup>20</sup>, a TB case was defined as an adult with symptoms of TB disease with sputum smear positive or negative for AFB, presenting abnormalities in CX-ray with no improvement under treatment with broad spectrum antibiotics for two weeks.

### *Enrolment in the IPT cohort*

Children less than 5 years of age living in the house when the adult TB case started treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the later of the following two dates: 3 month before treatment or date of registration. The children were followed until 5 years of age contributing follow-up time until the date of the last follow-up information. Children lost to follow-up were censored.

Prior to the initiation of IPT, the children were investigated for TB disease in a clinical examination for signs and symptoms using the Keith Edwards score<sup>21</sup>. If the investigation suggested TB disease the children were submitted to a careful and thorough assessment of all evidence from history, clinical examination and relevant investigations, e.g. laboratory examination, including HIV testing, and chest x-ray. Broad spectrum antibiotics were administered for 10-15 days. Children who failed to improve clinically and radiologically after 2 weeks of broad spectrum antibiotics, and without other



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3 explanatory disease were given a full TB treatment regimen according to the national  
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5 protocol. Antiretroviral treatment was not available at the time of the study and HIV  
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7 testing was therefore not generally performed. It was only performed when the  
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9 investigation suggested TB.  
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12 Children who developed signs and symptoms suggestive of TB disease while on IPT  
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14 were evaluated and treated in a similar way.  
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17 Children with TB disease, those who did not give consent, who were absent from first,  
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19 second or third visit or at enrolment consultation were excluded from the IPT cohort.  
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22 There were several steps in the enrolment procedure as depicted in Figure 1. Once an  
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24 adult TB case from the study area was identified a project assistant went to the patient's  
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26 house to update the census for the families living in the house, and socio-economic and  
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28 demographic information was noted on the questionnaire. Following the census-update, a  
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30 field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house  
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32 was visited by the nurse who read the TST and referred potential TB cases for further  
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34 clinical examination. Children without TB disease were eligible for enrolment in the IPT  
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36 cohort regardless of the TST result and were invited to attend the enrolment visit at the  
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38 local health centre. Eligible children who did not show up at inclusion were traced again.  
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40 If not found they were considered absent, but still followed up using basic census  
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42 information. Due to limited time frame, logistic reasons and limited funding they were  
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44 not included later.  
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51 For children enrolled in the IPT programme, isoniazid tablets were administered at 5  
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53 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6  
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55 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children  
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3 receiving >100 mg of isoniazid<sup>22</sup>. The medicine was provided at the house every two  
4 weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT.  
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8 The follow-up visits at 1 and 7 months were performed by the research clinician at the  
9 local health centre and at 4 and 9 months by a field assistant at the child's home.  
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12 Evaluation at follow-up visits included questions about side effects and a physical  
13 assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The  
14 cohort and study routines are described in detail elsewhere<sup>23</sup>. The initially intended IPT  
15 enrolment period was September 2005 to October 2007 with 9 months of follow-up to  
16 June 2008. However, children continued to be enrolled on IPT until the end of the study  
17 period in June 2008.  
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### 29 *Pre-IPT cohort*

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31 As previously described; children less than 5 years of age living in the same house as an  
32 adult index TB case at the time of initiation of treatment during the period May 1996 to  
33 July 1998 were retrieved from the BHP register<sup>19</sup>. To assess the impact of TB exposure at  
34 home in the absence of IPT, their mortality was compared with the mortality of children  
35 living in the study area who had not been exposed to TB at home, during the same period.  
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### 46 *Groups in the study*

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48 In the Pre-IPT cohort we had two groups: TB-exposed children and community control  
49 children. In the IPT cohort we intended also to have two groups: TB-exposed children on  
50 IPT and community control children. As we failed to include all exposed children in the  
51 IPT programme, a third group arose: TB-exposed children not on IPT.  
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### *Effect-size calculation*

For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses during a 4½ year period. An average of 3 children < 5 years of age per house and a mean follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children with approximately 4725 child years of observation. We anticipated the TB surveillance system to identify an increased number of TB patients during the IPT period. An estimated 300 index cases per year during a 2½ year period would give 750 index cases. An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25 years (half of the 2½ year study period) would yield 2250 children with approximately 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort we initially expected to be able to detect a 27% mortality reduction in the IPT cohort. Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau we limited follow-up for the pre-IPT cohort to the period from February 1996 to June 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT cohort lowering the number of identified children. In addition, <5-mortality dropped considerably more than we had anticipated.

### *Ethical approval*

The legal guardians (parents) or caregivers were informed about the study in writing (Portuguese) and verbally in the common language, Creol, before the child was enrolled in the IPT study. Informed consent was obtained from all the parents or caregivers before

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3 enrolment. The study protocol was approved by the Guinea-Bissau National Research  
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6 Coordination and Ethics Committee.  
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### 10 *Statistical analysis*

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12 Data regarding adult TB cases were obtained from the general TB identification system  
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14 in the study area while demographic information was taken from the basic surveillance  
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16 system of the BHP. Statistical analyses were conducted in STATA version 10.  
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22 Similar to the analysis of the impact of TB exposure in the absence of IPT, the average  
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24 delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months.  
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26 Hence, children registered in the same house as an adult index TB case 0-3 months before  
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28 treatment were considered exposed. The effect of exposure on mortality was evaluated by  
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30 rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in  
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32 Guinea-Bissau is very high. To be exposed a child had to be born and registered at the  
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34 time of exposure. Consequently only few children were exposed before 3 months of age.  
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37 Therefore we have chosen to commence the analyses at 3 months of age.  
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43 IPT treatment was initiated in September 2005. To allow exposure time before IPT,  
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45 follow-up started in July 2005. IPT enrolment for the present study ended in October  
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47 2007 with treatment ending in June 2008. Thus, the study period for the present study is  
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49 July 2005 to June 2008. Exposed children counted as unexposed controls until the time of  
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51 exposure. Exposed children never receiving IPT counted as exposed without IPT from  
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53 the start of exposure to the end of follow-up. Children subsequently enrolled on IPT  
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3 counted as exposed without IPT from the start of exposure until the start of IPT and then  
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5 as exposed with IPT to the end of follow-up. However, enrolment into the IPT program  
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7 continued after the enrolment period of the present study. Children enrolled on IPT after  
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9 October 2007 were also counted as exposed on IPT even though they did not finish the  
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11 treatment within the study period. Censoring the children enrolled on IPT after October  
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13 2007 at the time of IPT had little impact on the results. Some children who were present  
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15 when the TB team visited the TB case house (figure 1) were enrolled on IPT even though  
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17 they were not born or registered in the house before the TB case initiated treatment.  
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19 According to the epidemiological definitions these children were not exposed and they  
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21 have counted as unexposed with IPT. A separate analysis was conducted excluding these  
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23 subjects.  
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29 An adjusted analysis was conducted including possible confounders related to child  
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31 mortality: gender, ethnicity, district, socio-economic status, schooling of the mother,  
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33 child crowding (<5 years) and crowding among older individuals (>5 years). A score for  
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35 socio-economic status was calculated adding house indicators (yes=1; no/missing=0):  
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37 corrugated iron roof, electricity, television and in-door toilet. A separate “Missing”  
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39 category was constructed when information was missing on all four variables. Crowding  
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41 was defined as the number of individuals in the house of the TB case on January 1, 2007,  
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43 the mid-point of the examined period. Crowding was included in the analysis as a linear  
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45 predictor.  
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53 It was further examined whether differential mortality not related to TB exposure may  
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55 have existed in the exposed houses. Mortality was compared between children living in  
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3 the house of the adult TB case three years before TB exposure began and children living  
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5 in the remaining houses. As for the main study<sup>19</sup>, the comparison was made over a 3-year  
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8 period from July 2002 to June 2005. The period was chosen not to overlap the study  
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11 period.  
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**Results:***Index TB cases and included children*

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from “case houses with no inclusion” and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but > 5 years old at the time of enrolment. They did therefore not receive IPT. These children counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

*TB among exposed children*

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3 One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2  
4 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical  
5 and chest X-ray findings. One of these children tested HIV-positive and was enrolled in  
6 an antiretroviral (ARV) programme.  
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### 12 13 14 15 *TB exposure and mortality*

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17 Two children died during IPT. For a 6-month old boy, hospital records stated the cause of  
18 death as severe malaria and anaemia. The mother of a 2-year old girl, who died two  
19 months after the initiation of the IPT program, reported that the child had had diarrhoea,  
20 cough and fever prior to death. Antibiotics and other medications had been prescribed.  
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22 The research clinician had requested a chest X-ray, but the result was never received. No  
23 further IPT children died during the follow-up period.  
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34 In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of  
35 observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT  
36 contributed with 1023 PYO and the controls contributed with 30713 PYO.  
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40 Though not statistically significant, the exposed children receiving IPT had a lower  
41 mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-  
42 1.2) (Table 1). This estimate changed little when controlled for background factors  
43 (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and  
44 some exposed children did not receive IPT (Figure 1). In this group of TB-exposed  
45 children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7)  
46 (Table 1).  
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6 There were 68 children on IPT who were not formally exposed to TB because the child  
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8 was born or registered after exposure occurred in the house. Excluding these from the  
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10 IPT group we observed one death from 642 person years of observation giving a MRR of  
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12 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of  
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14 whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).  
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20 *Comparison of mortality among TB-exposed children in the absence and presence of IPT*

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22 We previously found that the excess mortality after exposure to TB only started 6 months  
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24 after exposure<sup>19</sup>. We made a similar analysis for the period 2005-2008; table 3 shows the  
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26 comparison of exposed children without IPT and unexposed community control children,  
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28 stratified by time since exposure and age at exposure.  
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32 Restricted to the period after 6 months, the mortality rate relative to community controls  
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34 in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children  
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36 without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-  
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38 1.1).  
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41 It was furthermore examined whether the effect in the exposed house was caused by  
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43 lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in  
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45 the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among  
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47 children in the houses which later had TB cases was the same as the mortality in the  
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49 control houses, the MRR being 1.04 (0.7-1.5) (Table 4).  
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3 In a previous study in the same community, exposure to TB at home was associated with  
4 an MRR of 1.66 (1.2-2.3) compared to community control children<sup>19</sup>. The present study  
5 assessed whether the excess mortality could be reduced by implementing an IPT  
6 programme. Both the MRR of 0.30 among children who received IPT and the overall  
7 MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower  
8 than the previously observed MRR of 1.66 (respective tests of interaction,  $p=0.01$  and  
9  $p=0.004$ ).

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11 It should be noted that the general child mortality declined markedly between the two  
12 periods studied (Table 5); among community controls mortality declined by more than  
13 50%. Given that excess mortality associated with TB exposure was 66% in the period  
14 from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total  
15 48/1000. If the impact of TB exposure had been similar during the period from 2005-  
16 2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as  
17 the unexposed children in the community, with an additional 19/1000 due to TB  
18 exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

## 41 Discussion

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43 In the present study we have shown an impact of the IPT programme on mortality among  
44 children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-  
45 1998, when IPT was not available in the area, suffered a 66% excess mortality compared  
46 with unexposed community control children. The mortality rate ratio was inverted in  
47 2005-2008 with markedly (though not significantly) lower mortality among exposed  
48 children on IPT compared to the community control children.. It should be emphasized  
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3 that comparing data from different time-periods is not straight-forward, as the conditions  
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5 have changed in many ways that cannot be completely deduced, and the results can be  
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7 biased. In our situation, the child mortality has dropped markedly between these two  
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9 time-periods. However, the excess mortality from 1996-1998 has changed to a marked  
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11 trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child  
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13 mortality, and our data suggest that this is partly due to the introduction of IPT.  
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### 20 **Unexpected observations**

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22 Mortality in the study area declined dramatically between the two study periods and  
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24 mortality declined more than expected among both exposed children receiving IPT and  
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26 exposed children not receiving IPT. Based on the experience from the 1996-1998 period,  
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28 TB exposure at home in the absence of IPT should have been associated with a 19/1000  
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30 person-years excess mortality.  
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34 Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa.  
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36 However, despite the recent HIV epidemic, mortality rates have decreased drastically  
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38 over the last decade following the same pattern observed in the other sub-Saharan  
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40 African countries<sup>24-26</sup>. The reasons for the mortality decline are not fully understood but  
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42 systematic annual vitamin A campaigns and the marked decrease in malaria incidence are  
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44 likely to have contributed.  
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48 The strong trend toward less mortality among IPT-treated children could possibly be  
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50 linked to increased attention to these children including easier access to other forms of  
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52 treatment and more attention from the parents. However, this would be unlikely to  
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54 explain why mortality also declined more than expected among the children who did not  
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3 receive IPT. This may suggest that all TB-associated mortality is not directly due to  
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5 clinical TB disease, but may be due to interactions with other infections. If the incidence  
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7 or severity of these other infections goes down, as has happened in Bissau, the mortality  
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9 associated with TB exposure would also decline.  
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### 12 13 14 15 **Interpretation and consistency with previous studies**

16  
17 Children exposed to TB at home had excess mortality from around 6 months after  
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19 exposure in the present study as well as in the previous study from 1996-1998. In both  
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21 studies, we showed that the TB houses 3 years earlier had exactly the same mortality as  
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23 community controls. It therefore seems unlikely that general social conditions in houses  
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25 with TB cases explain the higher mortality of TB-exposed children. Furthermore,  
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27 children enrolled on IPT in the IPT cohort (compared with community controls) had  
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29 significantly lower mortality than the TB exposed children not receiving IPT in the pre-  
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31 IPT cohort (compared with community controls). Hence, our results suggest that use of  
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33 isoniazid plays an important role in decreasing mortality in children exposed to TB.  
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35 Studies conducted in South Africa and Zambia have reported isoniazid to be highly  
36  
37 effective in reducing the mortality and incidence of TB in HIV-infected children and  
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39 adults living in an area with a high prevalence of TB<sup>14;27;28</sup>. Our findings support those  
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41 studies, but also observations made by Dr. Lincoln in the early 1950s showing that  
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43 isoniazid chemotherapy reduced the case fatality from primary TB among children<sup>29</sup>.  
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### 53 **Implications and conclusions**

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In the period 1996-1998 an excess mortality of 66% was found in children in TB households compared to controls. This excess mortality was reduced in the cohort of children receiving IPT from 2005-2008, and the all-cause mortality in children from TB households was lower than the controls, though not reaching statistical significance.

When comparing the mortality between the exposed children to the controls across the two different time periods, a significant difference in mortality was found. Furthermore, in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause mortality than exposed children not receiving IPT.

. All our data indicate that IPT should be part of the standard TB program and would have a large impact on child mortality in low-income countries.

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#### **Author's contributions**

PG, PA and VG designed the study. VG supervised and run data collection, AA run statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data handling, CW and PG carried out adult TB study, GL contributed in writing the article. VG drafted the article and all authors contributed to the final version.

**Conflict of interest:** There are no competing or conflicting interests.

**Table 1: The effect of exposure on mortality according to age**

Age Months	On IPT* D/PYO	Exposed No IPT	Unexposed D/PYO	MRR <sup>#1</sup> IPT/Unexposed	MRR <sup>#2</sup> IPT/Unexposed	MRR <sup>#1</sup> No	MRR <sup>#2</sup> No

		D/PYO				IPT/Unexposed	IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

\* Include unexposed children on IPT

#<sup>1</sup> Mortality Rate Ratio from a model with age as underlying time

#<sup>2</sup> Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

**Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008**

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

**Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.**

Months since exposure	Age at exposure (Months)			Total
	0-11	12-35	36-59	
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

**Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005**

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

**Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home**

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	2005-2008
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)



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4 **Figure 1: Illustration of the inclusion process**  
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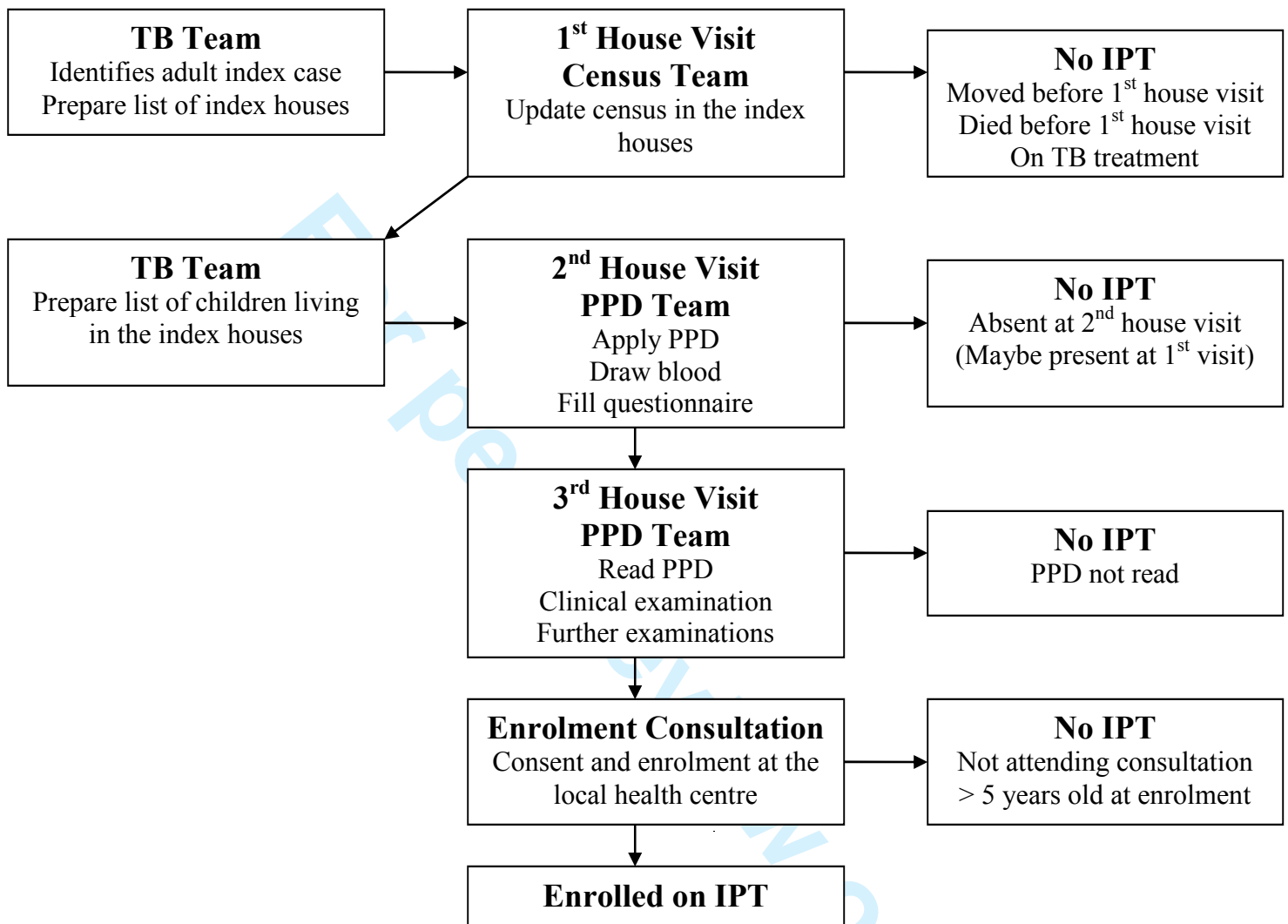
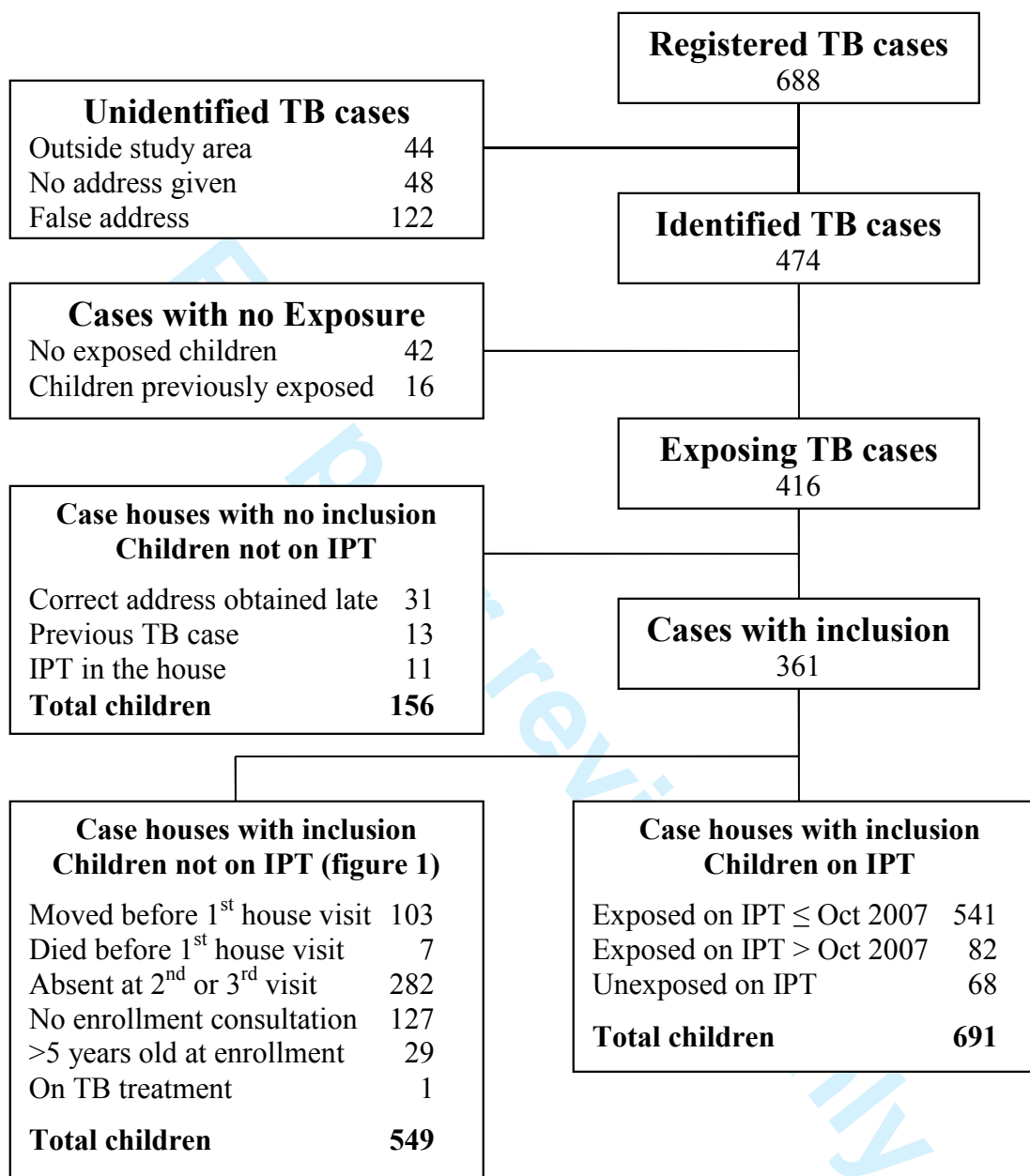


Figure 2: Flow chart of inclusion



**Figure 3: Person years of observation (PYO)**

<b>Children not on IPT</b>		<b>Children on IPT</b>	
<b>Exposed</b>		<b>Exposed</b>	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months at death/censoring		<b>Unexposed</b>	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64

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## Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancanha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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## Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau

Victor F. Gomes<sup>1</sup> MD, MSc, Andreas Andersen<sup>1</sup>, statistician, Grethe Lemvik<sup>1,2</sup> MD, Christian Wejse<sup>1,2</sup> MD, PhD, Ines Oliveira<sup>1</sup> MD, MSc, Fina J. Vieira<sup>3</sup> MD, Luis J. Carlos<sup>4</sup> MD, Cesaltina S. Vieira<sup>3</sup> MD, Peter Aaby<sup>1</sup> DSc, Per Gustafson<sup>5</sup> MD, PhD

- 1) Bandim Health Project, Indepth Network, Bissau, Guinea-Bissau, <http://www.bandim.org>, Statens Serum Institut, Copenhagen, Denmark
- 2) Department of Infectious Diseases, Aarhus University Hospital, Denmark
- 3) Hospital de Pneumologia “Raoul Follereau”, Bissau, Guinea-Bissau
- 4) Hospital Nacional Simao Mendes, Bissau, Guinea-Bissau
- 5) Infectious Diseases Research Group, Department of Clinical Sciences, Malmö, Lund University, Sweden

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Correspondence to:

Victor Francisco Gomes

PROJECTO DE SAÚDE DE BANDIM

APARTADO 861, 1004 BISSAU CODEX, GUINÉ-BISSAU

FAX No: +245 320 16 72

Phone No: +245 320 14 89/3204460; Mobile: +245 6658334

Email: [victorfranciscogomes@yahoo.co.uk](mailto:victorfranciscogomes@yahoo.co.uk)



## ABSTRACT

**Background and objective:** In a cohort of children less than 5 years old exposed to adult intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality compared with community controls. In 2005 we implemented isoniazid preventive therapy (IPT) for children exposed to TB at home, and the present study evaluates the effect of this intervention on mortality.

**Setting:** This prospective cohort study was conducted in six suburban areas, included in the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the capital city of Guinea-Bissau.

**Participants:** All children less than 5 years of age living in the same house as an adult with intrathoracic TB registered for treatment in the study area between 2005 and 2007 were evaluated for inclusion in the IPT programme.

**Main outcome measures (end points):** The all-cause mortality rate ratio (MRR) between exposed children on IPT and unexposed community control children.

**Results:** A total of 1396 children were identified as living in the same houses as 416 adult TB cases, of those 691 were enrolled in the IPT programme. Compared with community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).

The relative mortality in IPT children compared with community controls in 2005-2008 differed significantly from the relative mortality of exposed untreated children compared to the community controls in the 1996-1998 (test of interaction,  $p=0.01$ ).

**Conclusion:** In 2005-2008, exposed children on IPT had 70% lower mortality than the community control children, though not significantly. Relative to the community control

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children, the mortality among TB-exposed children on IPT in 2005-2008 was significantly lower than the mortality among TB-exposed children not on IPT in 1996-1998.

### Article summary

This article focuses on:

- impact of IPT on mortality among children exposed to an adult with intrathoracic TB at home and
- Mortality in children exposed to TB who were enrolled on IPT compared to those exposed but not receiving IPT in a previous study in the same setting.

### Strengths and limitations

Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills were taken. Given the low mortality in the cohort it was not possible to test to what extent adherence mattered for a beneficial effect of IPT.

Mortality in the study area declined dramatically between the two study periods, and the study therefore had much less power than originally expected. Nonetheless, results were so marked that it was still possible to show the hypothesized inversion of the mortality rate ratios between TB-exposed children and community controls between the pre-IPT period and the IPT period.

In an intervention study in an area with a very mobile population as in Bissau, it is not possible to enrol all eligible children. There are always some children travelling or absent

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3 at inclusion visits. This obviously opens for the possibility of selection biases as to who  
4 participated in the study. Due to the current [World Health Organisation \(WHO\)](#)  
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6 recommendation it was decided not to conduct a randomised study, hence, there are a  
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8 number of theoretical biases. Furthermore, the previous study was conducted 10 years  
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10 earlier than the present study and many things changed in the meantime. Another  
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12 limitation was that the children were not HIV tested, it might bias the results if there were  
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14 more HIV infected children in the IPT group compared to the no IPT group. However,  
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16 we would then expect higher mortality in the no IPT group compared to the community  
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## Introduction

Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited resources, mainly because children are considered to develop mild forms of disease and to contribute little to the maintenance of the TB epidemic<sup>1,2</sup>. However, recent studies indicate that children contribute a significant proportion of the disease burden and suffer severe TB-related morbidity and mortality<sup>2</sup>. Of the estimated 8.3 million new TB cases diagnosed in 2000, almost 900,000 were children<sup>3</sup>, and the proportion of children in the high-burden countries is estimated to be higher<sup>4,5</sup>.

Information on the cause of death among children in developing countries is difficult to ascertain. Most childhood deaths occur at home<sup>6</sup> and reliable medical information on causes of death is therefore lacking<sup>7</sup>. According to verbal autopsy studies, acute respiratory infection is one of the most important causes of mortality among children in low-income countries<sup>7,8</sup>. Necropsy studies conducted in Africa have shown that TB rivals acute bacterial and viral pneumonia as a major cause of death from respiratory disease in children from endemic areas<sup>9</sup>.

An intervention known to contribute to the reduction of morbidity and mortality due to TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive therapy during the 1960s after large well conducted randomised controlled trials (RCTs) that included a total of nearly 70,000 people of all ages<sup>10</sup>. After all this time, isoniazid continues to be the drug of choice<sup>11</sup>, and WHO recommends that all TB contacts under the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT for TB exposed children in general has been made; a recent Cochrane review concluded

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3 that there was not enough evidence for general recommendation of IPT for HIV infected  
4 children<sup>12</sup>. The use of isoniazid in low income countries is, however, limited by several  
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6 circumstances: difficulties of ruling out TB disease in children, mainly those infected  
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8 with HIV, before initiation of IPT<sup>13</sup>, liver toxicity<sup>14-16</sup>, and poor adherence<sup>17</sup>. These  
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10 circumstances may limit the widespread use of IPT in the resource-constrained settings,  
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12 where provision of TB care often falls short of internationally recommended standards<sup>13</sup>.  
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14 IPT has been shown to be effective in recent tuberculin skin test (TST) converters and  
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16 recent contacts of identified cases of TB disease<sup>17;18</sup>. In one study of IPT in HIV-positive  
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18 subjects, a 20% reduction of mortality was found in those with positive TST<sup>18</sup>. The effect  
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20 of such preventive therapy in children, however, is not well established.  
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29 The present study examined the impact of IPT on mortality in children less than 5 years  
30 of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous  
31 study in the same community, we found that exposure to TB at home was associated with  
32 66% excess mortality compared to community control children not exposed to TB at  
33 home<sup>19</sup>. The aim of the present study was to compare mortality between exposed children  
34 on IPT and community control children, and to compare this relative mortality to the  
35 previously observed excess mortality.  
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## Materials and methods

### *Setting*

The study was conducted as a prospective cohort study from 1 September 2005 to 31 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic surveillance site, located in Bissau, the capital city of Guinea-Bissau. The population which is currently around 102,000 is followed through regular censuses and registered with information on sex, ethnic background, date of birth, death and migration as well as additional data on socio-economic factors. Information regarding hospitalisations and deaths is collected every 3 months for children under 3 years of age. All paediatric hospitalisations from the study area have been registered since 1990. The incidence of adult intrathoracic TB in the area is high, 471 per 100,000 person years<sup>20</sup>.

Due to difficulties in obtaining specific causes of deaths in our setting the all cause mortality was used as the main outcome measurement of the effect of IPT.

The reliability of population and mortality data in the study setting is a huge strength, which makes this a unique and important study that would be difficult to duplicate in other TB endemic areas.

### *Houses and household contacts*

Houses in the study area are one-storey, rectangular constructions, usually with 6-8 rooms and are inhabited by 2 to 4 households (families), which can be extended families or not. The majority of houses do not have an internal ceiling, leaving a large gap between the internal walls and the roof. Households were defined as the extended family sharing the same space in the house, eating from the same pot.

## Recruitment of participants and patients

### *Identification of adult TB index cases*

Since May 1996 a TB surveillance system, implemented in collaboration with the national TB hospital (“Hospital Raoul Follereau”), has identified adult ( $\geq 15$  years) intrathoracic TB cases using passive and active case finding<sup>20</sup>. As previously described in more detail<sup>20</sup>, a TB case was defined as an adult with symptoms of TB disease with sputum smear positive or negative for AFB, presenting abnormalities in CX-ray with no improvement under treatment with broad spectrum antibiotics for two weeks. The procedures for identification and diagnosis of the cases within this TB surveillance system have previously been described<sup>20</sup>:

### *Enrolment in the IPT cohort*

Children less than 5 years of age living in the house when the adult TB case started treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the later of the following two dates: 3 month before treatment or date of registration. The children were followed until 5 years of age contributing follow-up time until the date of the last follow-up information. Children lost to follow-up were censored.

Prior to the initiation of IPT, the children were investigated for TB disease in a clinical examination for signs and symptoms using the Keith Edwards score<sup>21</sup>. If the investigation suggested TB disease the children were submitted to a careful and thorough assessment of all evidence from history, clinical examination and relevant investigations, e.g. laboratory examination, including HIV testing, and chest x-ray. Broad spectrum

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3 antibiotics were administered for 10-15 days. Children who failed to improve clinically  
4 and radiologically after 2 weeks of broad spectrum antibiotics, and without other  
5 explanatory disease were given a full TB treatment regimen according to the national  
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10 protocol. Antiretroviral treatment was not available at the time of the study and HIV  
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12 testing was therefore not generally performed. It was only performed when the  
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14 investigation suggested TB.

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17 Children who developed signs and symptoms suggestive of TB disease while on IPT  
18 were evaluated and treated in a similar way.

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21 Children with TB disease, those who did not give consent, who were absent from first,  
22 second or third visit or at enrolment consultation were excluded from the IPT cohort.

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27 There were several steps in the enrolment procedure as depicted in Figure 1. Once an  
28 adult TB case from the study area was identified a project assistant went to the patient's  
29 house to update the census for the families living in the house, and socio-economic and  
30 demographic information was noted on the questionnaire. Following the census-update, a  
31 field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house  
32 was visited by the nurse who read the TST and referred potential TB cases for further  
33 clinical examination. Children without TB disease were eligible for enrolment in the IPT  
34 cohort regardless of the TST result and were invited to attend the enrolment visit at the  
35 local health centre. Eligible children who did not show up at inclusion were traced again.

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48 If not found they were considered absent, but still followed up using basic census  
49 information. Due to limited time frame, logistic reasons and limited funding they were  
50 not included later.  
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3 For children enrolled in the IPT programme, isoniazid tablets were administered at 5  
4 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6  
5 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children  
6 receiving >100 mg of isoniazid<sup>22</sup>. The medicine was provided at the house every two  
7 weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT.  
8 The follow-up visits at 1 and 7 months were performed by the research clinician at the  
9 local health centre and at 4 and 9 months by a field assistant at the child's home.  
10 Evaluation at follow-up visits included questions about side effects and a physical  
11 assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The  
12 cohort and study routines are described in detail elsewhere<sup>23</sup>. The initially intended IPT  
13 enrolment period was September 2005 to October 2007 with 9 months of follow-up to  
14 June 2008. However, children continued to be enrolled on IPT until the end of the study  
15 period in June 2008.  
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### 36 *Pre-IPT cohort*

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38 As previously described; children less than 5 years of age living in the same house as an  
39 adult index TB case at the time of initiation of treatment during the period May 1996 to  
40 July 1998 were retrieved from the BHP register<sup>19</sup>. To assess the impact of TB exposure at  
41 home in the absence of IPT, their mortality was compared with the mortality of children  
42 living in the study area who had not been exposed to TB at home, during the same period.  
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### 51 *Groups in the study*

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In the Pre-IPT cohort we had two groups: TB-exposed children and community control children. In the IPT cohort we intended also to have two groups: TB-exposed children on IPT and community control children. As we failed to include all exposed children in the IPT programme, a third group arose: TB-exposed children not on IPT.

### *Effect-size calculation*

For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses during a 4½ year period. An average of 3 children < 5 years of age per house and a mean follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children with approximately 4725 child years of observation. We anticipated the TB surveillance system to identify an increased number of TB patients during the IPT period. An estimated 300 index cases per year during a 2½ year period would give 750 index cases. An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25 years (half of the 2½ year study period) would yield 2250 children with approximately 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort we initially expected to be able to detect a 27% mortality reduction in the IPT cohort. Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau we limited follow-up for the pre-IPT cohort to the period from February 1996 to June 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT cohort lowering the number of identified children. In addition, <5-mortality dropped considerably more than we had anticipated.

### *Ethical approval*

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3 The legal guardians (parents) or caregivers were informed about the study in writing  
4 (Portuguese) and verbally in the common language, Creol, before the child was enrolled  
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8 in the IPT study. Informed consent was obtained from all the parents or caregivers before  
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10 enrolment. The study protocol was approved by the Guinea-Bissau National Research  
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12  
13 Coordination and Ethics Committee.

### 14 15 16 17 18 *Statistical analysis*

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20 Data regarding adult TB cases were obtained from the general TB identification system  
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22 in the study area while demographic information was taken from the basic surveillance  
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24 system of the BHP. Statistical analyses were conducted in STATA version 10.  
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29 Similar to the analysis of the impact of TB exposure in the absence of IPT, the average  
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31 delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months.  
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33 Hence, children registered in the same house as an adult index TB case 0-3 months before  
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35 treatment were considered exposed. The effect of exposure on mortality was evaluated by  
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37 rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in  
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39 Guinea-Bissau is very high. To be exposed a child had to be born and registered at the  
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41 time of exposure. Consequently only few children were exposed before 3 months of age.  
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44 Therefore we have chosen to commence the analyses at 3 months of age.  
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50 IPT treatment was initiated in September 2005. To allow exposure time before IPT,  
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52 follow-up started in July 2005. IPT enrolment for the present study ended in October  
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54 2007 with treatment ending in June 2008. Thus, the study period for the present study is  
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3 July 2005 to June 2008. Exposed children counted as unexposed controls until the time of  
4 exposure. Exposed children never receiving IPT counted as exposed without IPT from  
5 the start of exposure to the end of follow-up. Children subsequently enrolled on IPT  
6 counted as exposed without IPT from the start of exposure until the start of IPT and then  
7 as exposed with IPT to the end of follow-up. However, enrolment into the IPT program  
8 continued after the enrolment period of the present study. Children enrolled on IPT after  
9 October 2007 were also counted as exposed on IPT even though they did not finish the  
10 treatment within the study period. Censoring the children enrolled on IPT after October  
11 2007 at the time of IPT had little impact on the results. Some children who were present  
12 when the TB team visited the TB case house (figure 1) were enrolled on IPT even though  
13 they were not born or registered in the house before the TB case initiated treatment.  
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15 According to the epidemiological definitions these children were not exposed and they  
16 have counted as unexposed with IPT. A separate analysis was conducted excluding these  
17 subjects.  
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20 An adjusted analysis was conducted including possible confounders related to child  
21 mortality: gender, ethnicity, district, socio-economic status, schooling of the mother,  
22 child crowding (<5 years) and crowding among older individuals (>5 years). A score for  
23 socio-economic status was calculated adding house indicators (yes=1; no/missing=0):  
24 corrugated iron roof, electricity, television and in-door toilet. A separate “Missing”  
25 category was constructed when information was missing on all four variables. Crowding  
26 was defined as the number of individuals in the house of the TB case on January 1, 2007,  
27 the mid-point of the examined period. Crowding was included in the analysis as a linear  
28 predictor.  
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6 It was further examined whether differential mortality not related to TB exposure may  
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8 have existed in the exposed houses. Mortality was compared between children living in  
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10 the house of the adult TB case three years before TB exposure began and children living  
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12 in the remaining houses. As for the main study<sup>19</sup>, the comparison was made over a 3-year  
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14 period from July 2002 to June 2005. The period was chosen not to overlap the study  
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16 period.  
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## Results:

### *Index TB cases and included children*

The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified cases there were no eligible children less than 5 years of age and for further 16 TB cases the children were previously exposed before the present study period began, and was therefore not included. No inclusion was conducted for a total of 55 TB cases with 156 exposed children; for 31 cases the correct address was only obtained long after treatment had been initiated and for 24 cases IPT enrolment had previously been initiated in the house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened when children born or registered after exposure were present at inclusion. A total of 705 exposed children never received IPT; 156 children from “case houses with no inclusion” and 549 children from case houses with inclusion (Figure 2). See baseline characteristics in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but > 5 years old at the time of enrolment. They did therefore not receive IPT. These children counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907 children, not registered as exposed or on IPT, entered the survival analysis as controls.

### *TB among exposed children*

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3 One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2  
4 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical  
5 and chest X-ray findings. One of these children tested HIV-positive and was enrolled in  
6 an antiretroviral (ARV) programme.  
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### 12 13 14 15 *TB exposure and mortality*

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17 Two children died during IPT. For a 6-month old boy, hospital records stated the cause of  
18 death as severe malaria and anaemia. The mother of a 2-year old girl, who died two  
19 months after the initiation of the IPT program, reported that the child had had diarrhoea,  
20 cough and fever prior to death. Antibiotics and other medications had been prescribed.  
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22 The research clinician had requested a chest X-ray, but the result was never received. No  
23 further IPT children died during the follow-up period.  
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34 In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of  
35 observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT  
36 contributed with 1023 PYO and the controls contributed with 30713 PYO.  
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40 Though not statistically significant, the exposed children receiving IPT had a lower  
41 mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-  
42 1.2) (Table 1). This estimate changed little when controlled for background factors  
43 (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and  
44 some exposed children did not receive IPT (Figure 1). In this group of TB-exposed  
45 children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7)  
46 (Table 1).  
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6 There were 68 children on IPT who were not formally exposed to TB because the child  
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8 was born or registered after exposure occurred in the house. Excluding these from the  
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10 IPT group we observed one death from 642 person years of observation giving a MRR of  
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12 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of  
13  
14 whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).  
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20 *Comparison of mortality among TB-exposed children in the absence and presence of IPT*

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22 We previously found that the excess mortality after exposure to TB only started 6 months  
23  
24 after exposure<sup>19</sup>. We made a similar analysis for the period 2005-2008; table 3 shows the  
25  
26 comparison of exposed children without IPT and unexposed community control children,  
27  
28 stratified by time since exposure and age at exposure.  
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32 Restricted to the period after 6 months, the mortality rate relative to community controls  
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34 in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children  
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36 without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-  
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38 1.1).  
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41 It was furthermore examined whether the effect in the exposed house was caused by  
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43 lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in  
44  
45 the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among  
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47 children in the houses which later had TB cases was the same as the mortality in the  
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49 control houses, the MRR being 1.04 (0.7-1.5) (Table 4).  
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~~The purpose of the present study was to assess whether the excess mortality associated with TB exposure at home could be reduced by implementing an IPT programme.~~ In a previous study in the same community, exposure to TB at home was associated with an MRR of 1.66 (1.2-2.3) compared to community control children<sup>19</sup>. The present study assessed whether the excess mortality could be reduced by implementing an IPT programme. ~~In the present study b~~Both the MRR of 0.30 among children who received IPT and the overall MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower than the previously observed MRR of 1.66 (respective tests of interaction,  $p=0.01$  and  $p=0.004$ ).

It should be noted that the general child mortality declined markedly between the two periods studied (Table 5); among community controls mortality declined by more than 50%. Given that excess mortality associated with TB exposure was 66% in the period from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total 48/1000. If the impact of TB exposure had been similar during the period from 2005-2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as the unexposed children in the community, with an additional 19/1000 due to TB exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

## Discussion

In the present study we have shown an considerable impact of the IPT programme on mortality among children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-1998, when IPT was not available in the area, suffered a 66% excess mortality compared with unexposed community control children. The mortality rate ratio

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3 was inverted in 2005-2008 with markedly (though not significantly) lower mortality  
4 among exposed children on IPT compared to the community control children. ~~This~~  
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6 ~~excess mortality was completely removed in the cohort of children receiving IPT from~~  
7 ~~2005-2008.~~ It should be emphasized that comparing data from different time-periods is  
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11 not straight-forward, as the conditions have changed in many ways that cannot be  
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13 completely deduced, and the results can be biased. In our situation, the child mortality  
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15 has dropped markedly between these two time-periods. However, the excess mortality  
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17 from 1996-1998 has changed to a marked trend of lower mortality in 2005-2008, which  
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19 cannot solely be tribute to the drop in child mortality, and our data suggest that this is  
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21 partly due to the introduction of IPT.  
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### 29 **Unexpected observations**

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31 Mortality in the study area declined dramatically between the two study periods and  
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33 mortality declined more than expected among both exposed children receiving IPT and  
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35 exposed children not receiving IPT. Based on the experience from the 1996-1998 period,  
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37 TB exposure at home in the absence of IPT should have been associated with a 19/1000  
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39 person-years excess mortality.  
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43 Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa.  
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45 However, despite the recent HIV epidemic, mortality rates have decreased drastically  
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47 over the last decade following the same pattern observed in the other sub-Saharan  
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49 African countries<sup>24-26</sup>. The reasons for the mortality decline are not fully understood but  
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51 systematic annual vitamin A campaigns and the marked decrease in malaria incidence are  
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53 likely to have contributed.  
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3 The strong trend toward less mortality among IPT-treated children could possibly be  
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5 linked to increased attention to these children including easier access to other forms of  
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7 treatment and more attention from the parents. However, this would be unlikely to  
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9 explain why mortality also declined more than expected among the children who did not  
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11 receive IPT. This may suggest that all TB-associated mortality is not directly due to  
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13 clinical TB disease, but may be due to interactions with other infections. If the incidence  
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15 or severity of these other infections goes down, as has happened in Bissau, the mortality  
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17 associated with TB exposure would also decline.  
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### 25 **Interpretation and consistency with previous studies**

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27 Children exposed to TB at home had excess mortality from around 6 months after  
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29 exposure in the present study as well as in the previous study from 1996-1998. In both  
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31 studies, we showed that the TB houses 3 years earlier had exactly the same mortality as  
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33 community controls. It therefore seems unlikely that general social conditions in houses  
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35 with TB cases explain the higher mortality of TB-exposed children. Furthermore,  
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37 children enrolled on IPT in the IPT cohort (compared with community controls) had  
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39 significantly lower mortality than the TB exposed children not receiving IPT in the pre-  
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41 IPT cohort (compared with community controls). Hence, our results suggest that use of  
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43 isoniazid plays an important role in decreasing mortality in children exposed to TB.  
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47 Studies conducted in South Africa and Zambia have reported isoniazid to be highly  
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49 effective in reducing the mortality and incidence of TB in HIV-infected children and  
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51 adults living in an area with a high prevalence of TB<sup>14;27;28</sup>. Our findings support those  
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3 studies, but also observations made by Dr. Lincoln in the early 1950s showing that  
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5 isoniazid chemotherapy reduced the case fatality from primary TB among children<sup>29</sup>.  
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### 10 **Implications and conclusions**

11  
12 In the period 1996-1998 an excess mortality of 66% was found in children in TB  
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14 households compared to controls. This excess mortality was reduced in the cohort of  
15  
16 children receiving IPT from 2005-2008, and the all-cause mortality in children from TB  
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18 households was lower than the controls, though not reaching statistical significance.  
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21  
22 When comparing the mortality between the exposed children to the controls across the  
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24 two different time periods, a significant difference in mortality was found. Furthermore,  
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26 in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause  
27  
28 mortality than exposed children not receiving IPT.  
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31  
32 . All our data indicate that IPT should be part of the standard TB program and would  
33  
34 have a large impact on child mortality in low-income countries.  
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### 48 **Author's contributions**

49  
50 PG, PA and VG designed the study. VG supervised and run data collection, AA run  
51  
52 statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data  
53  
54 handling, CW and PG carried out adult TB study, GL contributed in writing the article.  
55  
56 VG drafted the article and all authors contributed to the final version.  
57  
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**Conflict of interest:** There are no competing or conflicting interests.

**Table 1: The effect of exposure on mortality according to age**

Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR <sup>#1</sup> IPT/Unexposed	MRR <sup>#2</sup> IPT/Unexposed	MRR <sup>#1</sup> No IPT/Unexposed	MRR <sup>#2</sup> No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

D/PYO: Deaths/person years of observation

Unexposed: Community sample

\* Include unexposed children on IPT

<sup>#1</sup> Mortality Rate Ratio from a model with age as underlying time

<sup>#2</sup> Mortality Rate Ratio from a model with age as underlying time, adjusted for gender, ethnicity, district, socio-economic status, schooling of the mother and child crowding

**Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008**

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

**Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.**

Months since exposure	Age at exposure (Months)			Total
	0-11	12-35	36-59	
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

**Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005**

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

**Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home**

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	2005-2008
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)

Figure 1: Illustration of the inclusion process

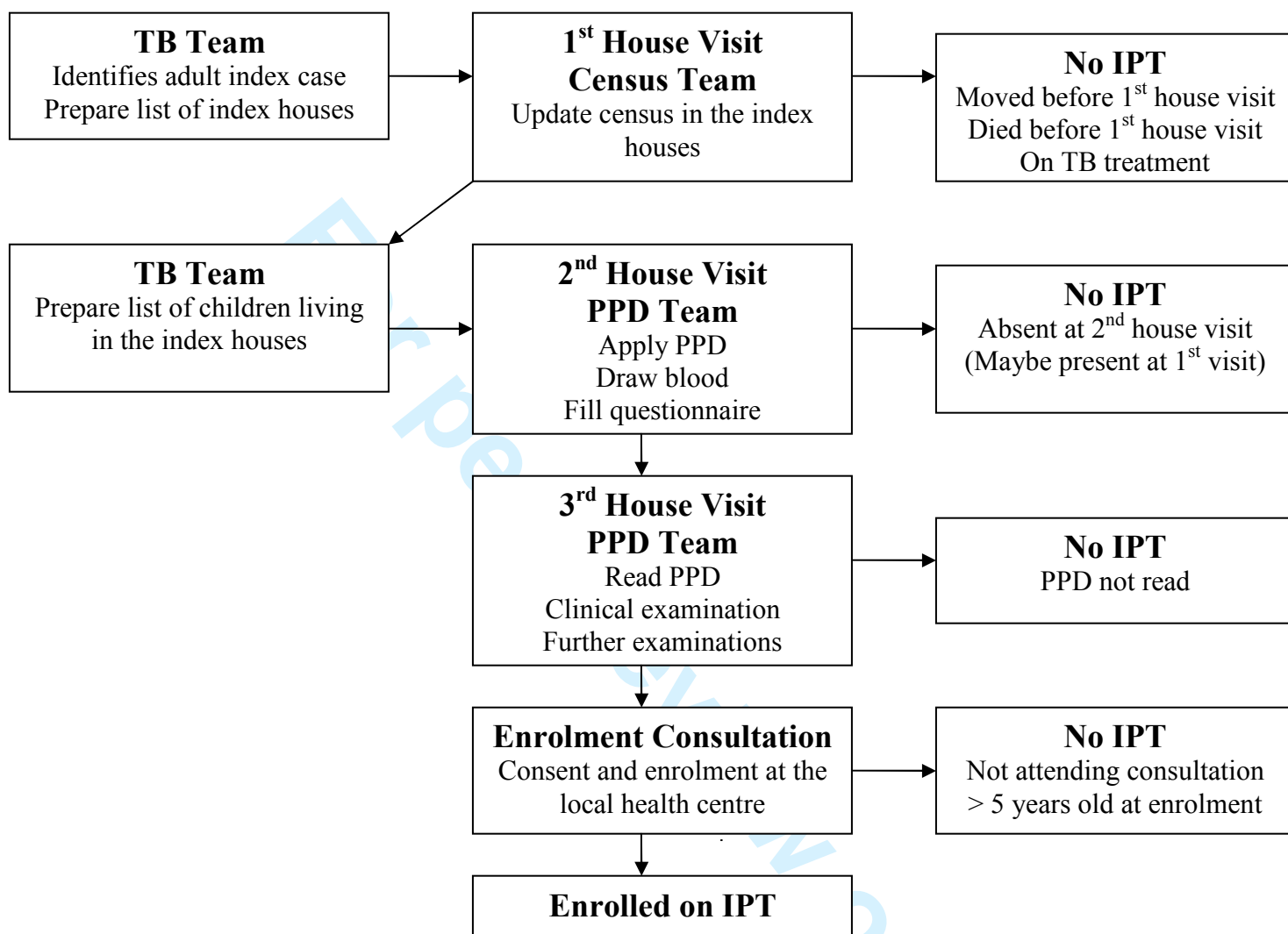
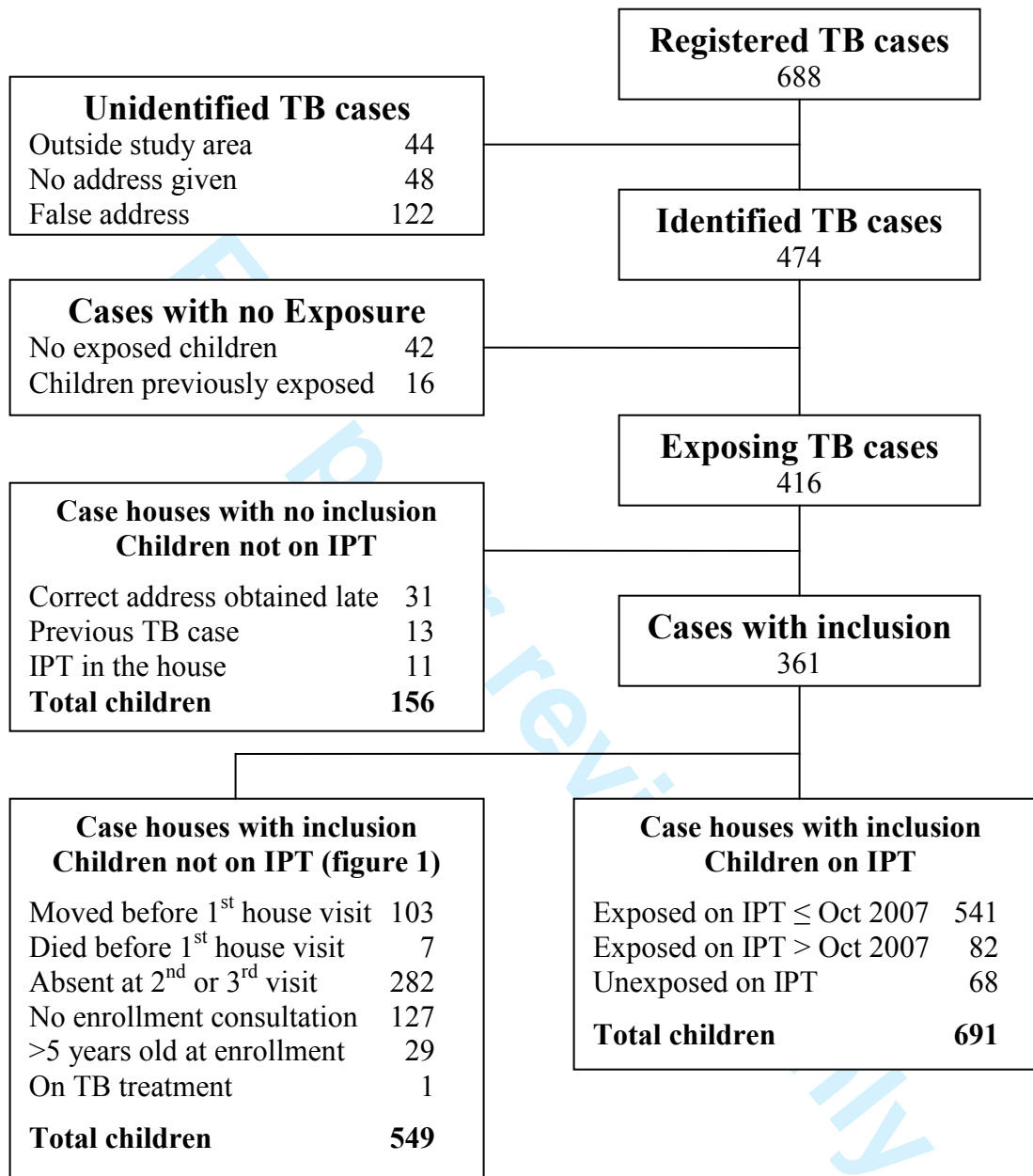




Figure 2: Flow chart of inclusion



**Figure 3: Person years of observation (PYO)**

<b>Children not on IPT</b>		<b>Children on IPT</b>	
<b>Exposed</b>		<b>Exposed</b>	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months at death/censoring		<b>Unexposed</b>	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64

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## Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancanha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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**Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau**

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1 **Impact of isoniazid preventive therapy on mortality among children less than 5**  
2 **years old following exposure to tuberculosis at home in Guinea-Bissau**

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4 Victor F. Gomes<sup>1</sup> MD, MSc, Andreas Andersen<sup>1</sup>, statistician, Grethe Lemvik<sup>1,2</sup> MD,  
5 Christian Wejse<sup>1,2</sup> MD, PhD, Ines Oliveira<sup>1</sup> MD, MSc, Fina J. Vieira<sup>3</sup> MD, Luis J. Carlos  
6 <sup>4</sup> MD, Cesaltina S. Vieira<sup>3</sup> MD, Peter Aaby<sup>1</sup> DSc, Per Gustafson<sup>5</sup> MD, PhD

- 7  
8 1) Bandim Health Project, Indepth Network, Bissau, Guinea-Bissau,  
9 <http://www.bandim.org>, Statens Serum Institut, Copenhagen, Denmark  
10 2) Department of Infectious Diseases, Aarhus University Hospital, Denmark  
11 3) Hospital de Pneumologia “Raoul Follereau”, Bissau, Guinea-Bissau  
12 4) Hospital Nacional Simao Mendes, Bissau, Guinea-Bissau  
13 5) Infectious Diseases Research Group, Department of Clinical Sciences, Malmö,  
14 Lund University, Sweden

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21 Correspondence to:

22 Victor Francisco Gomes

23 PROJECTO DE SAÚDE DE BANDIM

24 APARTADO 861, 1004 BISSAU CODEX, GUINÉ-BISSAU

25 FAX No: +245 320 16 72

26 Phone No: +245 320 14 89/3204460; Mobile: +245 6658334

27 Email: [victorfranciscogomes@yahoo.co.uk](mailto:victorfranciscogomes@yahoo.co.uk)



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3 31 **ABSTRACT**  
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6 32 **Background and objective:** In a cohort of children less than 5 years old exposed to adult  
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8 33 intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality  
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10 34 compared with community controls. In 2005 we implemented isoniazid preventive  
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12 35 therapy (IPT) for children exposed to TB at home, and the present study evaluates the  
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14 36 effect of this intervention on mortality.  
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17 37 **Setting:** This prospective cohort study was conducted in six suburban areas, included in  
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19 38 the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the  
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21 39 capital city of Guinea-Bissau.  
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24 40 **Participants:** All children less than 5 years of age living in the same house as an adult  
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26 41 with intrathoracic TB registered for treatment in the study area between 2005 and 2007  
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28 42 were evaluated for inclusion in the IPT program.  
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31 43 **Main outcome measures (end points):** The all-cause mortality rate ratio (MRR)  
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33 44 between exposed children on IPT, exposed without IPT and unexposed community  
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35 45 control children.  
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38 46 **Results:** A total of 1396 children were identified as living in the same houses as 416  
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40 47 adult TB cases, of those 691 were enrolled in the IPT program. Compared with  
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42 48 community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI  
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44 49 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).  
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46 50 The relative mortality in IPT children compared with community controls in 2005-2008  
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48 51 differed significantly from the relative mortality of exposed untreated children compared  
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50 52 to the community controls in the 1996-1998 (test of interaction,  $p=0.01$ ).  
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4 53 **Conclusion:** In 2005-2008, exposed children on IPT had 70% lower mortality than the  
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6 54 community control children, though not significantly. Relative to the community control  
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8 55 children, the mortality among TB-exposed children on IPT in 2005-2008 was  
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10 56 significantly lower than the mortality among TB-exposed children not on IPT in 1996-  
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12 57 1998.

### 14 58 15 16 17 59 **Article summary**

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19  
20 60 This article focuses on:

- 21  
22 61 • Impact of IPT on mortality among children exposed to an adult with intrathoracic  
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24 62 TB at home: comparing 1) exposed children who received IPT to unexposed  
25  
26 63 children and 2) exposed children who did not received IPT to unexposed children  
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28  
29 64 • Mortality in children exposed to TB who were enrolled on IPT compared to those  
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31 65 exposed but not receiving IPT in a previous study in the same setting.  
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### 35 36 37 67 **Strengths and limitations**

38  
39 68 Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills  
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41 69 were taken. Given the low mortality in the cohort it was not possible to test to what extent  
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43 70 adherence mattered for a beneficial effect of IPT.

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46 71 Mortality in the study area declined dramatically between the two study periods, and the  
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48 72 study therefore had much less power than originally expected. Nonetheless, results were  
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50 73 so marked that it was still possible to show the hypothesized inversion of the mortality  
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52 74 rate ratios between TB-exposed children and community controls between the pre-IPT  
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55 75 period and the IPT period.  
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In an intervention study in an area with a very mobile population as in Bissau, it is not possible to enrol all eligible children. There are always some children travelling or absent at inclusion visits. This obviously opens for the possibility of selection biases as to who participated in the study. Due to the current World Health Organisation (WHO) recommendation it was decided not to conduct a randomised study, hence, there are a number of theoretical biases. Furthermore, the previous study was conducted 10 years earlier than the present study and many things changed in the meantime. Another limitation was that the children were not HIV tested, it might bias the results if there were more HIV-infected children in the IPT group compared to the no IPT group. However, we would then expect higher mortality in the no IPT group compared with the community controls in the present IPT cohort.

## 89 Introduction

90 Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited  
91 resources, mainly because children are considered to develop mild forms of disease and  
92 to contribute little to the maintenance of the TB epidemic<sup>1,2</sup>. However, recent studies  
93 indicate that children contribute a significant proportion of the disease burden and suffer  
94 severe TB-related morbidity and mortality<sup>2</sup>. In 2011 TB incidence among children was  
95 estimate at 490,000, equivalent to about 6% of the total number 8.7 million incident cases  
96 <sup>3</sup>, and the proportion of children in the high-burden countries is estimated to be higher<sup>4,5</sup>.  
97 Information on the cause of death among children in developing countries is difficult to  
98 ascertain. Most childhood deaths occur at home<sup>6</sup> and reliable medical information on  
99 causes of death is therefore lacking<sup>7</sup>. According to verbal autopsy studies, acute  
100 respiratory infection is one of the most important causes of mortality among children in  
101 low-income countries<sup>7,8</sup>. Necropsy studies conducted in Africa have shown that TB rivals  
102 acute bacterial and viral pneumonia as a major cause of death from respiratory disease in  
103 children from endemic areas<sup>9</sup>.

104  
105 An intervention known to contribute to the reduction of morbidity and mortality due to  
106 TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive  
107 therapy during the 1960s after large well conducted randomised controlled trials (RCTs)  
108 that included a total of nearly 70.000 people of all ages<sup>10</sup>. After all this time, isoniazid  
109 continues to be the drug of choice<sup>11</sup>, and WHO recommends that all TB contacts under  
110 the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT  
111 for TB exposed children in general has been made; a recent Cochrane review concluded

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3 112 that there was not enough evidence for general recommendation of IPT for HIV-infected  
4  
5 113 children<sup>12</sup>. The use of isoniazid in low income countries is limited by difficulties of ruling  
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7  
8 114 out TB disease before initiation<sup>13</sup>, liver toxicity<sup>14-16</sup>, and poor adherence<sup>17</sup>. IPT has been  
9  
10 115 shown to be effective in recent tuberculin skin test (TST) converters and recent contacts  
11  
12 116 of identified cases of TB disease<sup>17;18</sup>. In one study of IPT in HIV-infected subjects, a 20%  
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14 117 reduction of mortality was found in those with positive TST<sup>18</sup>. The effect of such  
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16 118 preventive therapy in children, however, is not well established.  
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21  
22 120 The present study examined the impact of IPT on mortality in children less than 5 years  
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24 121 of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous  
25  
26 122 study in the same community, we found that exposure to TB at home was associated with  
27  
28 123 66% excess mortality compared to community control children not exposed to TB at  
29  
30 124 home<sup>19</sup>. The aim of the present study was to compare mortality between exposed children  
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32 125 on IPT and community control children, and to compare this relative mortality to the  
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34 126 previously observed excess mortality.  
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3 128 **Materials and methods**  
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5 129 *Setting*  
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8 130 The study was conducted as a prospective cohort study from 1 September 2005 to 31  
9  
10 131 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic  
11  
12 132 surveillance site, located in Bissau, the capital city of Guinea-Bissau, where the  
13  
14 133 prevalence of HIV1 and HIV2 among adults was 4.6% and 4.4%, respectively<sup>20</sup>. The  
15  
16 134 population which is currently around 102,000 is followed through regular censuses and  
17  
18 135 registered with information on sex, ethnic background, date of birth, death and migration  
19  
20 136 as well as additional data on socio-economic factors. Information regarding  
21  
22 137 hospitalisations and deaths is collected every 3 months for children under 3 years of age.  
23  
24 138 Information about children older than 3 years of age and adults is obtained from general  
25  
26 139 censuses carried out approximately every 3<sup>rd</sup> year. All paediatric hospitalisations from the  
27  
28 140 study area have been registered since 1990. The incidence of adult intrathoracic TB in the  
29  
30 141 area is high, 471 per 100,000 person years<sup>21</sup>.  
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36 142  
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38 143 Due to difficulties in obtaining specific causes of deaths in our setting the all-cause  
39  
40 144 mortality was used as the main outcome measurement of the effect of IPT.  
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42

43 145 The reliability of population and mortality data in the study setting is a huge strength,  
44  
45 146 which makes this a unique and important study that would be difficult to duplicate in  
46  
47 147 other TB endemic areas.  
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49 148  
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51 149 *Houses and household contacts*  
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53 150 Houses in the study area are one-storey, rectangular constructions, usually with 6-8  
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55 151 rooms and are inhabited by 2 to 4 households (families), which can be extended families  
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3 152 or not. The majority of houses do not have an internal ceiling, leaving a large gap  
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5 153 between the internal walls and the roof. Households were defined as the extended family  
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8 154 sharing the same space in the house, eating from the same pot.  
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## 12 156 **Recruitment of participants and patients**

### 13 157 *Identification of adult TB index cases*

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17 158 Since May 1996 a TB surveillance system, implemented in collaboration with the  
18  
19  
20 159 national TB hospital (“Hospital Raoul Follereau”), has identified adult ( $\geq 15$  years)  
21  
22 160 intrathoracic TB cases using passive and active case finding<sup>21</sup>. As previously described in  
23  
24 161 more detail<sup>21</sup>, an intrathoracic TB case was defined as an adult with symptoms of TB  
25  
26 162 with sputum smear microscopy positive or negative for acid-fast bacilli, presenting  
27  
28 163 abnormalities in the chest X-ray (CXR) with no improvement on treatment with broad  
29  
30 164 spectrum antibiotics for two weeks.  
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### 34 165

### 35 166 *Enrolment in the IPT cohort*

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37  
38 167 Children less than 5 years of age living in the house when the adult TB case started  
39  
40 168 treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the  
41  
42 169 later of the following two dates: 3 month before treatment or date of registration. The  
43  
44 170 children were followed until 5 years of age contributing follow-up time until the date of  
45  
46 171 the last follow-up information. Children lost to follow-up were censored.  
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49  
50 172 Prior to the initiation of IPT, the children were investigated for TB disease in a clinical  
51  
52 173 examination for signs and symptoms using the Keith Edwards score<sup>22</sup>. If the investigation  
53  
54 174 suggested TB disease the children were submitted to a careful and thorough assessment  
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3 175 of all evidence from history, clinical examination and relevant investigations, e.g.  
4  
5 176 laboratory examination, including HIV testing, and CXR. Broad spectrum antibiotics  
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7  
8 177 were administered for 10-15 days. Children who failed to improve clinically and  
9  
10 178 radiologically after 2 weeks of broad spectrum antibiotics, and without other explanatory  
11  
12 179 disease were given a full TB treatment regimen according to the national protocol.  
13  
14 180 Antiretroviral treatment was not available at the time of the study and HIV testing was  
15  
16 181 therefore not generally performed. It was only performed when the investigation  
17  
18 182 suggested TB. Children who developed signs and symptoms suggestive of TB disease  
19  
20 183 while on IPT were evaluated and treated in a similar way.  
21  
22 184 Children with TB disease, those who did not give consent, who were absent from first,  
23  
24 185 second or third visit or at enrolment consultation were excluded from the IPT cohort.  
25  
26 186 There were several steps in the enrolment procedure as depicted in Figure 1. Once an  
27  
28 187 adult TB case from the study area was identified a project assistant went to the patient's  
29  
30 188 house to update the census for the families living in the house, and socio-economic and  
31  
32 189 demographic information was noted on the questionnaire. Following the census-update, a  
33  
34 190 field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house  
35  
36 191 was visited by the nurse who read the TST and referred potential TB cases for further  
37  
38 192 clinical examination. Children without TB disease were eligible for enrolment in the IPT  
39  
40 193 cohort regardless of the TST result and were invited to attend the enrolment visit at the  
41  
42 194 local health centre. Eligible children who did not show up at inclusion were traced again.  
43  
44 195 If not found they were considered absent, but still followed up using basic census  
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46 196 information. Due to limited time frame, logistic reasons and limited funding they were  
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48 197 not included later.  
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3 198 For children enrolled in the IPT program, isoniazid tablets were administered at 5  
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5 199 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6  
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7  
8 200 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children  
9  
10 201 receiving >100 mg of isoniazid<sup>23</sup>. The medicine was provided at the house every two  
11  
12 202 weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT.  
13  
14 203 The follow-up visits at 1 and 7 months were performed by the research clinician at the  
15  
16 204 local health centre and at 4 and 9 months by a field assistant at the child's home.  
17  
18 205 Evaluation at follow-up visits included questions about side effects and a physical  
19  
20 206 assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The  
21  
22 207 cohort and study routines are described in detail elsewhere<sup>24</sup>. The initially intended IPT  
23  
24 208 enrolment period was September 2005 to October 2007 with 9 months of follow-up to  
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26 209 June 2008. However, children continued to be enrolled on IPT until the end of the study  
27  
28 210 period in June 2008.  
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#### 35 212 *Pre-IPT cohort*

36 213 As previously described; children less than 5 years of age living in the same house as an  
37  
38 214 adult index TB case at the time of initiation of treatment during the period May 1996 to  
39  
40 215 July 1998 were retrieved from the BHP register<sup>19</sup>. To assess the impact of TB exposure at  
41  
42 216 home in the absence of IPT, their mortality was compared with the mortality of children  
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44 217 living in the study area who had not been exposed to TB at home, during the same period.  
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#### 51 219 *Groups in the study*

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3 220 In the Pre-IPT cohort we had two groups: TB-exposed children and community control  
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5 221 children. In the IPT cohort we intended also to have two groups: TB-exposed children on  
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8 222 IPT and community control children. As we failed to include all exposed children in the  
9  
10 223 IPT program, a third group arose: TB-exposed children not on IPT.  
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15 225 *Effect-size calculation*  
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17 226 For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses  
18  
19 227 during a 4½ year period. An average of 3 children < 5 years of age per house and a mean  
20  
21 228 follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children  
22  
23 229 with approximately 4725 child years of observation. We anticipated the TB surveillance  
24  
25 230 system to identify an increased number of TB patients during the IPT period. An  
26  
27 231 estimated 300 index cases per year during a 2½ year period would give 750 index cases.  
28  
29 232 An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25  
30  
31 233 years (half of the 2½ year study period) would yield 2250 children with approximately  
32  
33 234 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort  
34  
35 235 we initially expected to be able to detect a 27% mortality reduction in the IPT cohort.  
36  
37 236 Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau  
38  
39 237 we limited follow-up for the pre-IPT cohort to the period from February 1996 to June  
40  
41 238 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT  
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43 239 cohort lowering the number of identified children. In addition, <5-mortality dropped  
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45 240 considerably more than we had anticipated.  
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55 242 *Ethical approval*  
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3 243 The legal guardians (parents) or caregivers were informed about the study in writing  
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5 244 (Portuguese) and verbally in the common language, Creol, before the child was enrolled  
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7  
8 245 in the IPT study. Informed consent was obtained from all the parents or caregivers before  
9  
10 246 enrolment. The study protocol was approved by the Guinea-Bissau National Research  
11  
12 247 Coordination and Ethics Committee.

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17 249 *Statistical analysis*

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20 250 Data regarding adult TB cases were obtained from the general TB identification system  
21  
22 251 in the study area while demographic information was taken from the basic surveillance  
23  
24 252 system of the BHP. Statistical analyses were conducted in STATA version 10.

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29 254 Similar to the analysis of the impact of TB exposure in the absence of IPT, the average  
30  
31 255 delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months.  
32  
33 256 Hence, children registered in the same house as an adult index TB case 0-3 months before  
34  
35 257 treatment were considered exposed. The effect of exposure on mortality was evaluated by  
36  
37 258 rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in  
38  
39 259 Guinea-Bissau is very high. To be exposed a child had to be born and registered at the  
40  
41 260 time of exposure. Consequently only few children were exposed before 3 months of age.  
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43 261 Therefore we have chosen to commence the analyses at 3 months of age.  
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50 262

51 263 IPT treatment was initiated in September 2005. To allow exposure time before IPT,  
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53 264 follow-up started in July 2005. IPT enrolment for the present study ended in October  
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55 265 2007 with treatment ending in June 2008. Thus, the study period for the present study is  
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3 266 July 2005 to June 2008. Exposed children counted as unexposed controls until the time of  
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5 267 exposure. Exposed children never receiving IPT counted as exposed without IPT from  
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7 268 the start of exposure to the end of follow-up. Children subsequently enrolled on IPT  
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9 269 counted as exposed without IPT from the start of exposure until the start of IPT and then  
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11 270 as exposed with IPT to the end of follow-up. However, enrolment into the IPT program  
12  
13 271 continued after the enrolment period of the present study. Children enrolled on IPT after  
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15 272 October 2007 were also counted as exposed on IPT even though they did not finish the  
16  
17 273 treatment within the study period. Censoring the children enrolled on IPT after October  
18  
19 274 2007 at the time of IPT had little impact on the results. Some children who were present  
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21 275 when the TB team visited the TB case house (figure 1) were enrolled on IPT even though  
22  
23 276 they were not born or registered in the house before the TB case initiated treatment.  
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25 277 According to the epidemiological definitions these children were not exposed and they  
26  
27 278 have counted as unexposed with IPT. A separate analysis was conducted excluding these  
28  
29 279 subjects.  
30  
31 280 An adjusted analysis was conducted including possible confounders related to child  
32  
33 281 mortality: gender, ethnicity, district, socio-economic status, schooling of the mother,  
34  
35 282 child crowding (<5 years) and crowding among older individuals (>5 years). A score for  
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37 283 socio-economic status was calculated adding house indicators (yes=1; no/missing=0):  
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39 284 corrugated iron roof, electricity, television and in-door toilet. A separate “Missing”  
40  
41 285 category was constructed when information was missing on all four variables. Crowding  
42  
43 286 was defined as the number of individuals in the house of the TB case on January 1, 2007,  
44  
45 287 the mid-point of the examined period. Crowding was included in the analysis as a linear  
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47 288 predictor.  
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6 290 It was further examined whether differential mortality not related to TB exposure may  
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8 291 have existed in the exposed houses. Mortality was compared between children living in  
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10 292 the house of the adult TB case three years before TB exposure began and children living  
11  
12 293 in the remaining houses. As for the main study<sup>19</sup>, the comparison was made over a 3-year  
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14 294 period from July 2002 to June 2005. The period was chosen not to overlap the study  
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17 295 period.  
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3 297 **Results:**  
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6 298 *Index TB cases and included children*  
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8 299 The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to  
9  
10 300 June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and  
11  
12 301 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified  
13  
14 302 cases there were no eligible children less than 5 years of age and for further 16 TB cases  
15  
16 303 the children were previously exposed before the present study period began, and was  
17  
18 304 therefore not included. No inclusion was conducted for a total of 55 TB cases with 156  
19  
20 305 exposed children; for 31 cases the correct address was only obtained long after treatment  
21  
22 306 had been initiated and for 24 cases IPT enrolment had previously been initiated in the  
23  
24 307 house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361  
25  
26 308 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened  
27  
28 309 when children born or registered after exposure were present at inclusion. A total of 705  
29  
30 310 exposed children never received IPT; 156 children from “case houses with no inclusion”  
31  
32 311 and 549 children from case houses with inclusion (Figure 2). See baseline characteristics  
33  
34 312 in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but  
35  
36 313 > 5 years old at the time of enrolment. They did therefore not receive IPT. These children  
37  
38 314 counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended  
39  
40 315 before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907  
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42 316 children, not registered as exposed or on IPT, entered the survival analysis as controls.  
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53 318 *TB among exposed children*  
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3 319 One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2  
4  
5 320 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical  
6  
7  
8 321 and CXR findings. One of these children was HIV-infected and was enrolled in an  
9  
10 322 antiretroviral (ARV) program.  
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15 324 *TB exposure and mortality*  
16

17 325 Two children died during IPT. For a 6-month old boy, hospital records stated the cause of  
18  
19 326 death as severe malaria and anaemia. The mother of a 2-year old girl, who died two  
20  
21 327 months after the initiation of the IPT program, reported that the child had had diarrhoea,  
22  
23 328 cough and fever prior to death. Antibiotics and other medications had been prescribed.  
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25  
26 329 The research clinician had requested a CXR, but the result was never received. No further  
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28 330 IPT children died during the follow-up period.  
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34 332 In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of  
35  
36 333 observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT  
37  
38 334 contributed with 1023 PYO and the controls contributed with 30713 PYO.  
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41 335 Though not statistically significant, the exposed children receiving IPT had a lower  
42  
43 336 mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-  
44  
45 337 1.2) (Table 1). This estimate changed little when controlled for background factors  
46  
47 338 (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and  
48  
49 339 some exposed children did not receive IPT (Figure 1). In this group of TB-exposed  
50  
51 340 children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7)  
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53 341 (Table 1).  
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7 There were 68 children on IPT who were not formally exposed to TB because the child  
8 was born or registered after exposure occurred in the house. Excluding these from the  
9 IPT group we observed one death from 642 person years of observation giving a MRR of  
10 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of  
11 whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).  
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20 349 *Comparison of mortality among TB-exposed children in the absence and presence of IPT*

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22 350 We previously found that the excess mortality after exposure to TB only started 6 months  
23 after exposure<sup>19</sup>. We made a similar analysis for the period 2005-2008; table 3 shows the  
24 comparison of exposed children without IPT and unexposed community control children,  
25 stratified by time since exposure and age at exposure.  
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32 354 Restricted to the period after 6 months, the mortality rate relative to community controls  
33 in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children  
34 without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-  
35 1.1).  
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41 358 It was furthermore examined whether the effect in the exposed house was caused by  
42 lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in  
43 the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among  
44 children in the houses which later had TB cases was the same as the mortality in the  
45 control houses, the MRR being 1.04 (0.7-1.5) (Table 4).  
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3 364 In a previous study in the same community, exposure to TB at home was associated with  
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5 365 an MRR of 1.66 (1.2-2.3) compared to community control children<sup>19</sup>. The present study  
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7  
8 366 assessed whether the excess mortality could be reduced by implementing an IPT  
9  
10 367 program. Both the MRR of 0.30 among children who received IPT and the overall MRR  
11  
12 368 of 0.71 for all exposed children in the 2005-2008 period were significantly lower than the  
13  
14 369 previously observed MRR of 1.66 (respective tests of interaction,  $p=0.01$  and  $p=0.004$ ).  
15  
16  
17 370 It should be noted that the general child mortality declined markedly between the two  
18  
19 371 periods studied (Table 5); among community controls mortality declined by more than  
20  
21 372 50%. Given that excess mortality associated with TB exposure was 66% in the period  
22  
23 373 from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total  
24  
25 374 48/1000. If the impact of TB exposure had been similar during the period from 2005-  
26  
27 375 2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as  
28  
29 376 the unexposed children in the community, with an additional 19/1000 due to TB  
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31 377 exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).  
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### 379 **Discussion**

380 In the present study we have shown an impact of the IPT program on mortality among  
381 children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-  
382 1998, when IPT was not available in the area, suffered a 66% excess mortality compared  
383 with unexposed community control children. The mortality rate ratio was inverted in  
384 2005-2008 with markedly (though not significantly) lower mortality among exposed  
385 children on IPT compared to the community control children. It should be emphasized  
386 that comparing data from different time-periods is not straight-forward, as the conditions

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3 387 have changed in many ways that cannot be completely deduced, and the results can be  
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5 388 biased. In our situation, the child mortality has dropped markedly between these two  
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8 389 time-periods. However, the excess mortality from 1996-1998 has changed to a marked  
9  
10 390 trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child  
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12 391 mortality, and our data suggest that this is partly due to the introduction of IPT.

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### 14 15 16 17 393 **Unexpected observations**

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19 394 Mortality in the study area declined dramatically between the two study periods and  
20  
21 395 mortality declined more than expected among both exposed children receiving IPT and  
22  
23 396 exposed children not receiving IPT. Based on the experience from the 1996-1998 period,  
24  
25 397 TB exposure at home in the absence of IPT should have been associated with a 19/1000  
26  
27 398 person-years excess mortality.

28  
29 399 Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa.  
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31 400 However, despite the recent HIV epidemic, mortality rates have decreased drastically  
32  
33 401 over the last decade following the same pattern observed in the other sub-Saharan  
34  
35 402 African countries<sup>25-27</sup>. The reasons for the mortality decline are not fully understood but  
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37 403 systematic annual vitamin A campaigns and the marked decrease in malaria incidence are  
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39 404 likely to have contributed.

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41 405 The strong trend toward less mortality among IPT-treated children could possibly be  
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43 406 linked to increased attention to these children including easier access to other forms of  
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45 407 treatment and more attention from the parents. However, this would be unlikely to  
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47 408 explain why mortality also declined more than expected among the children who did not  
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49 409 receive IPT. This may suggest that all TB-associated mortality is not directly due to  
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3 410 clinical TB disease, but may be due to interactions with other infections. If the incidence  
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5 411 or severity of these other infections goes down, as has happened in Bissau, the mortality  
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7 412 associated with TB exposure would also decline.  
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10 413 There may be a slight trend towards better outcome in the contacts receiving IPT,  
11  
12 414 although not significant. Travelling or absence was the main reason why exposed  
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14 415 children were not enrolled in the IPT program. These children may therefore have had  
15  
16 416 little contact with the TB case. Another possible reason may be selection bias so that the  
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18 417 group of children going through 9 months of treatment had mothers who were better able  
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20 418 to take care of them (although no differences in education level or socioeconomic index  
21  
22 419 was seen), or simply that the children at home at the time of inclusion were not exposed  
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24 420 to the possible dangers of travelling.  
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29 421 Table 3 showed no difference in overall mortality between unexposed controls and  
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31 422 exposed who did not get IPT, but did indeed show an effect among the oldest children  
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33 423 with +12 months since exposure. With the limited number of events available we were  
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35 424 not able to show a overall difference in mortality which would be expected. Yet despite  
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37 425 the limited number of events, it was possible to show a significant effect of exposure in a  
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39 426 subgroup of the un-treated children, namely among those most likely affected, ie the  
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41 427 mobile children + 3 years of age with the longest observation time since exposure.  
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#### 46 **Interpretation and consistency with previous studies**

47  
48 429 Children exposed to TB at home had excess mortality from around 6 months after  
49  
50 430 exposure in the present study as well as in the previous study from 1996-1998. In both  
51  
52 431 studies, we showed that the TB houses 3 years earlier had exactly the same mortality as  
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54 432 community controls. It therefore seems unlikely that general social conditions in houses  
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3 433 with TB cases explain the higher mortality of TB-exposed children. Furthermore,  
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5 434 children enrolled on IPT in the IPT cohort (compared with community controls) had  
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8 435 significantly lower mortality than the TB exposed children not receiving IPT in the pre-  
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10 436 IPT cohort (compared with community controls). Hence, our results suggest that use of  
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12 437 isoniazid plays an important role in decreasing mortality in children exposed to TB.  
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14 438 Studies conducted in South Africa and Zambia have reported isoniazid to be highly  
15  
16 439 effective in reducing the mortality and incidence of TB in HIV-infected children and  
17  
18 440 adults living in an area with a high prevalence of TB<sup>14;28;29</sup>. Our findings support those  
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20 441 studies, but also observations made by Dr. Lincoln in the early 1950s showing that  
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22 442 isoniazid chemotherapy reduced the case fatality from primary TB among children<sup>30</sup>.  
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#### 29 444 **Implications and conclusions**

30  
31 445 In the period 1996-1998 an excess mortality of 66% was found in children in TB  
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33 446 households compared to controls. This excess mortality was reduced in the cohort of  
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35 447 children receiving IPT from 2005-2008, and the all-cause mortality in children from TB  
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37 448 households was lower than the controls, though not reaching statistical significance.  
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39 449 When comparing the mortality between the exposed children to the controls across the  
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41 450 two different time periods, a significant difference in mortality was found. Furthermore,  
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43 451 in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause  
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45 452 mortality than exposed children not receiving IPT. All our data indicate that IPT should  
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47 453 be part of the standard TB program and would have a large impact on child mortality in  
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49 454 low-income countries.  
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12 462 **Author's contributions**

13  
14 463 PG, PA and VG designed the study. VG supervised and run data collection, AA run  
15 464 statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data  
16 465 handling, CW and PG carried out adult TB study, GL contributed in writing the article.  
17 466 VG drafted the article and all authors contributed to the final version.  
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21 468 **Conflict of interest:** There are no competing or conflicting interests.  
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25 470 **Data Sharing:** No additional unpublished data from the study  
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28 472

473 **Table 1: The effect of exposure on mortality according to age**

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Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR <sup>#1</sup> IPT/Unexposed	MRR <sup>#2</sup> IPT/Unexposed	MRR <sup>#1</sup> No IPT/Unexposed	MRR <sup>#2</sup> No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

475 D/PYO: Deaths/person years of observation

476 Unexposed: Community sample

477 \* Include unexposed children on IPT

478 <sup>#1</sup> Mortality Rate Ratio from a model with age as underlying time479 <sup>#2</sup> Mortality Rate Ratio from a model with age as underlying time, adjusted for gender,  
480 ethnicity, district, socio-economic status, schooling of the mother and child crowding

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**Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008**

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

**Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.**

Months since exposure	Age at exposure (Months)			Total
	0-11	12-35	36-59	
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

**Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005**

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

**Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home**

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	2005-2008
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)



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3 **FIGURE LEGENDS**  
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6 **Figure 1: Illustration of the inclusion process**  
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9 **Figure 2: Flow chart of inclusion**  
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11 **Figure 3: Person years of observation (PYO)**  
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8 **1 Impact of isoniazid preventive therapy on mortality among children less than 5**  
9 **2 years old following exposure to tuberculosis at home in Guinea-Bissau**  
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12 Victor F. Gomes<sup>1</sup> MD, MSc, Andreas Andersen<sup>1</sup>, statistician, Grethe Lemvik<sup>1,2</sup> MD,  
13 Christian Wejse<sup>1,2</sup> MD, PhD, Ines Oliveira<sup>1</sup> MD, MSc, Fina J. Vieira<sup>3</sup> MD, Luis J. Carlos  
14 <sup>4</sup> MD, Cesaltina S. Vieira<sup>3</sup> MD, Peter Aaby<sup>1</sup> DSc, Per Gustafson<sup>5</sup> MD, PhD  
15  
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19 1) Bandim Health Project, Indepth Network, Bissau, Guinea-Bissau,  
20 <http://www.bandim.org>, Statens Serum Institut, Copenhagen, Denmark  
21  
22 2) Department of Infectious Diseases, Aarhus University Hospital, Denmark  
23  
24 3) Hospital de Pneumologia “Raoul Follereau”, Bissau, Guinea-Bissau  
25  
26 4) Hospital Nacional Simao Mendes, Bissau, Guinea-Bissau  
27  
28 5) Infectious Diseases Research Group, Department of Clinical Sciences, Malmö,  
29  
30 Lund University, Sweden

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39 Correspondence to:

40 Victor Francisco Gomes

41 PROJECTO DE SAÚDE DE BANDIM

42 APARTADO 861, 1004 BISSAU CODEX, GUINÉ-BISSAU

43 FAX No: +245 320 16 72

44 Phone No: +245 320 14 89/3204460; Mobile: +245 6658334

45 Email: [victorfranciscogomes@yahoo.co.uk](mailto:victorfranciscogomes@yahoo.co.uk)  
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31 **ABSTRACT**

32 **Background and objective:** In a cohort of children less than 5 years old exposed to adult  
33 intrathoracic tuberculosis (TB) in 1996-1998 we found 66% increased mortality  
34 compared with community controls. In 2005 we implemented isoniazid preventive  
35 therapy (IPT) for children exposed to TB at home, and the present study evaluates the  
36 effect of this intervention on mortality.

37 **Setting:** This prospective cohort study was conducted in six suburban areas, included in  
38 the demographic surveillance system of the Bandim Health Project (BHP) in Bissau, the  
39 capital city of Guinea-Bissau.

40 **Participants:** All children less than 5 years of age living in the same house as an adult  
41 with intrathoracic TB registered for treatment in the study area between 2005 and 2007  
42 were evaluated for inclusion in the IPT programme.

43 **Main outcome measures (end points):** The all-cause mortality rate ratio (MRR)  
44 between exposed children on IPT, exposed without IPT and unexposed community  
45 control children.

46 **Results:** A total of 1396 children were identified as living in the same houses as 416  
47 adult TB cases, of those 691 were enrolled in the IPT programme. Compared with  
48 community controls, the IPT children had a mortality rate ratio (MRR) of 0.30 (95%CI  
49 0.1-1.2). The MRR comparing exposed children with and without IPT was 0.21 (0.0-1.1).  
50 The relative mortality in IPT children compared with community controls in 2005-2008  
51 differed significantly from the relative mortality of exposed untreated children compared  
52 to the community controls in the 1996-1998 (test of interaction, p=0.01).

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8 **Conclusion:** In 2005-2008, exposed children on IPT had 70% lower mortality than the  
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10 community control children, though not significantly. Relative to the community control  
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12 children, the mortality among TB-exposed children on IPT in 2005-2008 was  
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14 significantly lower than the mortality among TB-exposed children not on IPT in 1996-  
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16 1998.  
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### 19 **Article summary**

20 This article focuses on:

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24 • [Impact of IPT on mortality among children exposed to an adult with intrathoracic](#)  
25  
26 [TB at home: comparing 1\) exposed children who received IPT to unexposed](#)  
27  
28 [children and 2\) exposed children who did not received IPT to unexposed children](#)  
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- 30 • Mortality in children exposed to TB who were enrolled on IPT compared to those  
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32 exposed but not receiving IPT in a previous study in the same setting.  
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### 35 **Strengths and limitations**

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37 Overall, evaluation of compliance showed that around 79% of the possible isoniazid pills  
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39 were taken. Given the low mortality in the cohort it was not possible to test to what extent  
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41 adherence mattered for a beneficial effect of IPT.  
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43 Mortality in the study area declined dramatically between the two study periods, and the  
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45 study therefore had much less power than originally expected. Nonetheless, results were  
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47 so marked that it was still possible to show the hypothesized inversion of the mortality  
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49 rate ratios between TB-exposed children and community controls between the pre-IPT  
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51 period and the IPT period.  
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10 77 In an intervention study in an area with a very mobile population as in Bissau, it is not  
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12 78 possible to enrol all eligible children. There are always some children travelling or absent  
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14 79 at inclusion visits. This obviously opens for the possibility of selection biases as to who  
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16 80 participated in the study. Due to the current World Health Organisation (WHO)  
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18 81 recommendation it was decided not to conduct a randomised study, hence, there are a  
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20 82 number of theoretical biases. Furthermore, the previous study was conducted 10 years  
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22 83 earlier than the present study and many things changed in the meantime. Another  
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24 84 limitation was that the children were not HIV tested, it might bias the results if there were  
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26 85 more HIV-infected children in the IPT group compared to the no IPT group. However,  
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28 86 we would then expect higher mortality in the no IPT group compared ~~with~~ the  
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30 87 community controls [in the present IPT cohort](#).  
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## 89 Introduction

90 Childhood tuberculosis (TB) is to a large degree neglected in endemic areas with limited  
91 resources, mainly because children are considered to develop mild forms of disease and  
92 to contribute little to the maintenance of the TB epidemic<sup>1,2</sup>. However, recent studies  
93 indicate that children contribute a significant proportion of the disease burden and suffer  
94 severe TB-related morbidity and mortality<sup>2</sup>. In 2011 TB incidence among children was  
95 estimate at 490,000, equivalent to about 6% of the total number 8.7 million incident cases  
96 Of the estimated 8.3 million new TB cases diagnosed in 2000, almost 900,000 were  
97 children<sup>3</sup>, and the proportion of children in the high-burden countries is estimated to be  
98 higher<sup>4,5</sup>.

99 Information on the cause of death among children in developing countries is difficult to  
100 ascertain. Most childhood deaths occur at home<sup>6</sup> and reliable medical information on  
101 causes of death is therefore lacking<sup>7</sup>. According to verbal autopsy studies, acute  
102 respiratory infection is one of the most important causes of mortality among children in  
103 low-income countries<sup>7,8</sup>. Necropsy studies conducted in Africa have shown that TB rivals  
104 acute bacterial and viral pneumonia as a major cause of death from respiratory disease in  
105 children from endemic areas<sup>9</sup>.

106  
107 An intervention known to contribute to the reduction of morbidity and mortality due to  
108 TB is isoniazid preventive therapy (IPT). Isoniazid was recommended for TB preventive  
109 therapy during the 1960s after large well conducted randomised controlled trials (RCTs)  
110 that included a total of nearly 70,000 people of all ages<sup>10</sup>. After all this time, isoniazid  
111 continues to be the drug of choice<sup>11</sup>, and WHO recommends that all TB contacts under

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8 112 the age of 5 years should receive at least 6 months of IPT. No recent meta-analysis of IPT  
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10 113 for TB exposed children in general has been made; a recent Cochrane review concluded  
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12 114 that there was not enough evidence for general recommendation of IPT for HIV-infected  
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14 115 children<sup>12</sup>. [The use of isoniazid in low income countries is limited by difficulties of ruling](#)  
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16 116 [out TB disease before initiation<sup>13</sup>, liver toxicity<sup>14-16</sup>, and poor adherence<sup>17</sup>. The use of](#)  
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18 117 [isoniazid in low income countries is, however, limited by several circumstances:](#)  
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20 118 [difficulties of ruling out TB disease in children, mainly those infected with HIV, before](#)  
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22 119 [initiation of IPT<sup>13</sup>, liver toxicity<sup>14-16</sup>, and poor adherence<sup>17</sup>. These circumstances may](#)  
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24 120 [limit the widespread use of IPT in the resource constrained settings, where provision of](#)  
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26 121 [TB care often falls short of internationally recommended standards<sup>13</sup>.](#) IPT has been  
27  
28 122 shown to be effective in recent tuberculin skin test (TST) converters and recent contacts  
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30 123 of identified cases of TB disease<sup>17,18</sup>. In one study of IPT in HIV-positive-infected  
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32 124 subjects, a 20% reduction of mortality was found in those with positive TST<sup>18</sup>. The effect  
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34 125 of such preventive therapy in children, however, is not well established.  
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36 126  
37 127 The present study examined the impact of IPT on mortality in children less than 5 years  
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39 128 of age exposed to intrathoracic TB at home in an urban area of Bissau. In a previous  
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41 129 study in the same community, we found that exposure to TB at home was associated with  
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43 130 66% excess mortality compared to community control children not exposed to TB at  
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45 131 home<sup>19</sup>. The aim of the present study was to compare mortality between exposed children  
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47 132 on IPT and community control children, and to compare this relative mortality to the  
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49 133 previously observed excess mortality.  
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8 135 **Materials and methods**

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10 136 *Setting*

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12 137 The study was conducted as a prospective cohort study from 1 September 2005 to 31  
13  
14 138 October 2007 in six areas covered by the Bandim Health Project (BHP), a demographic  
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16 139 surveillance site, located in Bissau, the capital city of Guinea-Bissau, where the  
17  
18 140 prevalence of HIV1 and HIV2 among adults was 4.6% and 4.4%, respectively<sup>20</sup>. The  
19  
20 141 population which is currently around 102,000 is followed through regular censuses and  
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22 142 registered with information on sex, ethnic background, date of birth, death and migration  
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24 143 as well as additional data on socio-economic factors. Information regarding  
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26 144 hospitalisations and deaths is collected every 3 months for children under 3 years of age.  
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28 145 Information about children older than 3 years of age and adults is obtained from general  
29  
30 146 censuses carried out approximately every 3<sup>rd</sup> year. All paediatric hospitalisations from the  
31  
32 147 study area have been registered since 1990. The incidence of adult intrathoracic TB in the  
33  
34 148 area is high, 471 per 100,000 person years<sup>219</sup>.

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36 149  
37 150 Due to difficulties in obtaining specific causes of deaths in our setting the ~~all-cause~~  
38  
39 151 cause mortality was used as the main outcome measurement of the effect of IPT.

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41 152 The reliability of population and mortality data in the study setting is a huge strength,  
42  
43 153 which makes this a unique and important study that would be difficult to duplicate in  
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45 154 other TB endemic areas.

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47 155

48 156 *Houses and household contacts*

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50 157 Houses in the study area are one-storey, rectangular constructions, usually with 6-8  
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52 158 rooms and are inhabited by 2 to 4 households (families), which can be extended families

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8 159 or not. The majority of houses do not have an internal ceiling, leaving a large gap  
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10 160 between the internal walls and the roof. Households were defined as the extended family  
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12 161 sharing the same space in the house, eating from the same pot.  
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14 162

### 16 163 **Recruitment of participants and patients**

#### 17 164 *Identification of adult TB index cases*

18 165 Since May 1996 a TB surveillance system, implemented in collaboration with the  
19  
20 166 national TB hospital (“Hospital Raoul Follereau”), has identified adult ( $\geq 15$  years)  
21  
22 167 intrathoracic TB cases using passive and active case finding<sup>219</sup>. [As previously described](#)  
23  
24 168 [in more detail<sup>219</sup>, an intrathoracic TB case was defined as an adult with symptoms of TB](#)  
25  
26 169 [with sputum smear microscopy positive or negative for acid-fast bacilli, presenting](#)  
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28 170 [abnormalities in the chest X-ray \(CXR\) with no improvement on treatment with broad](#)  
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30 171 [spectrum antibiotics for two weeks. As previously described in more detail<sup>20</sup>, a TB case](#)  
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32 172 [was defined as an adult with symptoms of TB disease with sputum smear positive or](#)  
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34 173 [negative for AFB, presenting abnormalities in CX ray with no improvement under](#)  
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36 174 [treatment with broad spectrum antibiotics for two weeks.](#)  
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#### 40 176 *Enrolment in the IPT cohort*

41 177 Children less than 5 years of age living in the house when the adult TB case started  
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43 178 treatment, were eligible for inclusion in the IPT cohort. Exposure was set to start at the  
44  
45 179 later of the following two dates: 3 month before treatment or date of registration. The  
46  
47 180 children were followed until 5 years of age contributing follow-up time until the date of  
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49 181 the last follow-up information. Children lost to follow-up were censored.  
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9 182 Prior to the initiation of IPT, the children were investigated for TB disease in a clinical  
10 183 examination for signs and symptoms using the Keith Edwards score<sup>221</sup>. If the  
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12 184 investigation suggested TB disease the children were submitted to a careful and thorough  
13  
14 185 assessment of all evidence from history, clinical examination and relevant investigations,  
15  
16 186 e.g. laboratory examination, including HIV testing, and [CXRchest x-ray](#). Broad spectrum  
17  
18 187 antibiotics were administered for 10-15 days. Children who failed to improve clinically  
19  
20 188 and radiologically after 2 weeks of broad spectrum antibiotics, and without other  
21  
22 189 explanatory disease were given a full TB treatment regimen according to the national  
23  
24 190 protocol. Antiretroviral treatment was not available at the time of the study and HIV  
25  
26 191 testing was therefore not generally performed. It was only performed when the  
27  
28 192 investigation suggested TB. Children who developed signs and symptoms suggestive of  
29  
30 193 TB disease while on IPT were evaluated and treated in a similar way.  
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32 194 Children with TB disease, those who did not give consent, who were absent from first,  
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34 195 second or third visit or at enrolment consultation were excluded from the IPT cohort.  
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36 196 There were several steps in the enrolment procedure as depicted in Figure 1. Once an  
37  
38 197 adult TB case from the study area was identified a project assistant went to the patient's  
39  
40 198 house to update the census for the families living in the house, and socio-economic and  
41  
42 199 demographic information was noted on the questionnaire. Following the census-update, a  
43  
44 200 field assistant and a nurse visited the house to apply TSTs. 48-72 hours later the house  
45  
46 201 was visited by the nurse who read the TST and referred potential TB cases for further  
47  
48 202 clinical examination. Children without TB disease were eligible for enrolment in the IPT  
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50 203 cohort regardless of the TST result and were invited to attend the enrolment visit at the  
51  
52 204 local health centre. Eligible children who did not show up at inclusion were traced again.

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9 205 If not found they were considered absent, but still followed up using basic census  
10 206 information. Due to limited time frame, logistic reasons and limited funding they were  
11  
12 207 not included later.

13  
14 208 For children enrolled in the IPT programme, isoniazid tablets were administered at 5  
15  
16 209 mg/kg/day together with Pyridoxine (Vitamin B6) tablets for 9 months. The Vitamin B6  
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18 210 dosage was 25 mg for children receiving <100 mg of isoniazid and 50 mg for children  
19  
20 211 receiving >100 mg of isoniazid<sup>232</sup>. The medicine was provided at the house every two  
21  
22 212 weeks by a field assistant. Study children were visited after 1, 4, 7 and 9 months of IPT.  
23  
24 213 The follow-up visits at 1 and 7 months were performed by the research clinician at the  
25  
26 214 local health centre and at 4 and 9 months by a field assistant at the child's home.  
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28 215 Evaluation at follow-up visits included questions about side effects and a physical  
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30 216 assessment of signs and symptoms of hepatotoxicity, e.g. jaundice and vomiting. The  
31  
32 217 cohort and study routines are described in detail elsewhere<sup>243</sup>. The initially intended IPT  
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34 218 enrolment period was September 2005 to October 2007 with 9 months of follow-up to  
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36 219 June 2008. However, children continued to be enrolled on IPT until the end of the study  
37  
38 220 period in June 2008.

39 221

#### 40 222 *Pre-IPT cohort*

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43 223 As previously described; children less than 5 years of age living in the same house as an  
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45 224 adult index TB case at the time of initiation of treatment during the period May 1996 to  
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47 225 July 1998 were retrieved from the BHP register<sup>19</sup>. To assess the impact of TB exposure at  
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49 226 home in the absence of IPT, their mortality was compared with the mortality of children  
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51 227 living in the study area who had not been exposed to TB at home, during the same period.

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10 229 *Groups in the study*

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12 230 In the Pre-IPT cohort we had two groups: TB-exposed children and community control  
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14 231 children. In the IPT cohort we intended also to have two groups: TB-exposed children on  
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16 232 IPT and community control children. As we failed to include all exposed children in the  
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18 233 IPT programme, a third group arose: TB-exposed children not on IPT.

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22 235 *Effect-size calculation*

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24 236 For the pre-IPT cohort our study protocol initially estimated 700-index cases/houses  
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26 237 during a 4½ year period. An average of 3 children < 5 years of age per house and a mean  
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28 238 follow-up time of 2.25 years (half of the 4½ year study period) would yield 2100 children  
29  
30 239 with approximately 4725 child years of observation. We anticipated the TB surveillance  
31  
32 240 system to identify an increased number of TB patients during the IPT period. An  
33  
34 241 estimated 300 index cases per year during a 2½ year period would give 750 index cases.  
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36 242 An average of 3 children < 5 years of age per house and a mean follow-up time of 1.25  
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38 243 years (half of the 2½ year study period) would yield 2250 children with approximately  
39  
40 244 2813 child years of observation. Assuming a 7% annual mortality for the pre-IPT cohort  
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42 245 we initially expected to be able to detect a 27% mortality reduction in the IPT cohort.

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44 246 Several factors reduced the actual power of the study. Due to a civil war in Guinea Bissau  
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46 247 we limited follow-up for the pre-IPT cohort to the period from February 1996 to June  
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48 248 1998 before the war. Difficulties in locating the adult TB cases became greater in the IPT  
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50 249 cohort lowering the number of identified children. In addition, <5-mortality dropped  
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52 250 considerably more than we had anticipated.

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10 252 *Ethical approval*

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12 253 The legal guardians (parents) or caregivers were informed about the study in writing

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14 254 (Portuguese) and verbally in the common language, Creol, before the child was enrolled

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16 255 in the IPT study. Informed consent was obtained from all the parents or caregivers before

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18 256 enrolment. The study protocol was approved by the Guinea-Bissau National Research

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20 257 Coordination and Ethics Committee.

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24 259 *Statistical analysis*

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26 260 Data regarding adult TB cases were obtained from the general TB identification system

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28 261 in the study area while demographic information was taken from the basic surveillance

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30 262 system of the BHP. Statistical analyses were conducted in STATA version 10.

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34 264 Similar to the analysis of the impact of TB exposure in the absence of IPT, the average

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36 265 delay from onset of symptoms to initiation of TB treatment was assumed to be 3 months.

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38 266 Hence, children registered in the same house as an adult index TB case 0-3 months before

39  
40 267 treatment were considered exposed. The effect of exposure on mortality was evaluated by

41  
42 268 rate ratios from a Cox analysis with age as underlying time. Neonatal mortality in

43  
44 269 Guinea-Bissau is very high. To be exposed a child had to be born and registered at the

45  
46 270 time of exposure. Consequently only few children were exposed before 3 months of age.

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48 271 Therefore we have chosen to commence the analyses at 3 months of age.

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8 273 IPT treatment was initiated in September 2005. To allow exposure time before IPT,  
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10 274 follow-up started in July 2005. IPT enrolment for the present study ended in October  
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12 275 2007 with treatment ending in June 2008. Thus, the study period for the present study is  
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14 276 July 2005 to June 2008. Exposed children counted as unexposed controls until the time of  
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16 277 exposure. Exposed children never receiving IPT counted as exposed without IPT from  
17  
18 278 the start of exposure to the end of follow-up. Children subsequently enrolled on IPT  
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20 279 counted as exposed without IPT from the start of exposure until the start of IPT and then  
21  
22 280 as exposed with IPT to the end of follow-up. However, enrolment into the IPT program  
23  
24 281 continued after the enrolment period of the present study. Children enrolled on IPT after  
25  
26 282 October 2007 were also counted as exposed on IPT even though they did not finish the  
27  
28 283 treatment within the study period. Censoring the children enrolled on IPT after October  
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30 284 2007 at the time of IPT had little impact on the results. Some children who were present  
31  
32 285 when the TB team visited the TB case house (figure 1) were enrolled on IPT even though  
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34 286 they were not born or registered in the house before the TB case initiated treatment.  
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36 287 According to the epidemiological definitions these children were not exposed and they  
37  
38 288 have counted as unexposed with IPT. A separate analysis was conducted excluding these  
39  
40 289 subjects.  
41  
42 290 An adjusted analysis was conducted including possible confounders related to child  
43  
44 291 mortality: gender, ethnicity, district, socio-economic status, schooling of the mother,  
45  
46 292 child crowding (<5 years) and crowding among older individuals (>5 years). A score for  
47  
48 293 socio-economic status was calculated adding house indicators (yes=1; no/missing=0):  
49  
50 294 corrugated iron roof, electricity, television and in-door toilet. A separate "Missing"  
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52 295 category was constructed when information was missing on all four variables. Crowding  
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8 296 was defined as the number of individuals in the house of the TB case on January 1, 2007,  
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10 297 the mid-point of the examined period. Crowding was included in the analysis as a linear  
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12 298 predictor.

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16 300 It was further examined whether differential mortality not related to TB exposure may  
17  
18 301 have existed in the exposed houses. Mortality was compared between children living in  
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20 302 the house of the adult TB case three years before TB exposure began and children living  
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22 303 in the remaining houses. As for the main study<sup>19</sup>, the comparison was made over a 3-year  
23  
24 304 period from July 2002 to June 2005. The period was chosen not to overlap the study  
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26 305 period.

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307 **Results:**308 *Index TB cases and included children*

309 The surveillance system registered 688 adult intrathoracic TB cases from July 2005 to  
310 June 2008 (Figure 2). Of these, 44 lived outside the study area, 48 gave no address, and  
311 122 gave a false address. Hence, a total of 474 TB cases were identified. For 42 identified  
312 cases there were no eligible children less than 5 years of age and for further 16 TB cases  
313 the children were previously exposed before the present study period began, and was  
314 therefore not included. No inclusion was conducted for a total of 55 TB cases with 156  
315 exposed children; for 31 cases the correct address was only obtained long after treatment  
316 had been initiated and for 24 cases IPT enrolment had previously been initiated in the  
317 house and a new enrolment was not initiated. Inclusion was initiated in the houses of 361  
318 TB cases with 623 exposed and 68 unexposed children getting IPT. The latter happened  
319 when children born or registered after exposure were present at inclusion. A total of 705  
320 exposed children never received IPT; 156 children from “case houses with no inclusion”  
321 and 549 children from case houses with inclusion (Figure 2). See baseline characteristics  
322 in appendix table 1. Twenty-nine children were < 5 years old when exposure started, but  
323 > 5 years old at the time of enrolment. They did therefore not receive IPT. These children  
324 counted as exposed without IPT until end of follow-up at 5 years of age. Follow-up ended  
325 before 3 months of age for 14 children leaving 691 to enter the analysis. A total of 21907  
326 children, not registered as exposed or on IPT, entered the survival analysis as controls.

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328 *TB among exposed children*

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8 329 One child was on TB treatment at the time of inclusion. TB disease was diagnosed in 2  
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10 330 children after 3 and 5 weeks of IPT, respectively. Both diagnoses were based on clinical  
11  
12 331 and ~~chest X-ray~~CXR findings. One of these children ~~tested was~~ HIV-~~positive~~-infected  
13  
14 332 and was enrolled in an antiretroviral (ARV) programme.  
15  
16 333

#### 17 334 *TB exposure and mortality*

18  
19 335 Two children died during IPT. For a 6-month old boy, hospital records stated the cause of  
20  
21 336 death as severe malaria and anaemia. The mother of a 2-year old girl, who died two  
22  
23 337 months after the initiation of the IPT program, reported that the child had had diarrhoea,  
24  
25 338 cough and fever prior to death. Antibiotics and other medications had been prescribed.  
26  
27 339 The research clinician had requested a ~~chest X-ray~~CXR, but the result was never  
28  
29 340 received. No further IPT children died during the follow-up period.  
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32  
33 342 In all, the children on IPT (exposed and unexposed) contributed with 706 Person years of  
34  
35 343 observation (PYO) in the analysis (Figure 3). The children exposed but not on IPT  
36  
37 344 contributed with 1023 PYO and the controls contributed with 30713 PYO.

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39 345 Though not statistically significant, the exposed children receiving IPT had a lower  
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41 346 mortality rate than the controls of unexposed children, the MRR being 0.30 (95% CI 0.1-  
42  
43 347 1.2) (Table 1). This estimate changed little when controlled for background factors  
44  
45 348 (Tables 1 and 2). Some children had follow-up time as exposed before starting IPT and  
46  
47 349 some exposed children did not receive IPT (Figure 1). In this group of TB-exposed  
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49 350 children without IPT, the MRR was 0.97 compared to the controls (95% CI 0.6-1.7)  
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51 351 (Table 1).

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353 There were 68 children on IPT who were not formally exposed to TB because the child  
354 was born or registered after exposure occurred in the house. Excluding these from the  
355 IPT group we observed one death from 642 person years of observation giving a MRR of  
356 0.16 (0.0-1.1). Comparing with community controls all exposed children, irrespective of  
357 whether they received IPT or no IPT, had a MRR of 0.71 (0.4-1.2).

358

359 *Comparison of mortality among TB-exposed children in the absence and presence of IPT*

360 We previously found that the excess mortality after exposure to TB only started 6 months  
361 after exposure<sup>19</sup>. We made a similar analysis for the period 2005-2008; table 3 shows the  
362 comparison of exposed children without IPT and unexposed community control children,  
363 stratified by time since exposure and age at exposure.

364 Restricted to the period after 6 months, the mortality rate relative to community controls  
365 in 2005-2008 was 0.32 (0.1-1.3) for children with IPT and 1.49 (0.8-2.9) for children  
366 without IPT. The MRR comparing exposed children with and without IPT was 0.21 (0.0-  
367 1.1).

368 It was furthermore examined whether the effect in the exposed house was caused by  
369 lower mortality unrelated to TB exposure and IPT. The analysis comparing mortality in  
370 the exposed houses 3 years earlier (2002-2005) showed that the overall mortality among  
371 children in the houses which later had TB cases was the same as the mortality in the  
372 control houses, the MRR being 1.04 (0.7-1.5) (Table 4).

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8 374 In a previous study in the same community, exposure to TB at home was associated with  
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10 375 an MRR of 1.66 (1.2-2.3) compared to community control children<sup>19</sup>. The present study  
11  
12 376 assessed whether the excess mortality could be reduced by implementing an IPT  
13  
14 377 programme. Both the MRR of 0.30 among children who received IPT and the overall  
15  
16 378 MRR of 0.71 for all exposed children in the 2005-2008 period were significantly lower  
17  
18 379 than the previously observed MRR of 1.66 (respective tests of interaction,  $p=0.01$  and  
19  
20 380  $p=0.004$ ).

21  
22 381 It should be noted that the general child mortality declined markedly between the two  
23  
24 382 periods studied (Table 5); among community controls mortality declined by more than  
25  
26 383 50%. Given that excess mortality associated with TB exposure was 66% in the period  
27  
28 384 from 1996-1998, the mortality rate directly due to TB exposure was 19/1000 of the total  
29  
30 385 48/1000. If the impact of TB exposure had been similar during the period from 2005-  
31  
32 386 2008, the TB-exposed children without IPT should have had a mortality of 15/1000, as  
33  
34 387 the unexposed children in the community, with an additional 19/1000 due to TB  
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36 388 exposure, i.e. a total of 34/1000. However, their rate was only 13/1000 (Table 5).

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39 390 **Discussion**

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41 391 In the present study we have shown an impact of the IPT programme on mortality among  
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43 392 children less than 5 years of age exposed to adult TB. Children exposed to TB in 1996-  
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45 393 1998, when IPT was not available in the area, suffered a 66% excess mortality compared  
46  
47 394 with unexposed community control children. The mortality rate ratio was inverted in  
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49 395 2005-2008 with markedly (though not significantly) lower mortality among exposed  
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51 396 children on IPT compared to the community control children. It should be emphasized

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9 397 that comparing data from different time-periods is not straight-forward, as the conditions  
10 398 have changed in many ways that cannot be completely deduced, and the results can be  
11  
12 399 biased. In our situation, the child mortality has dropped markedly between these two  
13  
14 400 time-periods. However, the excess mortality from 1996-1998 has changed to a marked  
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16 401 trend of lower mortality in 2005-2008, which cannot solely be tribute to the drop in child  
17  
18 402 mortality, and our data suggest that this is partly due to the introduction of IPT.  
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20 403

#### 21 404 **Unexpected observations**

22  
23 405 Mortality in the study area declined dramatically between the two study periods and  
24  
25 406 mortality declined more than expected among both exposed children receiving IPT and  
26  
27 407 exposed children not receiving IPT. Based on the experience from the 1996-1998 period,  
28  
29 408 TB exposure at home in the absence of IPT should have been associated with a 19/1000  
30  
31 409 person-years excess mortality.

32  
33 410 Guinea-Bissau has had one of the highest levels of child mortality in sub-Saharan Africa.

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35 411 However, despite the recent HIV epidemic, mortality rates have decreased drastically  
36  
37 412 over the last decade following the same pattern observed in the other sub-Saharan

38  
39 413 African countries<sup>254-276</sup>. The reasons for the mortality decline are not fully understood but  
40  
41 414 systematic annual vitamin A campaigns and the marked decrease in malaria incidence are  
42  
43 415 likely to have contributed.

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45 416 The strong trend toward less mortality among IPT-treated children could possibly be  
46  
47 417 linked to increased attention to these children including easier access to other forms of  
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49 418 treatment and more attention from the parents. However, this would be unlikely to  
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51 419 explain why mortality also declined more than expected among the children who did not

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8 420 receive IPT. This may suggest that all TB-associated mortality is not directly due to  
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10 421 clinical TB disease, but may be due to interactions with other infections. If the incidence  
11  
12 422 or severity of these other infections goes down, as has happened in Bissau, the mortality  
13  
14 423 associated with TB exposure would also decline.

15  
16 424 There may be a slight trend towards better outcome in the contacts receiving IPT,  
17  
18 425 although not significant. Travelling or absence was the main reason why exposed  
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20 426 children were not enrolled in the IPT program. These children may therefore have had  
21  
22 427 little contact with the TB case. Another possible reason may be selection bias so that the  
23  
24 428 group of children going through 9 months of treatment had mothers who were better able  
25  
26 429 to take care of them (although no differences in education level or socioeconomic index  
27  
28 430 was seen), or simply that the children at home at the time of inclusion were not exposed  
29  
30 431 to the possible dangers of travelling.

31  
32 432 Table 3 showed no difference in overall mortality between unexposed controls and  
33  
34 433 exposed who did not get IPT, but did indeed show an effect among the oldest children  
35  
36 434 with +12 months since exposure. With the limited number of events available we were  
37  
38 435 not able to show a overall difference in mortality which would be expected. Yet despite  
39  
40 436 the limited number of events, it was possible to show a significant effect of exposure in a  
41  
42 437 subgroup of the un-treated children, namely among those most likely affected, ie the  
43  
44 438 mobile children + 3 years of age with the longest observation time since exposure.  
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#### 47 **Interpretation and consistency with previous studies**

48  
49 441 Children exposed to TB at home had excess mortality from around 6 months after  
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51 442 exposure in the present study as well as in the previous study from 1996-1998. In both  
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8 443 studies, we showed that the TB houses 3 years earlier had exactly the same mortality as  
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10 444 community controls. It therefore seems unlikely that general social conditions in houses  
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12 445 with TB cases explain the higher mortality of TB-exposed children. Furthermore,  
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14 446 children enrolled on IPT in the IPT cohort (compared with community controls) had  
15  
16 447 significantly lower mortality than the TB exposed children not receiving IPT in the pre-  
17  
18 448 IPT cohort (compared with community controls). Hence, our results suggest that use of  
19  
20 449 isoniazid plays an important role in decreasing mortality in children exposed to TB.  
21  
22 450 Studies conducted in South Africa and Zambia have reported isoniazid to be highly  
23  
24 451 effective in reducing the mortality and incidence of TB in HIV-infected children and  
25  
26 452 adults living in an area with a high prevalence of TB<sup>14,287,298</sup>. Our findings support those  
27  
28 453 studies, but also observations made by Dr. Lincoln in the early 1950s showing that  
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30 454 isoniazid chemotherapy reduced the case fatality from primary TB among children<sup>3029</sup>.  
31  
32 455

### 33 456 **Implications and conclusions**

34  
35 457 In the period 1996-1998 an excess mortality of 66% was found in children in TB  
36  
37 458 households compared to controls. This excess mortality was reduced in the cohort of  
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39 459 children receiving IPT from 2005-2008, and the all-cause mortality in children from TB  
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41 460 households was lower than the controls, though not reaching statistical significance.  
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43 461 When comparing the mortality between the exposed children to the controls across the  
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45 462 two different time periods, a significant difference in mortality was found. Furthermore,  
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47 463 in 2005-2008 there was a trend that exposed children receiving IPT had lower all-cause  
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49 464 mortality than exposed children not receiving IPT. All our data indicate that IPT should  
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8 465 be part of the standard TB program and would have a large impact on child mortality in  
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10 466 low-income countries.

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13 468  
14 469 **Funding:** This study was supported by Sida/SAREC (Swedish International  
15  
16 470 Development Cooperation Agency/Department for Research Cooperation), grant number  
17 471 SWE-2005-111 and Danish Agency For International Development (DANIDA).

18 472  
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20 473  
21 474 **Author's contributions**  
22 475 PG, PA and VG designed the study. VG supervised and run data collection, AA run  
23 476 statistical analysis, CV, FV and LC run inclusion and follow up, IO supported data  
24 477 handling, CW and PG carried out adult TB study, GL contributed in writing the article.  
25  
26 478 VG drafted the article and all authors contributed to the final version.

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29 479  
30 480 **Conflict of interest:** There are no competing or conflicting interests.

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485 **Table 1: The effect of exposure on mortality according to age**

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Age Months	On IPT* D/PYO	Exposed No IPT D/PYO	Unexposed D/PYO	MRR <sup>#1</sup> IPT/Unexposed	MRR <sup>#2</sup> IPT/Unexposed	MRR <sup>#1</sup> No IPT/Unexposed	MRR <sup>#2</sup> No IPT/Unexposed
3-11	1/28	2/138	184/6204	1.40 (0.2-9.8)	1.44 (0.2-11)	0.51 (0.1-2.0)	0.52 (0.1-2.1)
12-35	1/306	10/509	226/14620	0.23 (0.0-1.7)	0.23 (0.0-1.6)	1.29 (0.7-2.4)	1.28 (0.7-2.4)
36-60	0/371	1/376	46/9888	0	0	0.57 (0.1-4.1)	0.65 (0.1-4.6)
All	2/706	13/1023	456/30713	0.30 (0.1-1.2)	0.30 (0.1-1.2)	0.97 (0.6-1.7)	0.98 (0.6-1.7)

487 D/PYO: Deaths/person years of observation

488 Unexposed: Community sample

489 \* Include unexposed children on IPT

490 <sup>#1</sup> Mortality Rate Ratio from a model with age as underlying time491 <sup>#2</sup> Mortality Rate Ratio from a model with age as underlying time, adjusted for gender,  
492 ethnicity, district, socio-economic status, schooling of the mother and child crowding

493

**Table 2: Adjusted analysis on the overall effect of exposure on mortality from July 2005 to June 2008**

		MRR
Exposed with IPT vs controls		0.30 (0.1-1.2)
Exposed without IPT vs controls		0.98 (0.6-1.7)
Gender	Male	1
	Female	0.93 (0.8-1.1)
Ethnicity	Pepel	1
	Balanta	1.00 (0.7-1.4)
	Manjaco/Mancanha	0.84 (0.6-1.1)
	Mandinga/Fula	0.59 (0.5-0.8)
	Others	0.88 (0.7-1.2)
	Missing	2.32 (0.8-6.5)
District	Bandim 1	1
	Bandim 2	1.01 (0.7-1.4)
	Belem	0.64 (0.4-1.0)
	Mindara	0.72 (0.4-1.2)
	Cuntum 1	0.97 (0.8-1.2)
	Cuntum 2	0.92 (0.6-1.3)
Socio-economic Score	1	1
	2	0.89 (0.6-1.3)
	3	0.98 (0.6-1.6)
	4	0.83 (0.5-1.3)
	Missing	0.87 (0.4-1.9)
Schooling of Mother	0-3 years	1
	4-6 years	0.93 (0.7-1.2)
	7-9 years	0.72 (0.5-1.0)
	10+ years	0.46 (0.3-0.7)
	Missing	1.00 (0.7-1.4)
Crowding < 5 years		0.98 (0.9-1.1)
Crowding > 5 years		1.00 (1.0-1.0)

**Table 3: Illustrating Mortality Rate Ratios comparing exposed children without IPT and unexposed children. Exposure is stratified by time since exposure and age at exposure.**

Months since exposure	Age at exposure (Months)			Total
	0-11	12-35	36-59	
0-5 months	0	1.36 (0.5-3.7)	0	0.54 (0.2-1.5)
6-11 months	2.06 (0.7-6.5)	2.18 (0.5-8.8)	0	1.65 (0.7-4.0)
12+ months	1.93 (0.6-6.0)	0	13.1 (1.7-100)	1.32 (0.5-3.5)
Total	0.93 (0.4-2.1)	1.26 (0.6-2.8)	1.06 (0.1-7.6)	0.97 (0.6-1.7)

**Table 4: Comparing children living in the house of a TB case 3 years before exposure starts. The period is from July 2002 to June 2005**

Age Months	Exposed Deaths/PYO	Unexposed Deaths/PYO	MRR
3-11	6/151	286/5849	0.82 (0.4-1.8)
12-35	20/679	357/14020	1.20 (0.8-1.9)
36-60	7/710	127/11547	0.90 (0.4-1.9)
All	33/1540	770/31418	1.04 (0.7-1.5)

Mortality Rate Ratio from a model with age as underlying time

**Table 5: Mortality rates (MR) in the two periods with studies of the impact of TB exposure at home**

	MR per 1000 PYO (deaths/PYO)	
	1996-1998	2005-2008
Children without known TB exposure	35 (526/15100)	15 (456/30713)
Exposed children without IPT	48 (41/851)	13 (13/1023)
Exposed children with IPT		3 (2/706)

Figure 1: Illustration of the inclusion process

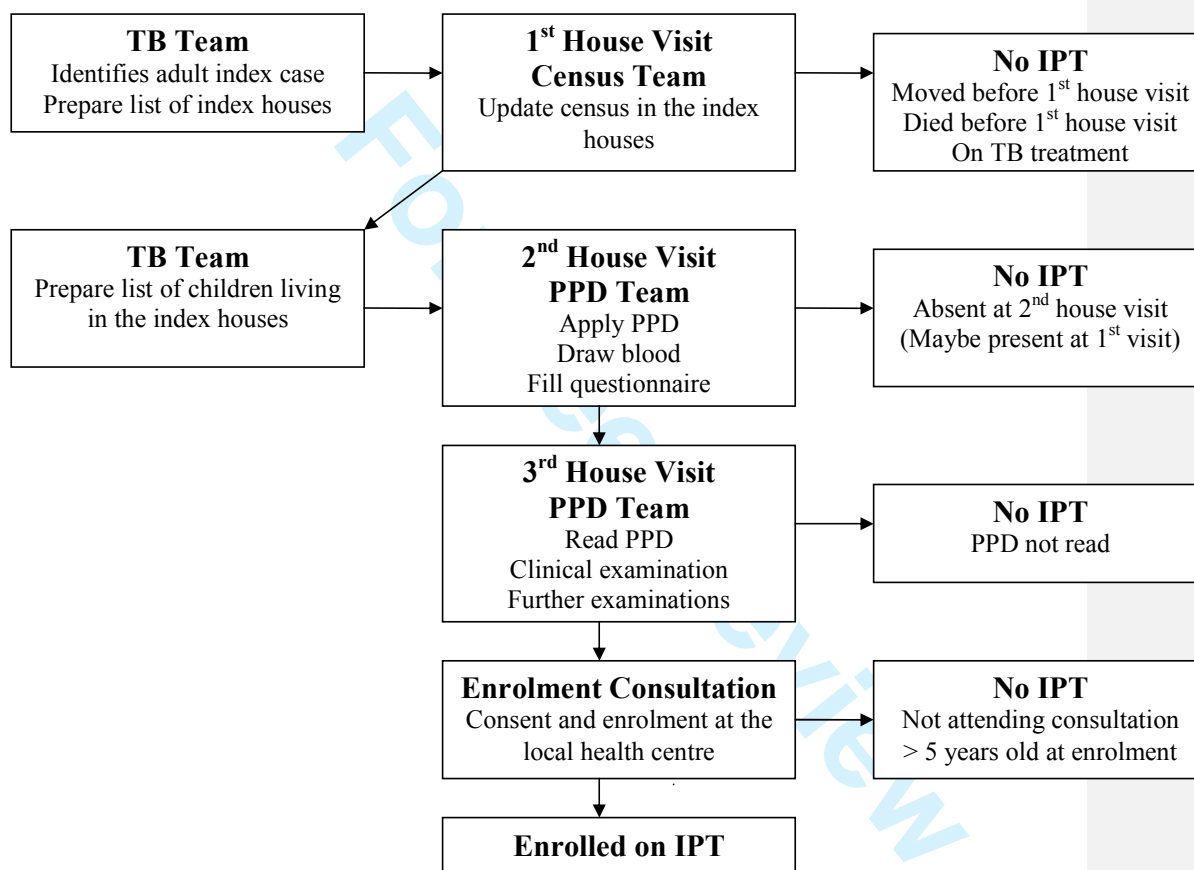
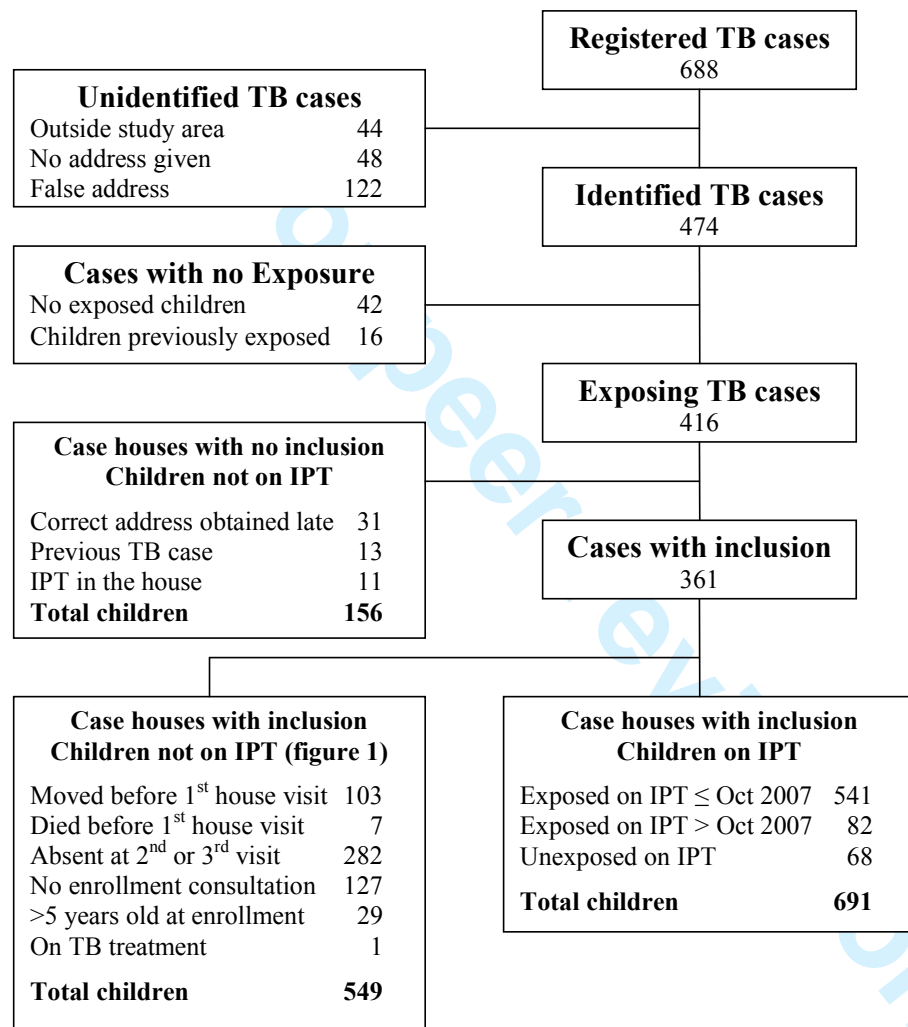


Figure 2: Flow chart of inclusion



**Figure 3: Person years of observation (PYO)**

<b>Children not on IPT</b>		<b>Children on IPT</b>	
<b>Exposed</b>		<b>Exposed</b>	
Deaths/Children	13/691*	Deaths/Children	1/623
PYO as exposed	739	PYO as exposed -IPT	284
		PYO as exposed +IPT	642
* 14 children excluded in the analysis: < 3 months at death/censoring		<b>Unexposed</b>	
		Deaths/Children	1/68
		PYO as unexposed +IPT	64



## Appendix

Table 1: Baseline characteristics

		Enrolment in the IPT program	
		Children not On IPT	Children On IPT
Number		50% (705)	50% (691)
Gender	Female	51% (337)	49% (326)
Ethnicity	Pepel	48% (178)	52% (195)
	Balanta	52% (89)	48% (81)
	Manjaco/Mancaha	48% (148)	52% (159)
	Mandinga/Fula	55% (174)	45% (141)
	Others	50% (112)	50% (113)
	Missing	67% (4)	33% (2)
District	Bandim 1	47% (220)	53% (244)
	Bandim 2	53% (96)	47% (85)
	Belem	43% (66)	57% (88)
	Mindara	43% (42)	57% (55)
	Cuntum 1	52% (139)	48% (130)
	Cuntum 2	61% (142)	39% (89)
Socio-economic Score	1	53% (40)	47% (35)
	2	53% (423)	47% (375)
	3	48% (76)	52% (81)
	4	51% (135)	49% (129)
	Missing	30% (31)	70% (71)
Schooling of Mother	0-3 years	56% (277)	44% (214)
	4-6 years	49% (155)	51% (162)
	7-9 years	52% (131)	48% (120)
	10+ years	47% (58)	53% (66)
	Missing	39% (84)	61% (129)
Crowding < 5 years	median (25 – 75 percentiles)	1 (0-2)	1 (0-2)
Crowding > 5 years	median (25 – 75 percentiles)	15 (4-22)	13 (5-24)

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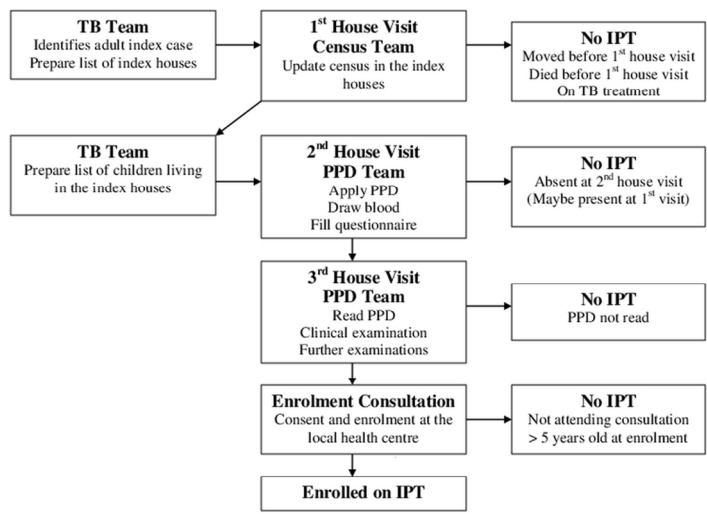
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Figure 1: Illustration of the inclusion process

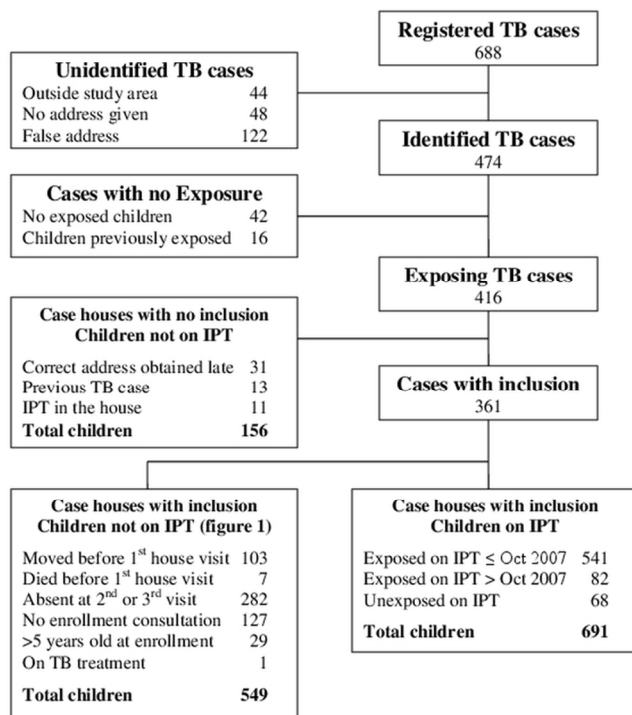


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Figure 2: Flow chart of inclusion



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**Figure 3: Person years of observation (PYO)**

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## Appendix

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