UNIVERSITAT DE BARCELONA

Epidemiological dynamics of Tuberculosis within the prison population and the community

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Epidemiological dynamics of Tuberculosis within the prison population and the community.

Doctoral thesis report dissertation presented by Victor Guillermo Sequera Buzarquis to apply for the degree of doctor at the University of Barcelona

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Llegar hasta aquí, es de afortunados.

Poder desarrollar una idea,
discutirla con expertos mundiales,
publicarla y defenderla como tesis,
es realmente de afortunados.

Esto no hubiese sido así
sin la familia que tengo,
padre, tíos, abuelos y hermanos
pero fundamentalmente la madre que me ha tocado.

Tampoco hubiese llegado hasta aquí
sin los buenos amigos,
de joven y de viejo
que yo mismo los he juntado.

Y, a los que aquí describo.
Porque esencialmente,
esta es una investigación
que trata de los no privilegiados.

Este compilado de hojas, no sería
una investigación rigurosa
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Pero definitivamente,
no iba a llegar hasta aquí,
sin Leticia a mi lado.

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Abbreviations and acronyms

AIDS	Acquired immunodeficiency syndrome
BCG	Bacillus Calmette-Guérin
COVID-19	Coronavirus disease
HIPP	WHO / Europe Health in Prisons Program
HIV	Human immunodeficiency virus
HR	Hazard ratio
INH	Isoniazid
IQR	Interquartile range
MDR-TB	Multidrug-resistant tuberculosis
MoH	Ministry of Health
MoJ	Ministry of Justice
Mtb	Mycobacterium tuberculosis
NTP	National Tuberculosis Programme
OR	Odds ratio
PAHO	Panamerican Health Organization
PC	Penitentiary Centres
PDL	People deprived of liberty
SNP	Single nucleotide polymorphism
TB	Tuberculosis
WHO	World Health Organization
XDR-TB	Extensively drug-resistant TB

List of the articles that comprise the thesis.

Thesis in compendium of publications format.

This thesis consists of 2 main objectives, 6 specific objectives, and 3 articles.

Three main articles with first authorship (In Material, Methods, and Results section).

Sequera, V. G., Aguirre, S., Estigarribia, G., Cellamare, M., Croda, J., Andrews, J. R., Martinez, L., & García-Basteiro, A. L. Increased incarceration rates drive growing tuberculosis burden in prisons and jeopardize overall tuberculosis control in Paraguay. *Scientific Reports*, (**2020)** *10* (1), 21247. https://doi.org/10.1038/s41598-020-77504-1

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3. Thesis summary

3.1 Summary in English

Epidemiological dynamics of Tuberculosis within the prison population and the community.

Introduction

The control of tuberculosis (TB) in Central America and South America has significantly progressed over the past 20 years. However, it has particularly stagnated during the second decade of the 21st century. Despite national and regional efforts, the stagnation of indicators has revealed a common pattern in some countries across the Latin American region. This pattern has been characterized by the concentration of the epidemic within prisons, coupled with a rising trend in incarceration rates across several countries in the region(1).

Despite the decline and subsequent rebound during and after the COVID-19 pandemic, as observed in several countries, Paraguay has maintained a stable TB incidence rate over the past 15 years (2). This rate typically ranges between 38 and 45 cases per 100,000 inhabitants, which translates to approximately 3,100 cases per year. During this period, healthcare institutions have made efforts to expand and universally provide free access to diagnostic, laboratory, and radiological services, as well as treatment, extending these services to the primary level of care(3).

Over the past decade, although the total number of cases in the country appears to be stable, there has been a concentration of the epidemic within the prison population. It was seen that the proportion of cases in prisons rise from approximately 7% of the total cases in 2009 to over 15% in 2018, and in recent reports, exceeding 20% of the total cases in the country (4). This concentration coincides with a doubling of the prison population, leading to overcrowding and exacerbating the TB concern. This situation may be contributing to the spread of TB to the rest of the population, undermining progress in TB control within the broader community.

Hypothesis

Thorough analysis of records from the National TB Program (NTP) and the Ministry of Justice, including the utilization of complete genomic sequencing of *Mycobacterium tuberculosis* (*Mtb*)

for cases occurring in prisons and surrounding communities, will provide us with a more precise understanding of the disease burden attributable to prisons and transmission patterns between these facilities and the broader community.

Main objective

The aim of this study is to describe and characterize the transmission dynamics of *Mtb* in penitentiary centres and the broader community in Paraguay, South America.

Methodology and Findings

In the initial stage, the thesis describes the phenomenon of TB concentration in prisons using data from the NTP. It is highlighted that the number of prisoners in Paraguay increased from 6,258 in 2009 to 14,627 in 2018 (incarceration rate, from 101 to 207 per 100,000 people), while the number of TB cases among prisoners increased by 250% (n = 192 in 2009 versus n = 480 in 2018). The annual TB notification rate among male inmates was 3,218 and 3,459 per 100,000 inmates in 2009 and 2018, respectively. The percentage of all TB cases occurring among inmates increased from 7.1% in 2009 to 14.5% in 2018. The relative risk of TB in prisons compared to the community was 70.3 (95% CI, 67.7–73.1), with a population-attributable fraction (PAF) to the general population of 9.5%. Among the 16 prisons in the country, two of them—Tacumbú (39.0%) and Ciudad del Este (23.3%)—account for two-thirds of all TB cases in prisons. TB among inmates is predominantly concentrated in those aged 20 to 34 years (77.3% of the total), double the percentage of cases for the same age group outside of prison.

At the next level, a retrospective cohort study of cases was conducted, linking the 2013 National Prison Census from the Ministry of Justice with TB notifications from the NTP spanning from 2010 to 2021. Cox proportional hazards models were used to quantify the risk of TB during and after incarceration and to identify intrinsic risk factors associated with the prison environment or the individual. During the census years, 11.6% (1,096/9,413) of individuals developed TB. Among them, 70.1% (768/1,096) were diagnosed with TB while incarcerated, and 29.9% (328/1,096) were diagnosed in the community, as per NTP records.

To estimate the risk of developing TB during and after incarceration, the study focused on five prisons, from which information on entry, release, and/or transfer dates were obtained. Within the five prisons studied, a total of 2,996 incarcerated individuals were included. The total follow-up time for this cohort was 15,341 person-years, with a median overall duration of 8.2 years (IQR: 5.63–9.01). The median time spent incarcerated was 2.25 years (IQR: 0.75–4.58). Nearly one-third (31.1%) of the prison population is renewed each year. Once released from these prisons, the median cumulative follow-up time outside of prison was 4.30 years (IQR: 0.92–7.08).

The overall notification rate was 2,940 TB cases per 100,000 person-years. We observed that 15.1% (451/2996) developed TB during the total follow-up years. Of these TB episodes, 58.1% (262/451) occurred during incarceration, and 29.7% (78/262) of them occurred during the first incarceration. More than two-fifths (41.9%, 189/451) of the recorded TB cases occurred outside of prison, with 35.4% (67/189) of them occurring after their first incarceration. The overall TB notification rate for the study population was 2,940 per 100,000 person-years. This rate increased with the duration of incarceration from 1,335 per 100,000 person-years in the first year of incarceration to 8,455 per 100,000 person-years after 8 years. Among individuals with a history of incarceration, the TB rate decreased from 1,717 in the first year after release to 593 per 100,000 person-years after 8 years of follow-up. However, it remained more than 10 times higher than the TB incidence rate of the general population.

In a third stage, a prospective genomic surveillance study was conducted, by sequencing *Mtb* genomes within and outside prisons in the two largest urban areas of Paraguay, Asunción, and Ciudad del Este. The study was carried out between 2016 and 2021. Of the samples meeting the criteria for genomic sequencing, 158 were from individuals diagnosed with TB while in prison, and 330 were from individuals diagnosed with TB in the community. Isolates of TB were collected in Asunción (274/488) and Ciudad del Este (214/488). There were 17 isolates excluded, with evidence of mixed infection with more than one detected sub-lineage, resulting in a final dataset of 471 *Mtb* isolates for analysis. Within these samples, genomic evidence was detected, indicating frequent recent transmission within prisons, as well as transmission connections extending across both prisons and adjacent populations. Moreover, a consistent signal of TB spread between urban areas was observed and emphasized the recent growth in population size within the three largest genomic transmission clusters. Around 78% (369/471) of all isolates were found within 26 genomic groups (each containing 2 to 159 isolates) defined by a threshold of 12 SNPs. In these samples, genomic evidence of frequent recent transmission within prisons and transmission links spanning both prisons and neighbouring populations was found. A signal of frequent spread of *Mtb* was identified, between urban areas and highlighted the recent expansion of the population size of the three largest genomic transmission clusters.

Conclusion

This thesis presents an in-depth epidemiological portrait supported by genomic sequencing, describing the transmission dynamics of *Mtb* within prisons and from prisons to the community, aiding in the identification of priorities for Paraguay's National TB Program (NTP).

The phenomenon of TB concentration within the country's prisons and how this pathology extends beyond the prison walls due to the high turnover of the prison population was described. Increases in TB in prisons determine TB epidemics globally. Through genomic sequencing, it was observed that most of TB cases included in the genomic study were likely attributable to recent transmission. There were identified three dominant clones that have expanded dramatically over the past twenty years, encompassing both prisons and surrounding communities.

It was found a pattern of close genomic relatedness between *Mtb* sampled within and outside of prisons. Together, the findings underscore the urgency of strengthening TB control programs with a high focus on reducing transmission risk both inside and outside of prisons. It is imperative to give due weight to the history of incarceration, where the incidence is several times higher than outside prisons, not only in Paraguay but also in various countries across Central and South America.

3.2 Resumen en Castellano

Dinámicas epidemiológicas de la Tuberculosis entre las prisiones y la comunidad.

Introducción

El control de la tuberculosis (TB) en Centroamérica y Sudamérica ha avanzado de manera considerable desde finales de los 90 y durante los últimos 20 años. Pero particularmente durante la segunda década del siglo XXI se ha estancado. Este estancamiento de los indicadores, a pesar de los esfuerzos nacionales y regionales, ha expuesto un patrón común en algunos países de la región latinoamericana. El patrón común ha sido la concentración de la epidemia en las prisiones, acompañado de un proceso de aumento de los niveles de tasas de encarcelamientos en varios países de la región(1).

A pesar del descenso y el repunte posterior durante y después de la Pandemia por COVID-19, como ocurrió en varios países, Paraguay es un país que tiene una tasa de incidencia de TB estable durante los últimos 15 años, que ronda entre 38 y 45 casos por 100.000 habitantes, lo que equivale a una media de 3100 casos por año(2). Durante este periodo desde las instituciones de salud se ha hecho el esfuerzo de expandir y universalizar gratuitamente el acceso al diagnóstico, laboratorial y radiológico, y al tratamiento, llegando hasta primer nivel de atención(3).

Durante la última década, aunque la cantidad de casos totales en el país pareciera ser estable, ocurrió una concentración de la epidemia en la población penal, pasando de valores de cercanos al 7% del total de casos en prisiones en el 2009 a superiores al 15% en el 2018, y en últimos informes por encima del 20% del total de casos del país(4). Esta concentración ocurre en un proceso también de duplicación de la población penal, generando hacinamiento y potenciando el problema de la TB, el cual puede estar condicionando un derrame de este problema al resto de la población, torpedeando los avances del control de la enfermedad en el resto de la comunidad.

La urgencia de focalizar estrategias para interrumpir la transmisión y prevenir nuevas infecciones dentro y fuera de los centros penales es fundamental. Sin embargo, aún se desconoce la dimensión que juegan las instituciones penitenciarias a sus poblaciones aledañas en la transmisión. En tal sentido, esta tesis tiene el objetivo de describir el riesgo para desarrollar TB en prisión, sus factores asociados, cómo persiste este riesgo una vez que se sale de prisión, además de detallar filogenéticamente cómo se generan clústeres comunes entre la prisión y la comunidad.

Hipótesis

Un análisis profundo de los registros del Programa Nacional de Tuberculosis (PNT) y del Ministerio de Justicia, incluyendo la secuenciación genómica completa de *Mycobacterium tuberculosis (Mtb)* para casos ocurridos en prisiones y comunidades circundantes, nos proporcionará una comprensión más precisa de la carga de enfermedad atribuible a las prisiones y los patrones de transmisión entre prisiones y comunidad en general.

Objetivo

El objetivo principal de esta tesis es caracterizar las dinámicas de transmisión del *Mtb* entre los centros penitenciarios y el resto de la comunidad en Paraguay, Sudamérica.

Metodología y Resultados

En una primera etapa de esta tesis, se describió el fenómeno de concentración de la TB en las prisiones con datos del PNT donde destacamos que el número de presos en Paraguay aumentó de 6.258 en 2009 a 14.627 en 2018 (tasa de encarcelamiento, de 101 a 207 por 100.000 personas), mientras que el número de casos de TB entre los presos aumentó en un 250% (n = 192 en 2009 versus n = 480 en 2018). La tasa anual de notificación de TB entre los reclusos varones fue de 3218 y 3459 por cada 100.000 reclusos en 2009 y 2018, respectivamente. El porcentaje de todos los casos de TB ocurridos entre reclusos aumentó del 7,1 % en 2009 al 14,5 % en 2018. El riesgo relativo de TB en las cárceles en comparación con la comunidad fue de 70,3 (IC del 95 %, 67,7–73,1); el riesgo atribuible poblacional (PAF) a la población general fue del 9,5%. Entre los 16 centros penitenciarios del país, dos de ellos -Tacumbú (39,0%) y Ciudad del Este (23,3%)- representan dos tercios de todos los casos de TB en las cárceles. La TB entre los reclusos se concentra predominantemente en los de 20 a 34 años (77,3% del total), el doble del porcentaje de casos para el mismo grupo de edad fuera de prisión.

En un siguiente nivel se realizó un estudio cohorte retrospectiva de casos donde se vinculó el Censo Nacional de Prisiones de 2013 del Ministerio de Justicia con las notificaciones de TB del PNT de 2010 a 2021. Usamos modelos de riesgos proporcionales de Cox para cuantificar el riesgo de TB durante y después del encarcelamiento e identificar factores de riesgo intrínsecos de la prisión o del individuo. Durante los años al censo, el 11,6% (1096/9413) enfermó de TB. De estos, el 70,1% (768/1096) fueron diagnosticados con TB en prisión y el 29,9% (328/1096) fueron diagnosticados en la comunidad, según los registros del PNT.

Para estimar el riesgo de enfermar de TB durante y después del encarcelamiento, el trabajo se concentró en cinco prisiones, de las cuales se contaba con información sobre las fechas de

ingreso, liberación y/o traslado. Dentro de las cinco cárceles del estudio estaban censadas 2996 personas privadas de libertad. El tiempo total de seguimiento de esta cohorte fue de 15.341 años-persona, con una duración mediana general de 8,2 años (RIC: 5,63–9,01). La mediana de tiempo de encarcelamiento fue de 2,25 años (RIC: 0,75-4,58). Casi un tercio (31,1%); de la población penitenciaria se renueva cada año. Una vez liberados de estas prisiones, la mediana de seguimiento acumulado fuera de prisión fue de 4,30 años (RIC: 0,92-7,08). La tasa general de notificación fue de 2.940 casos de TB por 100.000 personas-año. Observamos que el 15,1% (451/2996) desarrolló TB durante el total de años de seguimiento. De estos episodios de TB, el 58,1% (262/451) ocurrieron durante el encarcelamiento y el 29,7% (78/262) de ellos durante el primer encarcelamiento. Más de dos quintas partes (41,9%, 189/451) de los casos de TB registrados ocurrieron fuera de la prisión, y el 35,4% (67/189) de ellos ocurrieron luego de su primer encarcelamiento. La tasa general de notificación de TB para la población de estudio fue de 2.940 por 100.000 personas-año. Esta tasa aumentó con la duración del encarcelamiento de 1.335 por 100.000 personas-año en el primer año en prisión a 8.455 por 100.000 personas-año después de 8 años. Entre las personas con antecedentes de haber estado en prisión la tasa de TB disminuyó de 1.717 en el primer año después de la liberación, a 593 por 100.000 personas-año después de 8 años de seguimiento, pero se mantuvo más de 10 veces mayor que la tasa de incidencia de TB de la población general.

En una tercera etapa se realizó una vigilancia genómica de manera prospectiva, secuenciando genomas de *Mtb*, dentro y fuera de las prisiones en las dos áreas urbanas más grandes de Paraguay, Asunción y Ciudad del Este. Este trabajo fue realizado entre 2016 a 2021. De las muestras que cumplían los criterios para someter a secuenciación genómica, 158 eran de personas diagnosticadas de TB mientras estaban en prisión y 330 eran de personas diagnosticadas de TB en la comunidad. Se recolectaron aislamientos de TB en Asunción (274/488) y en Ciudad del Este (214/488). SE excluyeron 17 aislamientos con evidencia de infección mixta con más de un sublinaje detectado, lo que finalmente resultó en 471 aislamientos de *M. tuberculosis* con los cuales se realizaron los análisis. El 78% (369/471) de todos los aislamientos se encontraban dentro de 26 grupos genómicos (cada uno de los cuales incluía de 2 a 159 aislamientos) definidos por un umbral de 12 SNP. En estas muestras se encontró evidencia genómica de transmisión reciente frecuente dentro de las prisiones y vínculos de transmisión que abarcan cárceles y poblaciones aledañas. Se identificó una señal de propagación frecuente de *Mtb* entre áreas urbanas y se pudo señalar la expansión reciente del tamaño de la población de los tres grupos de transmisión genómica más grandes.

Conclusión

Esta tesis es un retrato epidemiológico profundo con apoyo de la secuenciación genómica que describe dinámicas de transmisión del *Mtb* en prisiones, y desde las prisiones a la comunidad, los cuales ayudan a identificar prioridades para el PNT de Paraguay y la región.

Se ha descrito el fenómeno de concentración de la TB en las prisiones en el país, sus determinantes y cómo esta patología se expande por fuera de la prisión, debido a la alta rotación de la población penal. El aumento de la TB en las prisiones determina el aumento de la epidemia de TB también en la población general.

A través de la secuenciación genómica se observó que la mayoría de los casos de TB incluidos en el estudio genómico probablemente eran atribuibles a una transmisión reciente y se identificaron tres clones dominantes, que se expandieron dramáticamente en los últimos veinte años abarcando prisiones y comunidades circundantes.

Se encontró un patrón de estrecha relación genómica entre *Mtb* muestreado dentro y fuera de las prisiones.

Juntos, los hallazgos resaltan la urgencia de fortalecer los programas de control de la TB con alto enfoque en reducir el riesgo de transmisión dentro y fuera de las cárceles, dando la dimensión que corresponde al antecedente de haber estado en prisión, donde tanto en Paraguay, como en diversos países de Centro América y Sudamérica, la incidencia es varias veces mayor que fuera de las cárceles.

4. Introduction

4.1 Background

TB in prisons has been a long-standing issue, but in recent decades, it has been observed to be increasingly prevalent worldwide. The prevalence of TB in prisons and its subsequent impact on the broader community is increasingly being elucidated, with certain regions of the world identifying it as the primary catalyst behind the epidemic. Reducing the factors that promote the transmission of TB within prisons has implications both within and beyond the prison system.

The approach should be broad, involving multiple sectors, rather than limited to the response of national TB control programmes. The objective of this section is to provide an overview of the current global status of this significant public health issue. I examine successful control strategies and highlight the primary challenges faced by national TB control programmes in mitigating the escalating trend of the TB epidemic within prisons.

4.2 TB and prisons: an enduring, pernicious partnership

The nexus between prisons and TB is not new. This ancient disease has been linked to prisons for >200 years. The industrial revolution brought with it the institutionalisation of prisons in many western cities, and here *Mtb* found the ideal conditions to flourish as one of the leading causes of disease and death until well into the 20th century (5–7) (Supplemental material 1).

The second half of the last century saw significant progress in TB control, but prisons were again at the centre of the TB epidemic in the last two decades of that century (8). The emergence of the HIV epidemic and the rise in illicit intravenous drug use exacerbated the issue, causing a resurgence of TB. The problem was further complicated by the emergence of drug-resistant cases, which coincided with the dissolution of the

Soviet Union and the birth of newly independent states lacking proper prison systems and effective national TB control programmes (9). All of this created a challenging situation for TB control, particularly in countries with large prison populations (10–12).

Due to these issues, in the final decade of the 20th century, 28 countries of the European region of the WHO formed a network dedicated to improving prison health. The Health in Prisons Program Europe is the only WHO network on this topic; it does not exist for other regions. It has greatly facilitated the exchange of experiences, the evaluation of the impact of various interventions and the development of best practices for the prison environment (13,14).

Asia houses most countries with the highest number of prisoners globally. The countries leading the list include China, India, Indonesia, Thailand, Iran, and the Philippines. However, the rate of incarceration is generally lower (15). Among the 30 countries with the highest incarceration rates globally, Turkmenistan and Thailand have high rates of 570 and 411 prisoners per 100 000 people, respectively, placing these two countries alone in that category. It is worth noting that the incidence rates of TB in prisons are high, especially in South-East Asian countries (Figure 1) (16,17).

The continent of Africa does not have the highest incarceration rates in the world. The countries of Rwanda, Namibia, Cabo Verde and South Africa have the highest rates in the region, ranging from 580 to 250 prisoners per 100 000 of the population (18–20). However, these countries are not among the top 30 nations with the highest incarceration rates globally. The incidence of TB in prisons is substantial, but the magnitude of the TB issue outside prisons is more significant. There are studies conducted in sub-Saharan countries that demonstrate how overcrowding and inadequate active case finding have amplified the risk of TB transmission in prisons (21,22).

In the Americas, especially in the USA, the problem is the high level of incarceration of its population, which has been growing steeply since the 1980s (23). It is currently the country with the second highest number of people deprived of liberty (PDL) behind China and has some of the highest incarceration rates in the world. Labelled the prison–industrial complex, the USA has ∼8% of its prison population in private prisons and the

rest in the federal system. In both, mass incarceration is associated with TB, and TB rates among incarcerated people are five times what they are outside prisons (24–26). Latin America and the Caribbean (LAC) have experienced the greatest increase in incarceration rates over the past decade, which has in turn amplified the TB epidemic in broader communities (1). LAC saw substantial progress in reducing the burden of the TB epidemic during the first 10 years of this century, but the increase in TB in prisons has been undermining the achievements of national TB control programmes in the region. In addition to the recent increase in the prison population in the last years, prisons in LAC are characterised by overcrowding, high levels of violence and subhuman conditions for prisoners, an explosive mix that today fuels the spread of TB in prisons and ultimately into their surrounding communities.

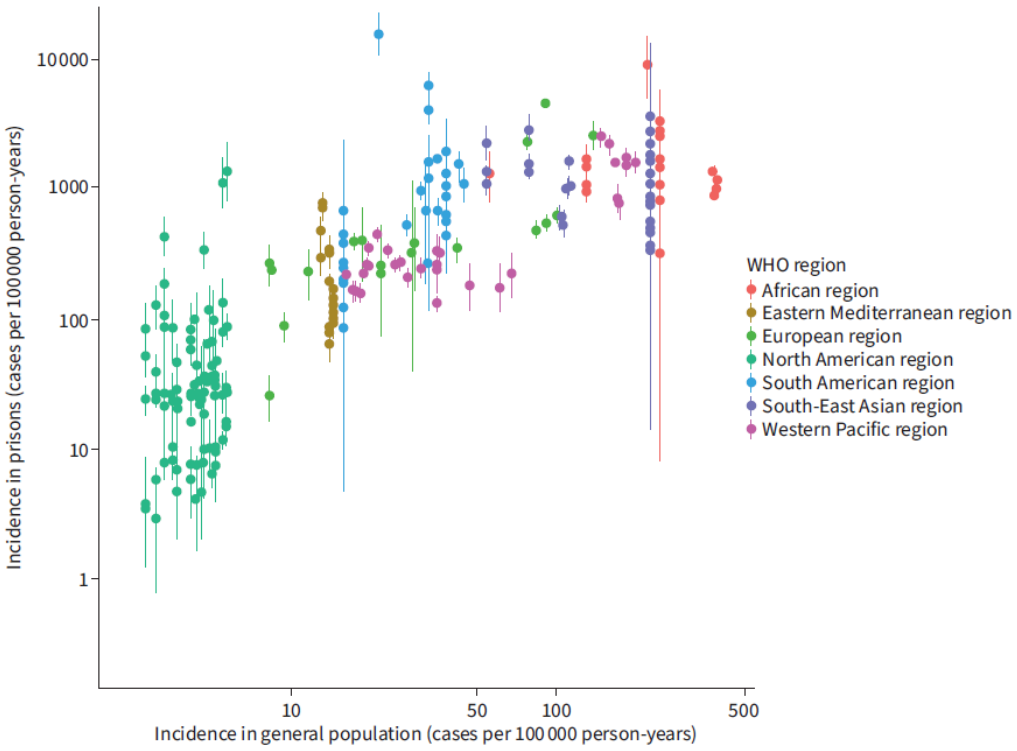

FIGURE 1. Relationship between study-specific TB incidence in prisons and TB incidence in the general population. Incident rate ratio in prisons versus the general population: 4.1 in North America, 12.6 in Africa, 11.7 in South-East Asia and 26.9 in South America (27)

A study conducted by Cords et al. (27) revealed an intriguing finding on the ratio between the incidence of TB in prisons and the general population. According to this study, LAC countries exhibit values that are two to five times higher than other regions

across the globe. These effects of prisons amplifying community TB epidemics, well described in Latin America, probably affect Asia and Africa as well (28,29).

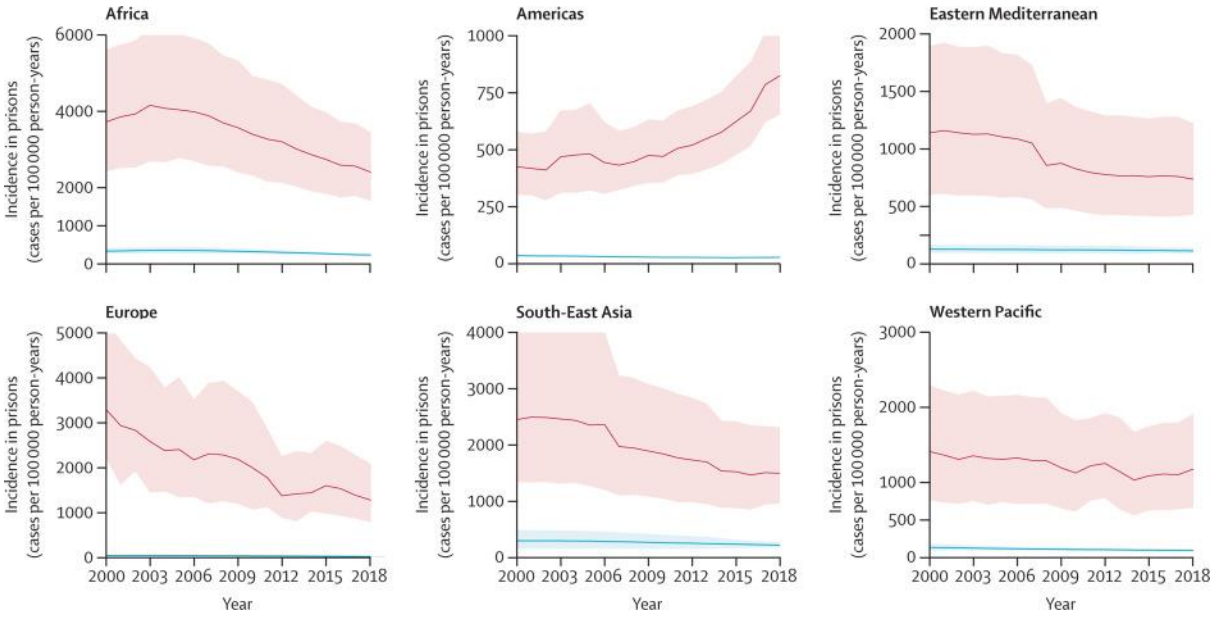

FIGURE 2 Trends in tuberculosis incidence estimates among incarcerated individuals compared with the general population for each WHO region from 2000 to 2019 (30).

4.3 Epidemiology of incarceration and TB

There were an estimated 11 million PDL in the world in 2022, but it is likely that two or three times this many people pass through prisons every year (31). In the last two decades, global incarceration increased by ~24%, but the region with the greatest increase was LAC, with an increase of >200% in the same period. The largest increases were seen in countries such as El Salvador (411%) and Ecuador (367%) (31). This sharp increase in incarceration has outpaced growth in the capacity of the prison environment and health services, leading to rising overcrowding and inadequate capacity to meet health needs (1,27) (Supplemental material 2).

The overall incidence of TB in prisons exceeds the incidence in the general population by 10 to 100 times, varying by country (Figure 2 and 3). Furthermore, data on TB in prisons are not always reported to ministries of health (32). South American countries,

on average, have more than 25 times the incidence of TB in their prisons than in the general population, followed by Africa, the Eastern Mediterranean and the South-East Asian region (27). The median estimated population attributable fraction of TB to prison exposures was 8.5% (interquartile range (IQR) 1.9–17.9%) in high-income countries and 6.3% (IQR 2.7–17.2%) in low–middle-income countries (LMICs) (1,33).

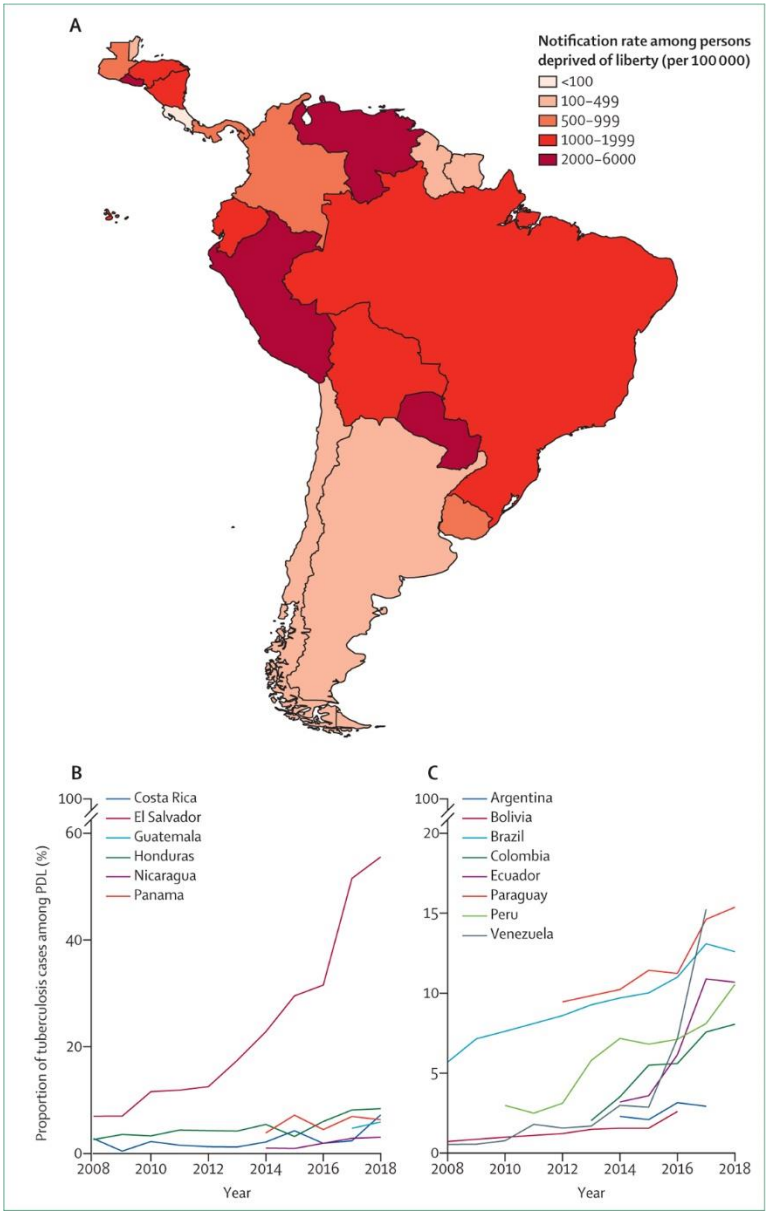

FIGURE 3. Increasing burden of tuberculosis among PDL in central and South America (A) TB case notification rate among PDL (from 2008 to 2018). The percentage of tuberculosis notifications among PDL in central America (B) and South America (C) from 2008 to 2018. Countries with more than ten cases among PDL are included for central America and more than 100 cases among PDL for South America. Time series of case notifications among PDL differ across countries. PDL=persons deprived of liberty (1).

As stated by Tavoshi et al. (34), “Confinement does not contain the disease”, based mainly on the fact that a prison population is characterised by high turnover. For instance, in an Irish prison, following the identification of only two TB cases among inmates, intense active case finding was undertaken, and molecular epidemiological evaluation led to the identification of more than 30 confirmed cases among prisoners, prison staff and in the community (35). In another study conducted in Uganda, it was shown that people with a history of imprisonment (even for a short time) were twice as likely to develop TB, and the population attributable fraction of incarceration-associated TB in this community was substantial (26%, 95% CI 20–32%) (36).

The incidence of TB among former prisoners is high. In a case–control study associated with molecular methods, Sacchi et al. (37) saw that, over the course of 4 years in a medium-sized city in Brazil, a quarter of TB cases were found to have occurred among prisoners, who made up <1% of the general population. This study revealed that former prisoners had 23% more TB cases in comparison with the general population. In the case of ex-prisoners who contracted TB, 83% (10 out of 12) were diagnosed within the first 2 years following their release from prison, indicating that they were probably infected in the prison environment (37). The same study showed that among the 97 M. tuberculosis isolates for which restriction fragment polymorphism (IS6110) genotyping was performed, 79 were classified into 17 clusters (37). Ten of these 17 groups (82%) were composed of PDL and community members, including ex-prisoners. About 41% of the community isolates were genetically like prisoner or ex-prisoner isolates, and Bayesian phylogenetic analysis showed a new clonal TB outbreak that spread to both prisons and the community. These results demonstrated a high degree of connectedness between M. tuberculosis strains circulating in prisons and in the community, indicating the role of a reservoir and transmission amplifier played by prisons, as proposed previously by Sacchi et al. (37) and Basu et al.(38).

The risk of developing TB increases during incarceration and remains elevated for years after release. A recent study revealed that TB incidence after 1 year in prison was 111 per 100 000 of the population, increasing to 1303 per 100 000 after 5.2 years in prison.

Once released, the rate declined to 229 per 100 000 and after 7 years, it declined to 42 per 100 000 but was still higher than the general population (Figure 4) (39).

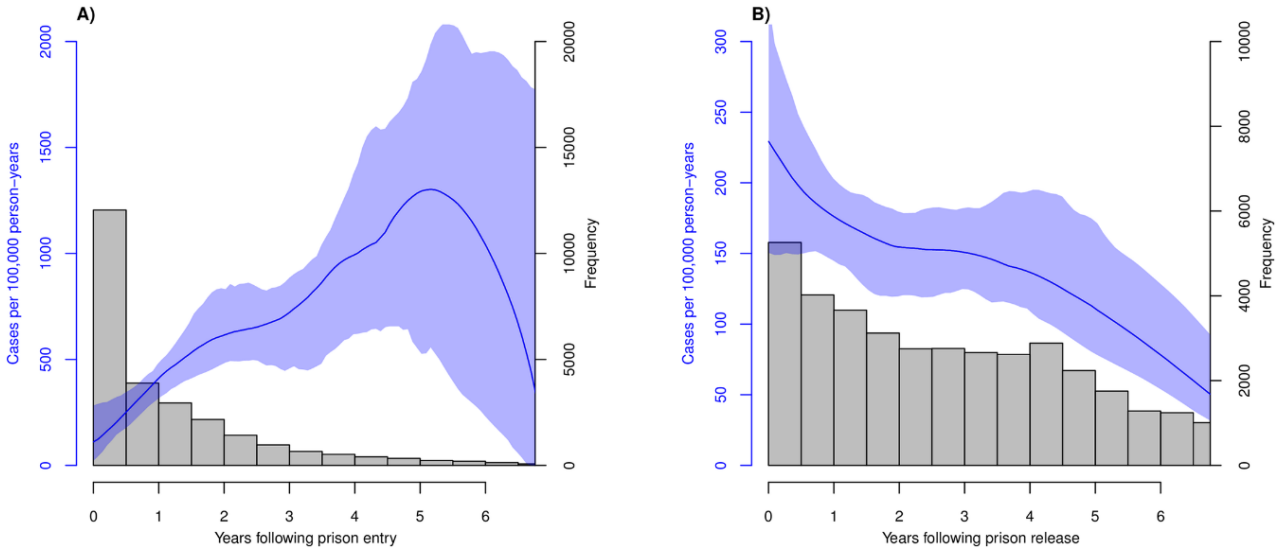

FIGURE 4. Incidence of TB among (A) Mato Grosso do Sul prisoners and (B) ex-prisoners based on length of incarceration and length of time following incarceration, respectively, with 95% bootstrap confidence intervals in blue shading. Histograms of individuals included in each survival analysis are overlaid (39).

The spillover of TB from prisons to the community is a phenomenon that has been well described in Brazil, Peru and Paraguay, (4,37,39–41). Studies reveal that the concentration of epidemics in prisons can cause a spillover effect on neighbouring communities, resulting in genetic epidemiology clusters among PDL, formerly incarcerated people and those who have never been to prison. In Paraguay, 50% of these clusters involved both PDL and non-PDL (including individuals with no prior incarceration), while former PDL accounted for 85% of TB cases with recent transmission evidence found within these cluster (42,43). Spillover also occurs with cases of MDR-TB, as described in Lima, where non-PDL patients have MDR-TB strains that are genetic matches to strains collected from current PDL with MDR-TB (40). A recent study in Thailand found that formerly incarcerated TB patients were 4.7 times more likely than nonincarcerated TB patients to be linked with other patients in large transmission clusters (29). This association suggests that their genotypes could have circulated in prisons and spread to the community.

Most TB cases in prison are the result of recent infection; genomic epidemiology studies conducted in Brazil and Paraguay have demonstrated the similarity between the clusters occurring in prison and explain the impact of recent transmission in this context (42,43).

The majority of PDL belong to groups that are already more vulnerable in society, including the poor, people who use drugs, indigenous populations, LGBTQ+ minorities, and immigrants (44,45). This is exacerbated by the fact that their pre-incarceration circumstances already place them in a group with a higher incidence than the general population. Additionally, it should be noted that, in many instances, incarceration is the first opportunity for an individual to interact with the health system(44,46).

Beyond individual risk factors, the prison environment contributes greatly to the transmission of TB because of structural factors, whereby people are confined in overcrowded, poorly ventilated cells and are underfed, with limited access to medical care and poor diagnostic capacity. An ecological study with data from more than 5500 Brazilian municipalities showed that prison is an independent factor associated with a higher incidence of the disease and is as important as income inequality and poverty (47).

To evaluate the role of the prison environment in TB transmission, Urrego et al. (48)collected architectural and environmental data from 141 cells in three prisons in Brazil. Using a Wells–Riley-based model of TB transmission, they found that enhanced passive diagnosis alone would have minimal impact on transmission. By aggregating inmate movement data in two of the study prisons, they constructed dynamic contact networks, combined with TST conversion and TB incidence. The risk of TST conversion was determined primarily by: 1) daily TB exposure in a cell; and 2) the smear result of the case, with a three times higher risk for smear-positive case contacts. This confirmed the influence of the prison environment on disease transmission (48).

Re-entry to prison is also very frequent, and some studies show that one-third of the prison population have been in prison previously (49). The flow in and out of a prison can reach three to five times the stable size of the prison in a single year (50). This

explains how prisons are intensely linked to the population to which they belong (figure 5) (51–53).

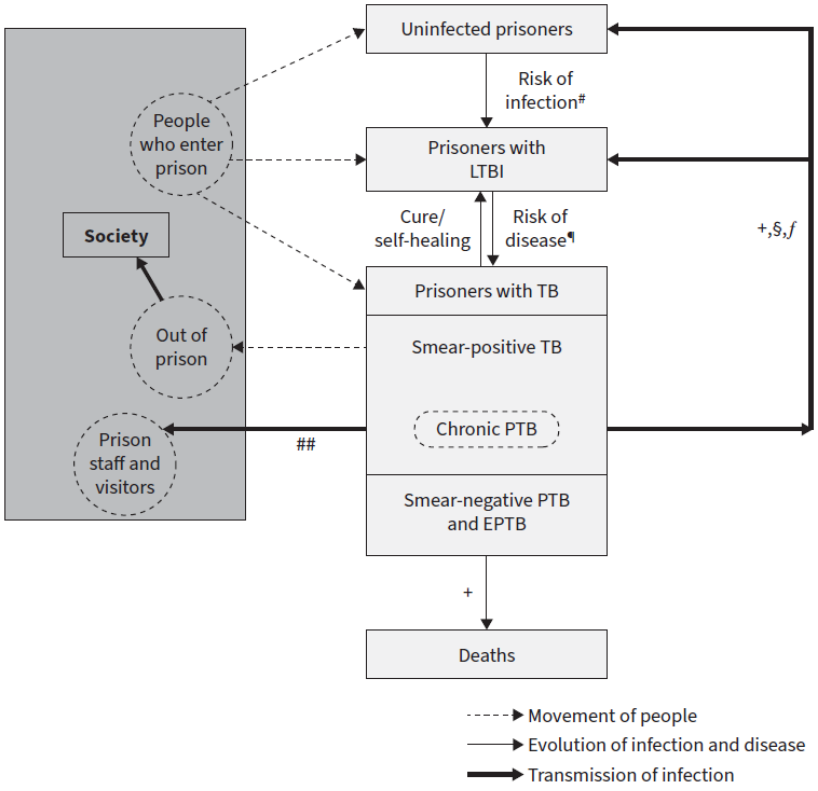

FIGURE 5. Transmission of TB inside and beyond prison. #: prison conditions increase the risk of TB transmission; ¶: disadvantaged socioeconomic background, HIV infection and prison conditions (e.g. poor nutrition); +: result of delayed diagnosis and treatment; §: overcrowding, poor ventilation, and poor hygiene; ƒ: transfer between and within prisons; ##: lack of coordination between prison and general health services. (50)

4.4 TB genomic surveillance to prisons and community

Mtb subtyping has been possible since 1990 using various DNA fingerprinting technologies(54,55). The identification of closely related strains makes possible to detect clusters of cases that may be linked to recent transmission, cases requiring additional investigation and potential intervention. Whole Genome Sequencing (WGS) offers significantly superior subtyping resolution than older equipment, thus providing greater confidence in involved case relationships(56).

Using WGS for TB investigations has also been proven to be more effective in several high-income countries such as: UK, Canada, and Netherlands(54,57,58). WGS helps define outbreaks more precisely, provides insights into transmission dynamics, and sometimes identifies previously undetected cases, as well as possible "super-spreaders," that should be isolated and treated first. Among other things, WGS can be used to determine whether recurrent cases are the result of reactivation or reinfection, which is helpful in the evaluation of a NTP's effectiveness(54,59).

Among moderate to high burden TB countries that includes mainly low an medium-income levels, the introduction of this capability can prove highly beneficial in prioritizing specific investigations(60). In particular, those related to next-generation sequencing (NGS) for expanded or mass use from sputum samples with positive bacilloscopy, which are already being conducted in many research fields(61,62). However, their costs and technical complexity avoid them from being routinely used in some places. By facilitating implementation and making it more cost-effective, this technology can enable rapid inference of susceptibility to certain drugs, as well as identify recent transmission phenomena and estimate the impact of certain lineages at the population level. In addition to supporting early treatment with appropriate medications, techniques such as NGS will reduce the need for routine phenotypic testing, which is complex, slow, and difficult to maintain in laboratory settings with limited funds and resources(54).

The significant gaps in TB incidence between prisons and surrounding communities are well-documented. However, directly linking TB cases in the community to transmission from prisons presents challenges. Most prior studies addressing this issue have relied on classical epidemiological associations. Genomic surveillance in these settings assists to identify overflow events more accurately, allowing for a better assessment of the impact of high transmission environments (such as hospitals, mining work, and prisons) on TB transmission in the general population.

In Central West Brazil, close to Paraguay, during 2014 to 2019 were performed an interesting experience about linked *Mtb* genomic surveillance to detailed individual-level incarceration background, to build high-resolution population-wide TB

transmission, in a prison-to-community TB disparity of extreme proportions(42). This study evidenced that the genome sequences of TB cases among the incarcerated population and the community exhibit significant similarity, closely aligning in evolutionary terms, and sometimes even reaching identical matches. The *Mtb* clones identified encompassed both incarcerated individuals and the community, while the genomic clusters included individuals with and without a history of incarceration (Figure 6).

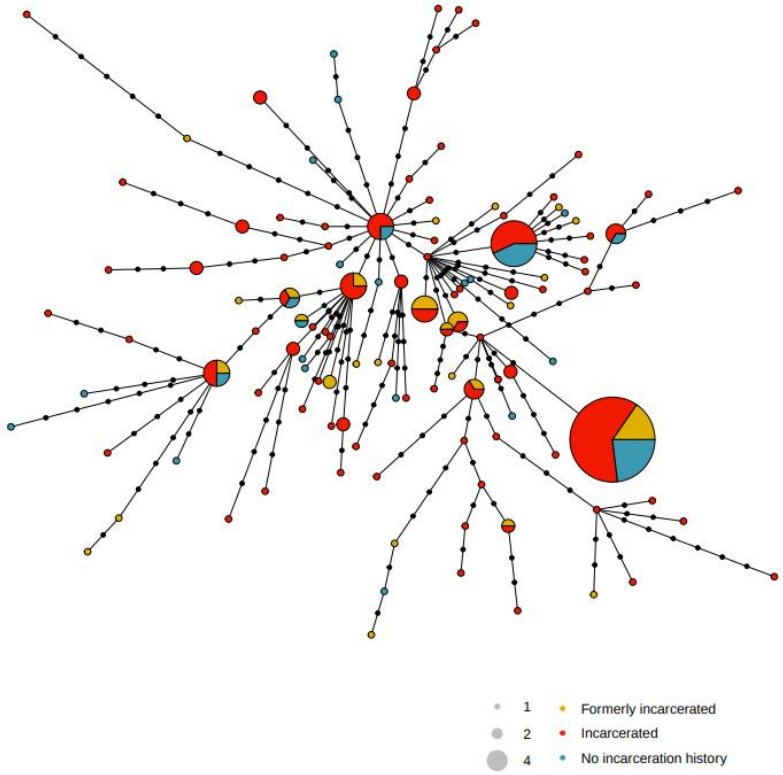

FIGURE 6. Central West Brazil whole genomic sequencing for prisons-to-community tuberculosis transmission study, 2014-2019. A haplotype network of the single largest genomic cluster, including 170 isolates. Nodes represent unique haplotypes and are scaled to number of isolates. Points along branches indicate SNP distances between haplotypes. Node colour indicates incarceration status at the time of diagnosis (63)

Within the genomic transmission trees, evidence revealed recurrent infection spread across all major cities within the state and throughout the sampling years, indicating that transmission is not confined to a single prison but rather extends to a broader incarceration system.

The movements of individuals within the Brazilian penal system form a tightly interlinked network of contacts, which accelerates the spread of *Mtb* between prisons and heightens the risk of infection in the broader region. This, in turn, extends the impact to the wider community. Consequently, this genomic study highlights the pivotal role of penitentiary transfers in *Mtb* dissemination within prisons, leading to the emergence of new disease clusters. This case may exemplify the broader dynamics observed with a range of other pathogens.

FIGURE 7. Phylogenetic tree of patients with pulmonary tuberculosis of *Mycobacterium tuberculosis* lineage in study of risk for prison-to-community tuberculosis transmission, Chiang Rai Province, Thailand, 2017–2020. Scale bar indicates 0.01 substitutions per site SNP, single-nucleotide polymorphism.

In recent years, from 2017 to 2020, a study was conducted in Thailand involving the whole genome sequencing of *Mtb* to identify clusters and their correlation with a history of incarceration(29). The Thailand TB burden is high, and in 2021 the country had the largest inmate population in Southeast Asia (411 inmates/100,000 population). In this study four major genotypic clusters were identified, with 28% of patients within these clusters having a history of incarceration. Patients with prior TB-related incarceration

were more probable to be included in larger clusters than non-incarcerated. Furthermore, patients with tuberculosis within these major genotypic clusters were geographically dispersed throughout the same province (Figure 7).

The Thailand study found that formerly incarcerated TB patients were 4.7 times more likely than nonincarcerated TB patients to be linked with other patients in large transmission clusters. The association between being in a large cluster and having a previous incarceration history suggests that these genotypes could have circulated in prisons and spread to the community(29).

In this both countries, the rapid rise in incarceration rates and absolutely discrepancies in tuberculosis incidence between inside and outside prisons, suggests that spillover of infection could contribute to community TB transmission. A previous study in Georgia estimated that 31% of all multidrug resistant TB cases were linked to prisons directly or indirectly(58). But both studies cited before (Brazil and Thailand) had a conservative transmission inference framework, because identifies a minimum contribution of spillover to total community transmission, even so, it still appears to be in line with the Georgia finding.

Prisons and other detention centres have served as reservoirs for other pathogens including meningococcus and SARS-CoV-2(64,65). Prison transfers have also spread of SARS-CoV-2 across prisons in California, resulting in widespread outbreaks(66). TB is just one example of several pathogens, so Brazil and Thailand studies are only indicative.

The doubt in the number of spillover events reveals the complexity in transmission reconstruction in an endemic situation where *Mtb* sampling is partial. The findings emphasize the potential for connecting additional information to reduce uncertainty in transmission inferences. Moreover, additional approaches are reasonable to enhance the accurate representation of variation within individual *Mtb* infections. This can be achieved by integrating genomic variation of *Mtb* within the host or by capturing variation across the whole *Mtb* genome, including the varied PE/PPE genes, which were omitted from these analyses(42).

So far, genomic evidence has been presented that the risk of tuberculosis contagion from prisons to surrounding communities has been very high in Brazil and Thailand. The dramatic expansion of incarceration in recent decades has put a growing population at extremely high risk of contracting tuberculosis; This risk extends to surrounding communities. Therefore, reducing the excessive risk of tuberculosis transmission within prisons and other detention facilities is an urgent public health priority.

4.5 Screening TB in prisons

In most prisons, TB is discovered through passive surveillance, through the recognition of respiratory symptoms among PDL presenting for evaluation to prison health units, or with diagnosis by sputum smear microscopy and/or sputum culture and, where available, CXR. However, in the majority of LMIC prisons, the TB diagnostic infrastructure is limited, and delays are the norm. Often, samples or PDL must be transported to facilities with diagnostic capacity, which adds to costs and results in delays or missed diagnoses (67).

Involving trained incarcerated peer educators in TB control in Ethiopian prisons significantly improved TB case detection rates (68). Several LMICs have made important progress in incorporating more sensitive methods for diagnosing TB and detecting drug resistance, such as rapid molecular tests (e.g. Xpert *MTB*/RIF; Cepheid, Sunnyvale, CA, USA), which are recommended by the WHO, as smear microscopy only detects 50–60% of all TB cases. However, interventions are still needed to shorten the time between disease onset, diagnosis and initiation of treatment, because delays potentiate transmission in the prison environment (69). Therefore, it is essential to seek new strategies for TB control in prisons that are cost-effective and locally feasible.

In 2021, the WHO updated the TB management guidelines, and in its module 2 on Systematic Screening for TB Disease, it strongly recommends that the Ministries of Health and Justice invest in TB screening in prisons (70). They propose that screening should always be performed on entry, annually and on exit to prevent TB transmission both in the prison and in the community at large. This seems an excellent

recommendation but does not consider that in many LMICs it may not be feasible to perform screening at three time points without increased financing for TB programmes. Amid resource constraints, there is a lack of evidence about which screening strategies should be prioritised.

Existing reports on mass TB screening in prisons are not clear about the costs involved. Some cross-sectional studies show a high yield from annual TB screening; however, the impact on reducing transmission and on the cost-effectiveness of this activity is not well understood. Due to the combination of the high burden of disease in LMIC prisons and the typically short period of incarceration, identifying cost-effective strategies for mass diagnosis, as well as preventing TBIs during incarceration, may be keys to disease control (71–74).

Mathematical models have predicted that a combination of different strategies could considerably reduce TB incidence in prisons. Mabud et al. (39) proposed through modelling that combined interventions reduce TB incidence in PDL by 75% and by 35% in the community after 10 years of interventions. In their proposal, it is striking that exit screening is more cost-effective than entry screening. By returning PDL to the community, an individual is moved from a high-incidence environment to a low incidence environment; focusing efforts on people leaving high-incidence settings can have a greater impact on community transmission. However, it also generates an important impact inside the prison, due to the high percentage of prison re-entries that may occur. It should be noted that this model did not consider the transitional states between infection and active disease and their role in the chain of transmission (Figure 8) (39).

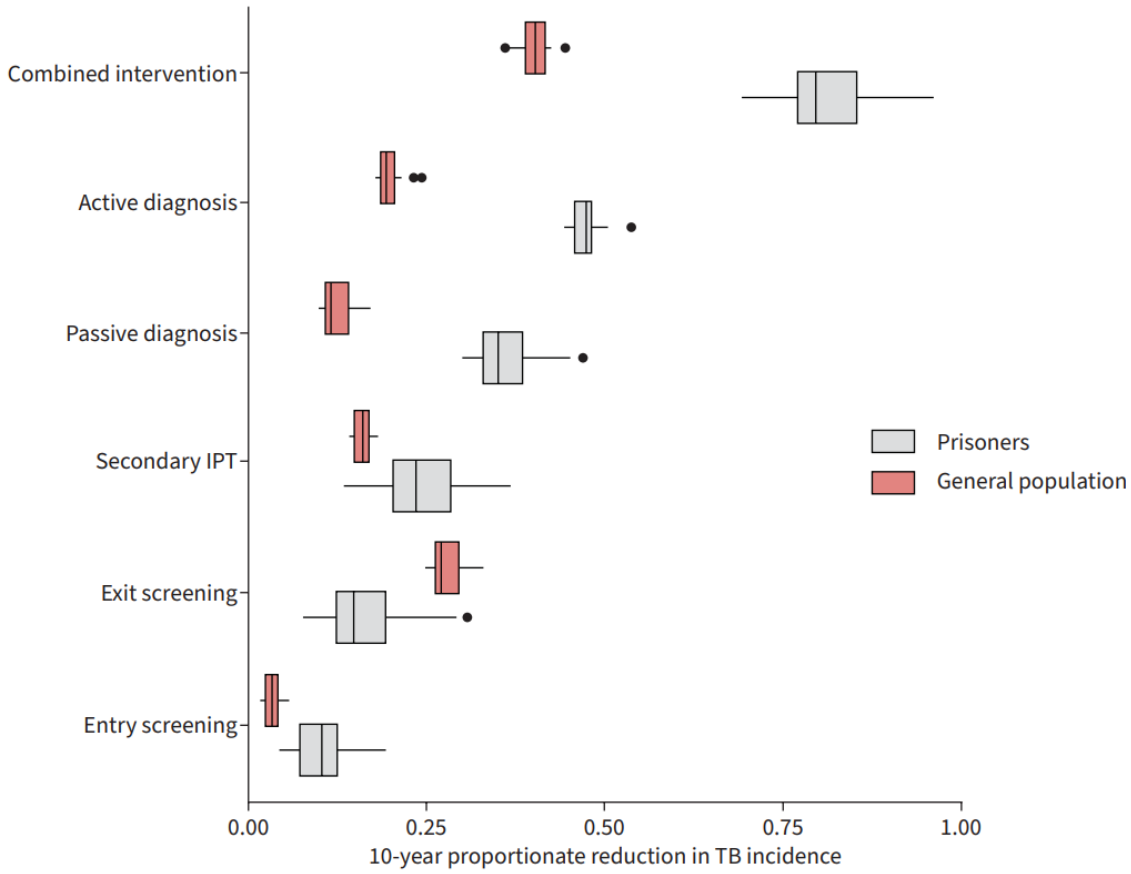

FIGURE 8. Proportionate decrease in TB incidence over 10 years according to various prison-based interventions. The box and whisker plots describe uncertainty in intervention effectiveness produced by Latin hypercube sampling analysis; the boxes characterise 25th, 50th and 75th percentile values, and whiskers characterise a range of values up 1.5 times the interquartile range. Dots represent outliers beyond this range. IPT: isoniazid preventive therapy (39).

There are still open questions about how to reduce the exorbitantly high rate of TB in prisons and define the best intervention. An annual mass screening study in Brazil has shown that mass active searches in prison double the incidence found in prisons that only detect TB passively (75). In addition, this study showed that more non coughing or totally asymptomatic cases tended to be picked up as screening rounds progressed (75).

Da Silva Santos et al. (76) evaluated the performance and cost of mass screening in prisons, comparing different screening strategies for all PDL. No one strategy was far superior to the others, so the main message of the study, and for public health in general, is that there is no best strategy, but there are strategies available and their

implementation will depend on the characteristics of the prison and the country (Table 1) (76,77).

Because mass screening of all PDL can be very costly, one alternative is to pool sputum samples for testing by molecular diagnostics, followed by individual confirmatory testing of samples from pools that screen positive. A recent study in Brazil compared individual testing with the Xpert *MTB*/RIF Ultra test with pooled sputum sampling using Xpert *MTB*/RIF Ultra; the sensitivity and specificity of sputum pooling were high (sensitivity 94%, 95% CI 88–98%; specificity 100%, 95% CI 84–100%), regardless of the pool size.

Strategies	Cases Diagnosed	Missed Cases	% Yield (95% CI)	Participants Screened with Xpert Nº	Mean Cost per Case Detected US$
All cases	214	…	…	…	485
Comparator groups					
Strategy 1: Sputum Xpert for all participants	160	54	74 (68-71)	1452	249
Strategy 2: Symptom screening	141	73	65 (59-71)	1163	255
If positive: Xpert					
Strategy 3: Chest radiography (CAD4TB)	138	76	64 (57-70)	383	370
If score 60+: Xpert					
Strategy 4: Symptom screening	163	51	76 (70-81)	1248	395
If positive: Xpert					
If negative, CXR (CAD4TB) followed bt					
Xpert of score 60+					

Abbreviations: CAD4TB, ComputerAided Detection for Tubertculosis; CI, confidence interval; CXR, chest radiograph;
MTB, Mycobacterium tubertculosis; RIF, rifampicin; US, United States;
Xpert, *MTB*/RIF assay.

TABLE 1. *Yield and Cost Diagnosed for 4 Tuberculosis Screening Strategies. From Da Silva Santos et al, 2021.* (76)

This approach was more efficient than individual testing across a broad range of simulated TB prevalence settings and enabled active case finding to be scaled up while containing costs (Figure 9) (77).

Regarding CXR, computer-aided detection technology can be useful when established equipment is not available in prisons and when a trained technician is lacking, which is

common in prison settings. A study by Soares(78) et al. found that three commercially available systems all had high accuracy (area under the curve >0.87) compared with sputum Xpert *MTB*/RIF testing, with two of the systems exceeding the WHO target product profile benchmarks for a nonsputum-based screening test. In addition, the automated CXR scores were strongly correlated with bacillary burden, suggesting CXR-based screening may identify participants who transmit the most. Similarly, the investment of automatic radiography equipment for mass screening is recommended, as it ends up lowering the costs of screening in the long term (Table 2) (78–81). The limitation is that these radiographs lose their sensitivity and specificity with increasing age. However, CXR-based diagnosis may still be useful in prisons, where the average age is <35 years. However, specificity is also lost if the individual has previously had TB, which is more common in prisons.

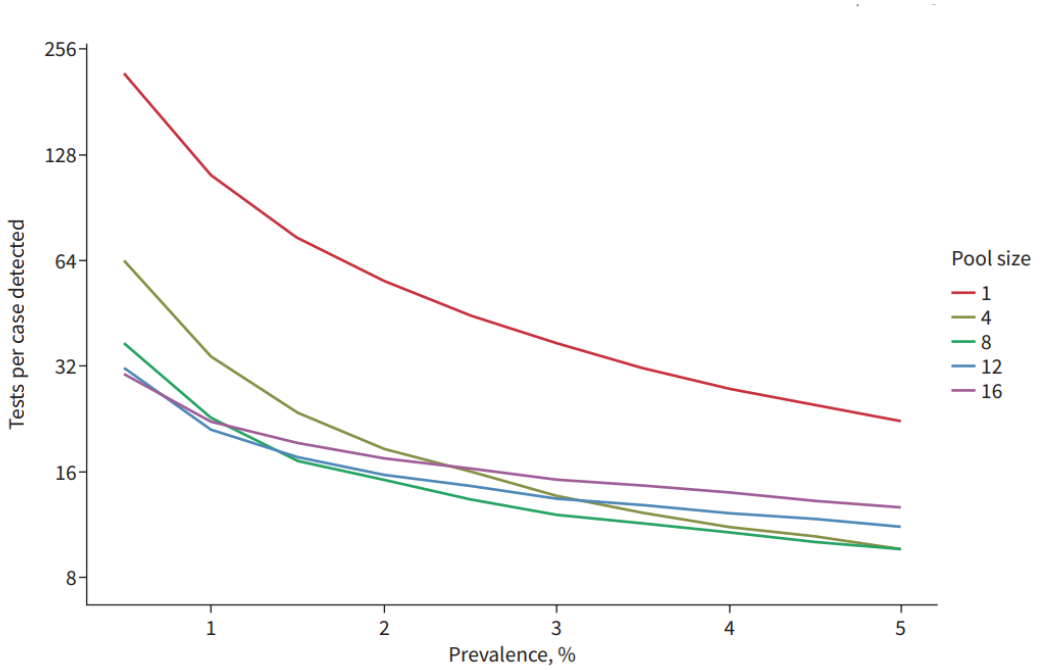

FIGURE 9 Number of Xpert *MTB*/RIF Ultra cartridges needed per TB case detected according to pool size and population TB prevalence (77).

A recent review study found that screening tools with higher sensitivity, such as Xpert tests and CXR, are linked to lower estimates of the number needed to screen (NNS). However, the NNS for active case-finding strategies varies greatly among adult risk groups, particularly in prisons.

Across screening methods, there was no significant difference in the NNS for prison inmates between low/moderate- and medium/high-incidence settings. This suggests that prison inmates are at a high risk for TB, regardless of the local TB incidence, and should be prioritised for high-sensitivity screening by TB management programmes (82). However, a study conducted in Malaysia found that among all risk groups for developing TB, such as diabetes patients, smokers, drug users, haemodialysis patients and those with rheumatoid arthritis, the three most cost-effective strategies for mass screening were screening of prison populations, individuals >60 years of age and HIV patients (83).

System	AUC (95% CI)	At pre-defined thresholds		At 90% sensitivity, 4% prevalence		
		Sensitivity % (95% CI)	Specificity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %
CAD4v6	0.87 (0.85-0.90)	80.7 (75.4-85.3)	82.7 (80.8-84.4)	62.3 (52.0-73.1)	9	99.3
LunitTB	0.91 (0.88-0.93)	79.9 (74.5-84.6)	89.8 (88.3-91.2)	83.7 (72.4-87.3)	18.7	99.5
qXR	0.90 (0.87-0.92)	74.5 (68.8-79.7)	89.4 (87.9-90.8)	74.2 (60.2-81.3)	12.7	99.4

TABLE 2. Sensitivity, Specificity, Area Under the Curve (AUC), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of each algorithm at pre-defined thresholds or with thresholds adjusted to 90% sensitivity as specified by the WHO Target Product Profile minimum target. From Soares et al, 2023. (78)

Finally, to date, there is no evidence that more than once-a-year mass screening would be preferred, by whichever technique. Simultaneously, the role of asymptomatic individuals as important players in transmission of the disease is increasingly evident, which encourages mass screening to include this group of individuals actively, rather than waiting for TB symptomatology, in settings with a high TB incidence (79).

The WHO has prioritised the search for TB diagnostics other than those based on sputum, such as exhaled air, oral fluids and blood. Currently, the electronic nose (eNose), which has previously demonstrated specificity and sensitivity to diagnose or rule out TB, was not as good as expected in prison populations (84). Cox et al. (85) showed that use of the Xpert *MTB*/RIF Ultra with oral swab specimens provides poor yield for microbiological PTB confirmation in children. When Lima et al. (86) tested prison populations using swab-obtained oral fluid with the Xpert *MTB*/RIF Ultra for

diagnosis of PTB, they found comparable sensitivity to sputum when the individual's semi-quantitative mycobacterial load was medium and high (86). A case–control study of point-of-care C-reactive protein in blood and the Xpert *MTB*-Host-Response test did not meet with the WHO target product profile benchmarks for a triage test (87). In conclusion, these studies have not yet demonstrated sufficient superiority over sputum molecular testing and are affected by the person's semi-quantitative mycobacterial load.

4.6 LTBI in prisons: the great challenge

LTBI is often cited as the reservoir of the TB epidemic. Worldwide, an estimated 1.7 billion people are living with LTBI, usually without symptoms (88). Without treatment, 5–10% of people with LTBI will develop active TB at some point in their lives. This scenario can be much worse in prisons. The greatest risk of progression to active disease is known to occur in the first 2 years after infection. Considering that most people serve an average of 48 months in prison, reducing infection through TPT could also have an impact on the incidence of the disease (89,90).

Currently, TB control in the penitentiary system is based on the screening of persons with active TB and their respective treatment, with little or nothing done about LTBI. The new WHO guidelines only briefly mention PDL among other risk groups and mention the diagnosis and treatment of LTBI in a practically conditional manner (70). Thus, no country will routinely make the effort to find and treat LTBI in prisons, especially in LMICs where the problem is greatest.

LTBI diagnosis is difficult, with the diagnosis being made by in vivo or in vitro stimulation with *Mtb* antigens, used in a TST or IGRA.

The TST is widely used and inexpensive but has low specificity in BCG-vaccinated populations, and it is subject to cross-reactivity with nontuberculous mycobacteria and has low sensitivity in immunocompromised individuals. The low cost makes the use of the TST advantageous, as does the relative simplicity of the technique, which requires only personnel experienced in intradermal applications. However, the number of false-

positive diagnoses (up to 30%) induced by the booster effect and the need for two visits with a 2–5-day interval for induration reading must be considered, as self-reading is associated with a high error rate. However, revisiting PDL may not be as difficult in prisons as it can be in the community (91–93).

The IGRA has a higher specificity than a TST, but recent studies have shown that conversions (from a negative to a positive result) and reversals (from a positive to a negative result) are more common with the IGRA than with the TST. In addition, the IGRA is more expensive and requires laboratory support, so there is currently no ideal method for diagnosing LTBI, especially in LMICs (93–95).

HIV testing should also be performed when looking for TB and LTBI. Most of the time, the presence of HIV influences the detection of symptoms and interpretation of the diagnostic test results of TB and LTBI (96).

Few studies have been carried out on LTBI in prisons. However, it seems clear that in high-incidence populations or people who have been in prison, the prevalence of LTBI is elevated compared with the general population. Furthermore, in prisons with high infection rates, these individuals may be at higher risk of developing TB due to their susceptibility to infection (92). Therefore, it is proposed that LTBI screening should be performed at prison entry and perhaps periodically to assess for recent infection. In settings with a high incidence of TB, screening for active TB and recent infections could be performed more than once a year to identify newly infected persons and thus prevent the disease (97).

Diagnosis and treatment of LTBI are crucial for disease control, especially in countries with high prevalence or simply in highly endemic settings such as prisons. Mathematical models show that if 8% of people with LTBI could be permanently protected each year, the global incidence in 2050 would be 14 times lower than the incidence in 2013, without the need for additional intervention (98).

In Pakistan, a programme for TB prevention in prisons provided universal TPT without LTBI testing. The justification is because in high-transmission settings: 1) the risk of a false-negative TST/IGRA result is high; 2) TPT prevents infections while it is being

performed; and 3) the costs of TPT alone are already sufficiently lower than any IGRA test (99). This reflects the global scarcity of tuberculin at times.

Regarding treatment of LTBI, the historical regimen has been isoniazid daily dosing for 6–9 months for TPT in prison settings. However, it is well known that this has low levels of adherence and less than half of cases complete the treatment, mainly because of its length. The new shorter regimens have shown superiority to the longer ones, simply because of higher adherence and even lower toxicity. Short weekly treatment regimens of isoniazid with rifapentine for 3 months (3HP) are an excellent opportunity for use in these settings. Recent studies in prisons in the USA, Pakistan and Malawi have shown a >86% completion rate of short-course TPT (3HP) among those who initiated treatment. Although the cost of the 3HP regimen is much higher, the fully treated patient cost is lower than isoniazid treatment, making it a cost-effective action for providing LTBI treatment in prisons (99–103).

In August 2022, Unitaid, The Aurum Institute, Clinton Health Access Initiative and MedAccess announced new agreements to reduce the price of rifapentine-based treatments to prevent TB in LMICs. This should be a catalyst for the implementation of the new reduced schemes for prison settings. The new affordable prices will be available in 138 LMICs, including those with the highest TB burdens globally (104).

New ultra-short 1-month TPT regimens of isoniazid and rifapentine appear to be successful for people with HIV, and their use in people without HIV is still being studied (104,105).

4.7 Prison health and public health

Given the important role prisons play in amplifying TB epidemics in the broader population, TB interventions performed in prisons are likely to confer broad health benefits in the general population (106).

Identifying a strategy for reducing TB in penitentiary institutions is not simply a matter of selecting a method as in a usual health service, focused on cost-effectiveness.

Because it will be carried out in a socially difficult environment, in which certain social relationships are often imposed, this ostensibly makes it more difficult (107). Even the movement of PDL from their cell to a particular location for a Chest X ray is governed by these relationships, mediated on the one hand by the set of rules imposed by the prison system and on the other by the PDL themselves (108). Studies in prisons also raise ethical issues to be considered, as research actions or new massive measures are often confused with coercive measures against someone who is in a situation of vulnerability, serving a sentence. Adherence to high ethical values and the preservation of human rights is extremely important in designing and conducting research involving PDL (109,110).

The most impactful solution for the crisis of TB in prisons is likely to be addressing the rising rates of incarceration through criminal justice reforms and decarceration (25). Paraguay has the highest number of pre-trial detainees in Central and South America (Figure 10). However, while we await legal reforms, departments of health should provide and take responsibility for prison health services and advocate for healthy prison conditions (111).

More recent data confirm that the performance of prison health services can be significantly improved after transfer to departments of health, and that such transfers can support the development of prison health indicators, the evaluation of service performance and the integration of prison health data into national health statistics (112,113).

Greater insight into what is happening in prisons with TB, as well as the results of interventions in prisons, with an innovative approach, resourced by WHO and other stakeholders, will be crucial to generate the evidence needed to address the growing TB crisis in prisons. Unfortunately, the WHO did not include the increasing burden of TB in prisons in its Global TB Report 2022 (114) . Recently, in the Global TB Report 2023, although weakly addressed, concerns regarding TB in prisons were addressed(2). Detailed disaggregation of data for prisons in national and global reports is essential for achieving improvements in tackling the burden of TB in prisons (28).

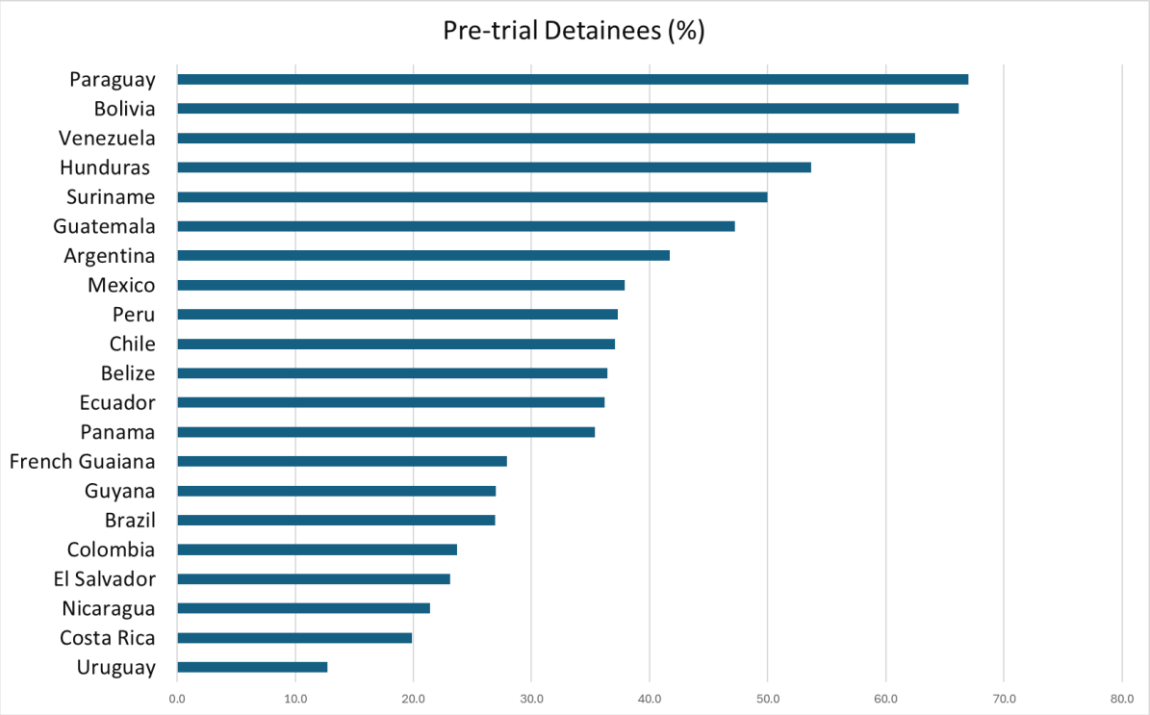

FIGURE 10. Percentage of Pre-trial detainee among PDL of Central and South American countries. Source: Word Prison Brief data (30).

4.8 Setting the stage: TB in Paraguay and its prisons

The entirety of the research undertaken in this thesis is founded upon data and fieldwork conducted in Paraguay, South America. A portion of the analysis was conducted using national data provided by the National TB Program (NTP) and the Ministry of Justice, while other aspects were approached with a specific focus on the largest prisons and their surrounding areas. As such, obtaining a comprehensive understanding of the epidemiological context is essential for this research.

Paraguay exhibits a moderate burden of TB, with an average of 2950 reported cases per year and an annual incidence rate fluctuating between 29.9 and 43.6 cases per 100,000 inhabitants over the past 15 years (Figure 11 and 12) (2). Significant strides have been made in the country's TB control efforts during this period, including the expansion and universal access to free diagnosis and treatment, the decentralization of TB clinical management to primary care, and the strengthening of health policies targeting specific vulnerable TB groups in recent years (such as indigenous populations, prison population,

individuals with diabetes, immunocompromised individuals, and those living with HIV)(115). Despite these advancements, the incidence rate has remained relatively stable, except for a decline and subsequent resurgence in TB cases observed during and after the SARS-CoV2 pandemic, as documented in various regions worldwide (116,117). What changed during this period is the concentration of the TB epidemic within the prison population, obscuring the progress made among other at-risk groups and the broader community.

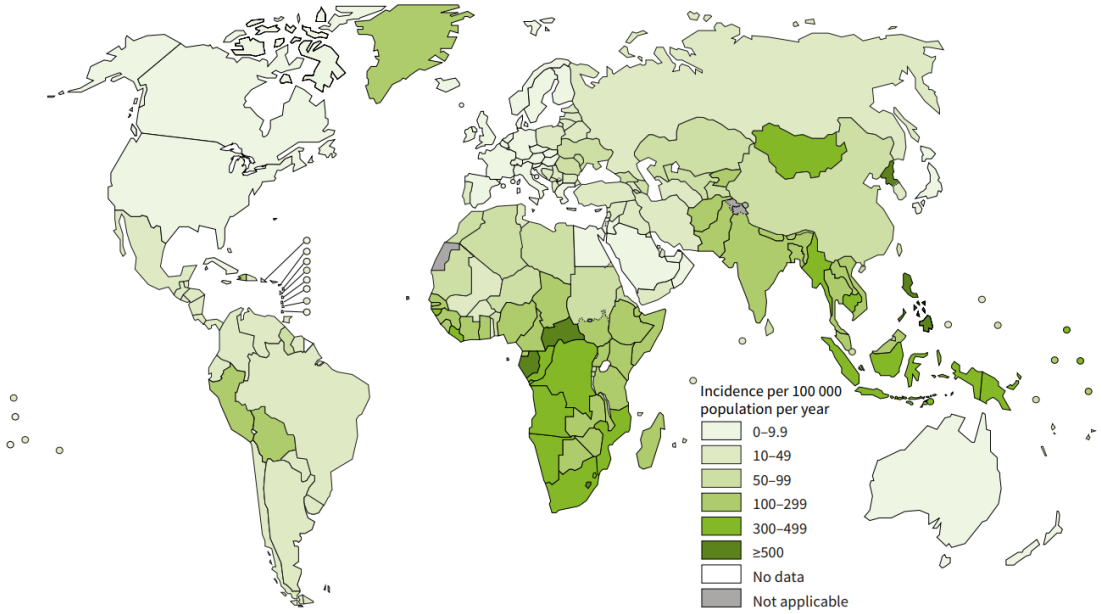

FIGURE 11. Estimated TB incidence rates, 2022. Global TB Report 2023(2).

Currently, Paraguay hosts 18 penitentiary centres accommodating a prison population of 17,712 individuals as of 2023 (118). This predominantly male population (97%) has doubled over the past decade, resulting in unprecedented levels of overcrowding. Paraguay's total incarceration rate stands at 220 per 100,000 inhabitants(31). However, the extent of overcrowding varies across the country's prisons, with facilities such as Tacumbú and Ciudad del Este Penitentiary Centres experiencing higher levels of overcrowding. Both prisons are situated in the country's two most populous territories, the first in the metropolitan area of Asunción, the capital city, and the second in Ciudad del Este, the second most populous city, located 300 km from the capital in a border area known as the Triple Frontier due to its shared boundaries with Argentina and Brazil (Figure 13). These prisons together house 24.5% of the country's prison population in 2023. Despite efforts by the Paraguayan Ministry of Justice to address this issue, both

facilities operate at more than double their intended capacity, exceeding 200%, according to official records. However, when evaluated against international human rights standards, their overcrowding levels surpass 600% of their designed capacity(118,119). One positive aspect regarding tuberculosis in Paraguayan prisons is that inmates exhibit the highest treatment success rates, attributed to the effective implementation of directly observed treatment(120).

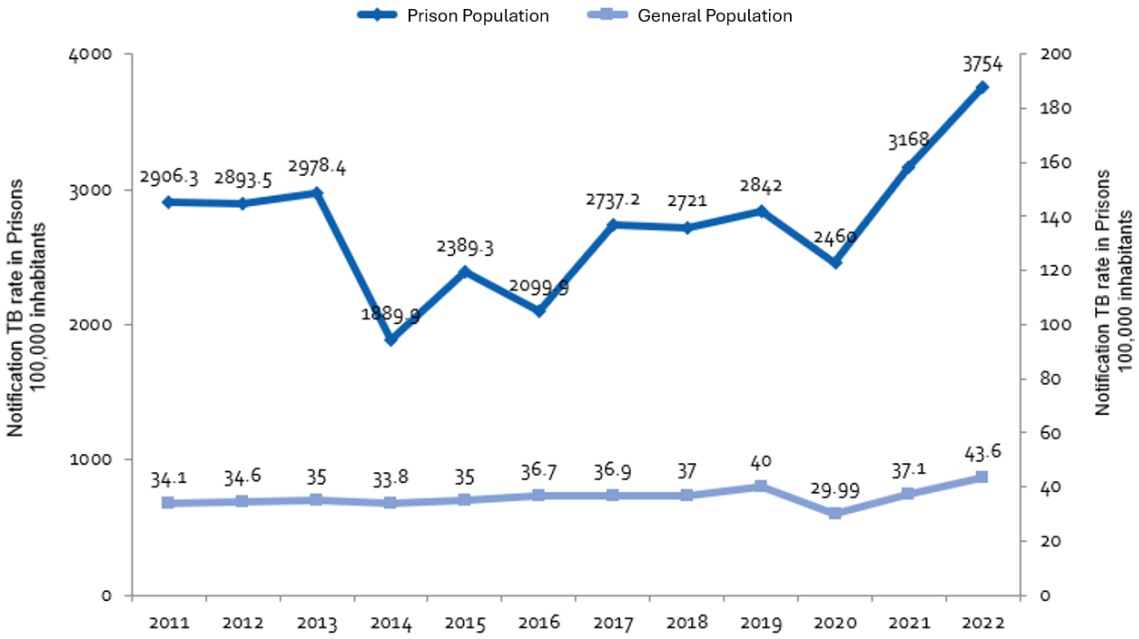

FIGURE 12. Notification TB rate among prisons population and general population during 2011 to 2022 in Paraguay. Adapted from NTP, Ministry of Health, Paraguay.

The TB incidence rate in Paraguayan prisons is 70 times higher than in the general population (4). It is well recognized that TB is influenced by individual risk factors and determinants such as poverty, substance abuse, smoking, diabetes, HIV, among others. These factors are also concentrated and disproportionately represented within the prison population compared to the general population(121). However, perhaps the primary driver of this epidemiological phenomenon is the poor structural conditions of the prisons, which have not kept pace with the population growth of the last decade. Currently, overcrowding, and inadequate ventilation in cells and pavilions may be the primary factors contributing to the high transmission of TB within prisons.

This phenomenon, which it will explore and delve into within this thesis, aims not only to describe the dynamics of the concentrated epidemic within the prison population but also to explore the impact of that epidemic on neighbouring territories, affecting the communities where these prisons are situated. It is important to recognize that the prison population is not static; turnover rates can range from 2 to 5 times the total population of the facility within a year, considering transfers, visits, permits, and prison staff(44). Consequently, the significant burden of TB within penitentiary centres extends beyond their confines, becoming a broader public health concern that spills over into the wider community.

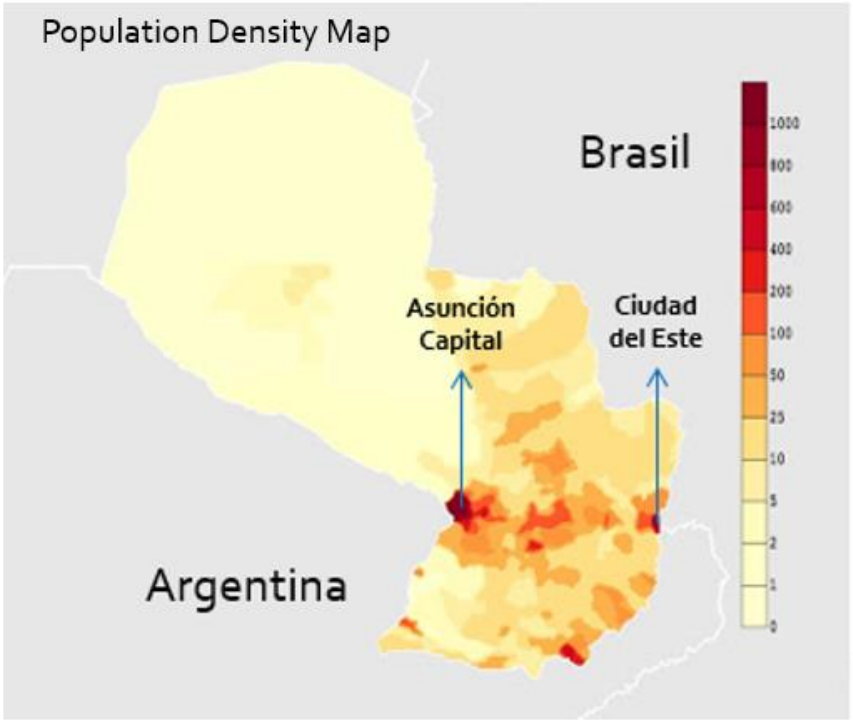

FIGURE 13. Population Density Map of Paraguay, peoples per km^2. Total Population 2022: 7,1 million inhabitants. Adapted from NTP, Ministry of Health, Paraguay.

5. Hypotheses

5.1 Overall hypothesis of this thesis

Thorough analysis of historical data from the National TB Program (NTP) and the Ministry of Justice, including the utilization of complete genomic sequencing of *Mycobacterium tuberculosis* for cases occurring in prisons and surrounding communities, will provide us with a more precise understanding of the disease burden attributable to prisons and transmission patterns between these facilities and the broader community. This information will be instrumental in redirecting efforts, strategies, and funding toward TB control in the country.

5.2. Specific hypothesis of this thesis

- Latin America and Paraguay seem to be showing signs of a prison-based tuberculosis epidemic in the last decade.
- The risk of developing TB in prisons is several times higher than in the community. The incarcerated population faces the highest risk of TB development.
- Structural building and social conditions within prisons exert stronger influence than individual determinants in the development of TB.
- A significant proportion of TB cases occurring in the community originate from transmission within the prison setting.
- Individuals with no history of incarceration, current inmates, or former inmates all may share clusters with signs of recent transmission of TB.

6. Objectives

6.1 General objectives

The aim of this study is divided into two general objectives:

- To describe the burden and determinant factors of TB disease in both penitentiary centres and the community using official data from the country.
- To describe the transmission patterns of *Mycobacterium tuberculosis* in penitentiary centres and the community through the utilization of genomic analysis.

6.2 Specific objectives

1.- To characterize the TB epidemic in prisons, focusing on Paraguay, and identify changes over the past decade.

2.- To analyse the risk of TB in prisons compared to the general population.

3.- To identify risk factors associated with the development of TB disease in prisons.

4.- To identify the proportion of prison-associated TB cases in the community.

5.- To determine the lineages of *Mycobacterium tuberculosis* existing in prisons and their surrounding communities.

6.- To identify clusters and recent transmission of *Mycobacterium Tuberculosis* through whole genome sequencing in both prison and community cases.

7. Material, Methods, and Results

7.1- Article 1

Increased incarceration rates drive growing tuberculosis burden in prisons and jeopardize overall tuberculosis control in Paraguay.

Sequera, V. G., Aguirre, S., Estigarribia, G., Cellamare, M., Croda, J., Andrews, J. R., Martinez, L., & García-Basteiro, A. L. (**2020**). *Scientific Reports*, *10* (1), 21247.

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Q1 (Multidisciplinary)

Specific Objectives

1.- Characterize the TB epidemic in prisons, focusing on Paraguay, and identify changes over the past decade.

2.- Analyse the risk of TB in prisons compared to the general population.

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Increased incarceration rates drive growing tuberculosis burden in prisons and jeopardize overall tuberculosis control in Paraguay

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Incarcerated populations are at high-risk to develop tuberculosis (TB), however their impact on the population-level tuberculosis epidemic has been scarcely studied. We aimed to describe the burden and trends of TB among incarcerated populations over time in Paraguay, its clinical and epidemiological differences and the population attributable fraction. This is an observational, descriptive study including all TB cases notified to the National TB control Program in Paraguay during the period 2009–2018. We also used case registries of prisoners diagnosed with tuberculosis from the Minister of Justice. The population attributable fraction of TB in the community due to incarcerated cases was estimated through Levin's formula. The characteristics of TB cases in and outside of prison were compared as well as the characteristics of TB in prisons were modified over time. During 2009–2018, 2764 (9.7%) of the 28,534 TB reported cases in Paraguay occurred in prisons. The number of prisoners in Paraguay increased from 6258 in 2009 to 14,627 in 2018 (incarceration rate, 101 to 207 per 100,000 persons) while the number of TB cases among prisoners increased by 250% (n = 192 in 2009 versus n = 480 in 2018). The annual TB notification rate among male prisoners was 3218 and 3459 per 100,000 inmates in 2009 and 2018, respectively. The percentage of all TB cases occurring among prisoners increased from 7.1% in 2009 to 14.5% in 2018. The relative risk of TB in prisons compared to community was 70.3 (95% CI, 67.7–73.1); the overall population attributable risk was 9.5%. Among the 16 penitentiary centers in the country, two of them—Tacumbú (39.0%) and Ciudad del Este (23.3%)—represent two thirds of all TB cases in prisons. TB among inmates is predominantly concentrated in those 20–34 years old (77.3% of all), twice the percentage of cases for the same age group outside of prison. Our findings show that the TB epidemic in prisons represents one of the most important challenges for TB control in Paraguay, especially in the country's largest cities. Appropriate TB control measures among incarcerated populations are needed and may have substantial impact on the overall TB burden in the country.

Since the beginning of the twenty-first century, various national and international legal transformations in the penal system led to increases in mass incarceration in the Latin America. Paraguay is no exception to this problem[1,2]. Prison conditions, such as overcrowding, poor ventilation and limited access to health services, encourage the transmission of several diseases, including tuberculosis (TB)[3,4]. In addition, other risk factors conducive for the development of TB infection and active disease are also common among prisoners including alcohol or drug use, homelessness, mental illness, smoking, malnutrition, HIV-related immunosuppression, prior tuberculosis exposure, among others[5–7]. These combinations of individual and environmental risk factors lead to an extraordinary high force of infection and burden of disease in many prisons[5,8].

In addition, prisons represent a reservoir for the spread of diseases to the rest of the community[5,9]. TB infection can be spread to the general population after prisoners are released, through the prisons staff who have permanent contact with the community, and also through visits of family and friends of inmates to prisons.

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Therefore, transmission dynamics between prisoners and the general population play a key role in driving disease in the general population[10–14]. The prison environment, more so than the prison population itself, drives TB incidence, and directed interventions within prisons could have an important outcome on the broader TB epidemic[5,14,15].

Priority TB control interventions among inmates include the early diagnosis and treatment of TB cases. Regular screening for TB symptoms and the performance of smear microscopy, culture, or rapid molecular diagnostics are important to improve case detection. However, TB prevention and control efforts in prisons are often insufficient, such as in Paraguay. The lack of optimal measures for TB control at the national level may lead to increasing trends in prison settings[5,14,15].

In recent years, the Paraguayan TB Control Program (NTP) has reported high rates of tuberculosis among inmates[16]. This information has been critical to foster control efforts of the penitentiary TB epidemic since 2014, with coordinated strategies between the Ministry of Health, Penitentiary System and Ministry of Justice. These strategies included the creation of isolation rooms for new cases, increased implementation of systematic screening for TB symptoms, and prioritization of molecular TB diagnostics, such as Xpert MTB/RIF (Xpert hereinafter) in prisons, implemented before the broader roll-out in the rest of the country.

This study aims to characterize trends in TB incidence among inmates of all sixteen penitentiary centers in Paraguay, to identify the sub-populations of prisoners with high risk of TB, in addition to differences and clinical trends and by gender of TB outside and inside prisons, and thus estimate the population attributable fraction (PAF) of TB prisons in relation to the burden of TB in the general population of the country.

Methods

This is a retrospective analysis study of de-identified, secondary data obtained through an official requests to the Penitentiary System, NTP and the national database of demographic statistics of the country from the General Directorate of Statistics Surveys and Censuses (DGEEC in Spanish), covering the period from 2009 to 2018. All sixteen prisons in Paraguay were included, two of which are exclusively for women.

TB diagnosis and reporting procedures in prisons. TB diagnosis, treatment, and surveillance among inmates and prison staff are based on NTP guidelines[17]. Each prison has a professional health care worker responsible for TB control. Screening for respiratory symptoms (i.e., cough of more than 2 weeks, coughing up blood, unintentional weight loss, fever, night sweats) is an active strategy. Active screening was performed through regular screening by prison health workers and volunteers responsible for health within each pavilion. Patients with presumptive TB are examined and sputum samples tested with Ziehl–Neelsen microscopy. According to historical data of each prison, a minimum number of respiratory symptoms is expected, as a search indicator, prior to number of reported cases. Xpert testing is being progressively implemented by prisons since 2015. In the most recent year of the study (2018), Xpert was used to diagnose less than 15% of TB cases. Each prison has a network of laboratories where their samples are processed belonging to the national public health system. All procedures, including medical evaluation, diagnosis and treatment, was provided without cost to the patient. For admission to the prison, only a standard clinical review is performed, without a TB infection test. A chest X-ray is only performed if the doctor considers it necessary in the evaluation. Contact tracing in prisons is not good, only active search of cases in the immediate environment of the confirmed case. Among inmates, TB cases were defined as persons who were diagnosed with TB by clinical or bacteriological criteria and started anti-tuberculosis treatment during their period of incarceration.

Data collection and analysis. Individual data were obtained from all TB cases reported during 2009–2018 in the NTP Surveillance System. In 2009, a new case-notification form was implemented in this system, which led to improved documentation and quality of the data according to their incarceration status and the type of penitentiary center. At the same time, the health department of prisons started a monthly report of prison population health focused on communicable diseases, including HIV and TB cases diagnosed and put under treatment. Therefore, our analysis focuses in the period after which this new system was implemented. The variables included in this analysis were the place of residence, sex, age, as well as clinical presentation, HIV status and sputum smear positivity. We calculated the population attributable fraction of incarceration to the general population tuberculosis epidemic. The population attributable fraction expresses the proportion of new tuberculosis infections that would be eliminated at the population-level had all cases among prisoners not occurred. The population attributable fraction of TB cases in the community due to prisoners' infection for all period was estimated using the Levin formula[18]:

$$\text{(Incident rate in total population} - \text{Incident rate unexposed/Incident rate in total population}$$

Where the whole population corresponds to the total population of the country taking into account the prisoners and the unexposed is the population that is out of prison. To calculate annual TB notification rates (cases per 100,000 population), we used population estimates for Paraguay of the 2012 census and the corresponding annual projections of the General Direction of Statistics, Surveys and Censuses as well as the mean number of prisoners mid-year reported by the Ministry of Justice to the National TB Program. The number of reported cases corresponds to the official data of the National Tuberculosis Control Program. To compare annual TB notification rates among prisoners and non-prisoners, we used the chi square test to compare the groups of prisoners and non-prisoners according to their clinical and epidemiological characteristics. A linear regression test was performed to analyse the ratios throughout the decade. The statistical analyses were performed in SPSS version 22. The level of statistical significance used was $p < 0.05$. It must be taken into account that this formula cannot estimate the transmission effect.

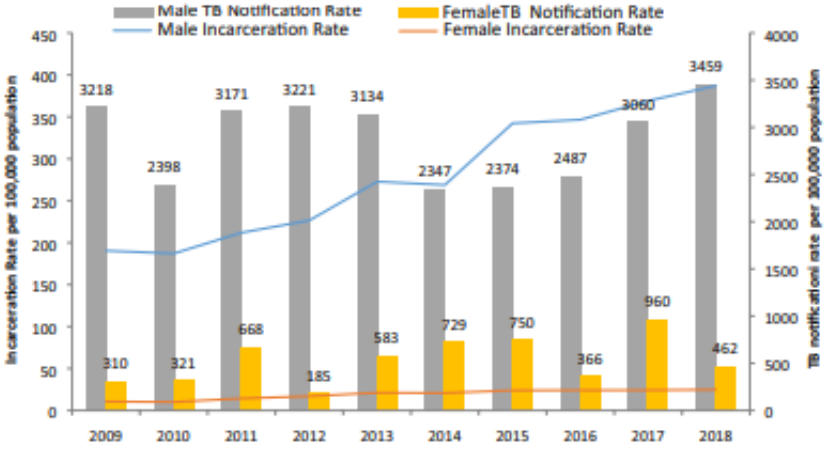

Figure 1. Distribution of the incarceration rate and TB notification rates in the penal system by sex, Paraguay 2009–2018.

Ethics. The Ethics Evaluation Committee of the Central Public Health Laboratory of the Ministry of Public Health and Social Welfare of Paraguay (International Certification FWA N° FWAOOO20088) approved this work with the code CEI-LCSP 91/010217.

Results

During the period from 2009 to 2018, 28,534 TB cases were reported to the NTP of Paraguay, and 2764 (9.7%) of these cases occurred in prisons. The contribution of TB notifications in prisons to the overall country TB case notification increased by more than 100% from 2009 to 2018 (7.1% in 2009 to 14.5% in 2018) (Fig. 1). During the study period, the prison population grew progressively more than 2.3 times, from 6258 to 14,627 people and the national incarceration rate of Paraguay increased from 101 to 207 per 100.000. There was a greater increase in rates of female incarceration (11 to 27 cases/100,000 individuals; 134% increase) than male incarceration rates (190 to 411 cases/100.000 individuals; 103% increase), though 94.1% of all inmates in 2018 were men (Fig. 1).

The annual number of TB cases reported in prisons increased by 2.5 times during the decade, from 192 cases in 2009 to 480 cases in 2018. TB notification rate among inmates has shown a non-significant increase, from 3068/100,000 in 2009 to 3281/100,000 in 2018 ($p = 0.988$). Similarly, the national TB notification rate remained stable at around 40 per 100,000 (40.7/100,000 in 2009 to 40.0/100,000 in 2018, p value 0.578). The relative risk of TB for incarcerated people for the entire time period, compared to general population, was 70.3 (95% CI 67.7–73.1) and did not significant change during the period ($p = 0.872$; relative risks of 75.4, 58.3, 75.7, 74.4, 79.9, 61.5, 60.6, 59.7, 73.9, 82.0 in each year from 2009 to 2018). The mean overall population attributable fraction was 9.5%, increasing significantly from 7.0% in 2009, to 14.4 in 2018 ($p = 0.001$ for linear trend). Figure 2 shows the progressive increase in the percentage of TB cases over total cases and the stable incidence rate in the decade.

Men represented 93.7% of prison population and 98.7% of TB cases in prison. TB notification rate of men in prison was 5.1 times higher than women (2887.7 vs 570 per 100 thousand prisoners)". HIV status was available for 87% of patients with TB in prison and 66% of TB patients in the general population. Availability of information about HIV status among TB cases increased considerably during the decade, from 34.4% to 89.4% and 11.1 to 85.4% in prisons and general population, respectively. The prevalence of HIV among TB cases in inmates was 4.8% and 7.2% among TB cases in non-prisoners. The prevalence of HIV among TB patients in prisons was 4.8% in men and 8.3% in female, while outside of prisons was 7.9% and 5.7% in male and female patients, respectively. Bacteriological confirmation was higher among inmates (91.0%) compared to the general population (68.9%). The percentage of prisoners with extra-pulmonary TB was lower than the general population (2.9% vs 9.3%) and treatment success rates were higher (77.7% vs 67.9%) (Table 1).

The TB case notification rate outside prisons was 2.2 higher for men than women, but in prisoners, this difference was 5.1 times. Inmates between 20 and 34 years of age accounted for 77.3% of TB cases in prisons, twice the percentage (38.2%) that this age group accounted for among non-prisoner TB cases. The age distributions of TB case notifications in prisons and the general population were relatively stable over the decade of the study.

Notification rates varied substantially among the country´s departments. The highest notification rates were concentrated in the three regions with the largest indigenous population, all in the Chaco region. We must remember that the indigenous population of Paraguay is less than 1.8% of the population (117,150 people) and the indigenous people in prison correspond to less than 1.5% of the population in prisons.

The highest absolute number of new TB cases were concentrated in cities with the largest population and incarceration centers. Two penitentiary centers located in large population centers accounted for nearly two-thirds of all TB cases in the country's prisons: Tacumbú (39.0%) in the capital city of Asunción and Penitentiary Center of Ciudad del Este (23.3%). Likewise, the territories where these penitentiaries are located also have higher

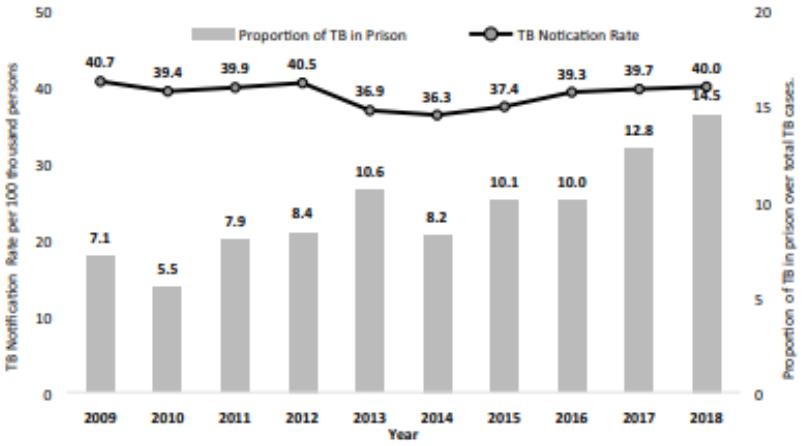

Figure 2. National tuberculosis notification rate and proportion of tuberculosis in prisons among total tuberculosis cases in the country, Paraguay 2009–2018.

Category	General Population			Penitentiary Centers			p. value, men vs women*
	Total	Male	Female	Total	Male	Female	
Total population, thousands	66,092	33,356	32,735	101	94	6	<0.0001
Number of TB cases	25,770	17,891	7879	2764	2728	36	<0.0001
Notification rate, TB cases /100.000 hab	39.0	53.6	24.1	2742.4	2887.7	570.0	<0.0001
HIV status reported, %**	66.2	67.8	62.7	87.0	87.1	80.6	0.853
Co-infection TB/VIH, %***	7.2	7.9	5.7	4.8	4.8	8.3	0.342
Bacteriologically confirmed pulmonary, %	68.9	71.6	62.9	91.0	91.1	77.8	0.652
Clinically diagnosed pulmonary, %	21.8	19.7	26.5	6.2	6.0	16.7	0.809283
Extrapulmonary, %	9.3	8.7	10.6	2.9	2.9	5.6	0.362
Treatment success,%	67.9	66.3	71.6	77.7	77.5	66.7	0.481

Table 1. Characteristics of Tuberculosis case-notifications in the General Population and Penitentiary Centers during the period 2009–2018. *p values calculated with the Chi-square test comparing prisoners' data by sex. **HIV status based on a positive or negative status result or AIDS diagnosis from the time of tuberculosis notification. ***% HIV co-infection calculated as percentage positive HIV + results among those with HIV status reported.

number of reported TB cases at the general population (Fig. 3). Observation of the data in the territory gives a dimension of the TB problem in large cities and their corresponding prisons. Furthermore, TB in prisons is not a "rural or indigenous" problem; it is a problem in large cities.

Colors reflect the number of all TB cases during 2009–2018 in the country that were diagnosed in each department, and the black circles are proportion of TB cases notified among prisoners in each prison (some of the prisons are overlapping). More than 2/3 of cases were notified at the two biggest prisons in the country: Tacumbú Prison and Penitentiary Center of Ciudad del Este. These two prisons are located in the largest cities of Paraguay, Asunción and Ciudad del Este. These territories also have the largest population and number of cases of TB cases among all departments. Figure 3 has been generated by on elf the author (MC) with the Open Source software R version 3.5.3 (https://cran.r-project.org/bin/windows/base/old/3.5.3/). The Shapefile at administrative level 2 freely available at https://data.world/ocha-fiss/212cd82f-bcb8-445f-8b32-aa194387f6c3.

Discussion

Although national TB incidence rates in Paraguay remained unchanged over the past decade, the number of TB cases in prisons doubled during this period. These increases among this high-risk population spoil gains from TB control efforts among the general population. This trend threatens achievement of the END TB and Sustainable Development Goals TB control targets[19]. These findings highlight the importance of prioritizing national TB control efforts on prisoner populations.

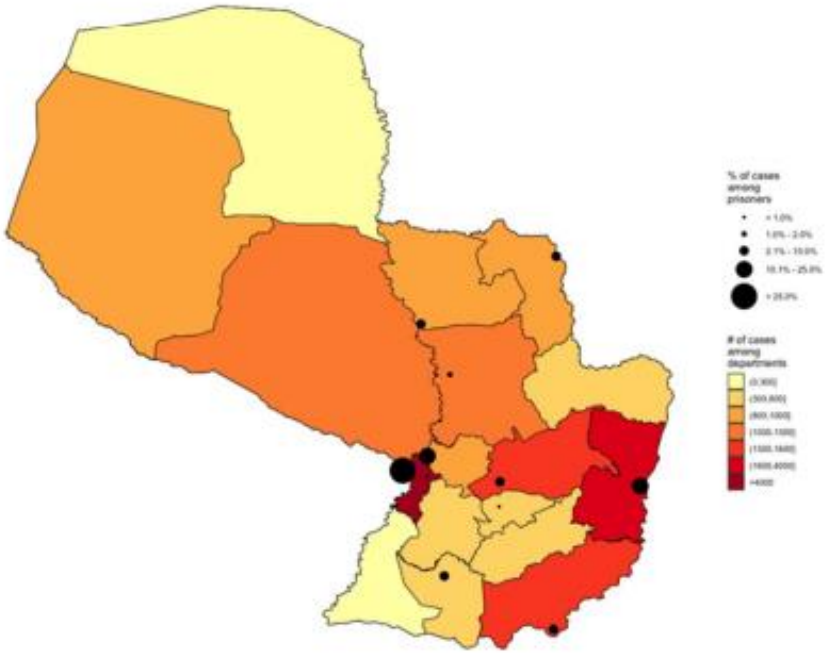

Figure 3. Percentage of tuberculosis notifications in the general population by departments and among prisoners by penitentiary centers of Paraguay, 2009–2018.

The percentage of all TB cases in Paraguay among incarcerated persons doubled in the ten-year period, from 7.1 to 14.5% of the total TB cases in the country. Consistent with recent findings from Brazil, the overall TB incidence rate in Paraguay was stable despite the rising incarceration rate[9]. These results differ from the findings of a project with data of 26 eastern European and central Asian countries, which showed that each percentage point of increase in incarceration rates relates to an increased TB incidence of 0.34% for the whole country[20]. Several factors, such as different prisons infrastructure; HIV prevalence; or changes in surveillance, economic, demographic, and political indicators might explain this finding. Also, there could be a trend towards decline in Paraguay because the improvement in other health-related indicators, but incarceration offset is such that the national trend was stable instead of decreasing. Thus, it is likely that national TB figures might not decline until a considerable reduction in the TB burden is prisons is achieved[21].

Inmates, as well as indigenous populations, are recognized as a high-risk TB group in Paraguay, but the assessment of the contribution of TB from indigenous or prisons to the overall TB burden in the community is not well known[22]. The high PAF of prison's TB to overall TB burden (14.4%) indicates that a considerable weight of national TB burden occurs precisely in prisons. Although the PAF formulae has been used by landmark studies for global TB burden in prisons, such as a 2010 systematic review (Baussano, 2010)[5]. It does not take into account the cases of disease that arise in the community which are connected to transmission in prisons: in relatives following infections during prison visits or disease in ex-prisoner after released[5,23]. A recent study from Brazil found that TB incidence among ex-prisoners is higher than that of the general population several years following their release, suggesting that including only cases arising duration incarceration results in an underestimate of the true effect of prisons on the TB epidemic[14]. Nonetheless, a high PAF emphasizes the need for continuous efforts to prevent the spread of TB within prisoners in order to reduce the overall TB burden.

In prison, individuals that are diagnosed are more likely to have bacteriological confirmation compared with cases occurring outside of prisons. This is similar to results observed in Brazil over a similar period[9,23–25]. Prison conditions in Brazil are likely to be similar to those in Paraguay, especially in the states or departments bordering the country[26]. Therefore, there is a need to work on joint transborder TB strategies for prisoners in Brazil and Paraguay[14].

The high TB rates observed among inmates in recent years, along with increases in mass incarceration and overcrowding, triggered several interventions and policies on national and international institutions. There were some reforms implemented in 2014, reinforcing the coordination and governance of the NTP to the staff of health care workers from the penitentiary centers through an inter-institutional agreement, which have increased the detection rate in the last years, however, the incidence trend did not change that much. International institutions such as the Global Fund helped with the creation of exclusive rooms for the isolation of TB cases for the first months of treatment, but currently this is not enough for the number of cases diagnosed. Due to this collaboration, the roll out of GeneXpert platforms in laboratories near the penitentiary centers was also initiated, but

the number of platforms in the country is still limited. Other strategies have been suggested, such as prisoner training to recognize their own respiratory symptoms, improved quality of sputum or other samples, massive periodic X-ray screening, enhanced surveillance among former prisoners once released[3,27]. Given the increasing incarceration rates in Paraguay, a comprehensive reform of the penal system, which does not create conditions of vulnerability and risk for TB needs to be considered. As an example, acceleration of trial decisions and limiting the number of prisoners without sentence, could likely contribute to reduced TB numbers[28,29].

Multi-Drug-Resistance TB (MDR-TB) incidence is low in Paraguay (0.99 per 100,000 population) and, although there is no data in prisons, it seems to be lower in this population group[19]. Importantly, a majority of the TB burden in the penal system is concentrated in two prisons where more than half the national prison population resides. The remaining prisons have an average of 7 (range, 0–29) TB cases per year, and there is a large discrepancy in the size and prisoner concentration between prisons. Thus, although the tuberculosis epidemic among prisoners in increasing in Paraguay as a whole, we are able to identify the prisons where enhanced control efforts should be concentrated, and resources should be allocated.

This study has limitations. First, we observed substantial heterogeneity mainly in the level of overcrowding and diagnostic capacity in differing geographical regions and prisons. Other relevant data, such as molecular characterization of strains from prisoners are needed to understand TB transmission networks in prisons, including internal outbreaks. There is likely under-diagnosis of tuberculosis mainly in extra-pulmonary TB (three times less in prisons than outside), as well as variation in reporting over time, which influences notification trends. This degree of under-diagnosis is distinct inside and outside of prisons. The case detection rate is likely much lower in prisons than the general population, which suggests that the contribution of tuberculosis from prisoners to the national tuberculosis epidemic that we report in this study is likely an underestimate. Third, TB cases occurring in recently released prisoners could not be identified, contributing to poor TB case ascertainment, specially because the NTP does not collect data concerning to prison records[17]. Lastly, the PAF obtained through the Levin formula is an underestimation. Levin's formula is oriented towards non-communicable disease phenomena and this formula does not take into account transmission that occurs when prisoners leave prison and infect people in the general population[18]. More complex PAF modeling techniques were recently proposed which show higher PAFs for communicable disease than those obtained with traditional analyses, but still need to be validated[30].

In conclusion, these findings alert us to the magnitude of TB crisis within the prison system in Paraguay. They are useful to guide the development of TB control policies and national strategic plans in order to improve TB programs in penal institutions. Prisons act as "institutional amplifiers" or "reservoirs" of the disease on community[13,31]. Special attention needs to be paid about including physical prison infrastructure, early detection of cases, isolation rooms and timely treatment initiation. This is a first baseline epidemiological assessment for the country, and next studies should assess whether measures addressing prison specific TB drivers (implementation of mass screening strategies, introduction of molecular diagnostic tools) are effective. Future studies should aim to understand where *M. tuberculosis* transmission occurs, which community territories are overrepresented in the prison population and therefore expresses more cases of tuberculosis and what the foci are to improve our understanding on the contribution of TB in prisons to the national TB burden.

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Author contributions

V.G.S., S.A. and G.E. developed the primary question and overall plan for this study. V.G.S., S.A., G.E. and M.C. designed the original study and collected the data analyzed here. V.G.S., S.A. and G.E. wrote the first draft of the paper. V.G.S., S.A., G.E., J.C., J.R.A., L.M. and A.G.B. provided feedback and key revisions to the initial draft. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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7.2- Article 2

Excess tuberculosis risk during and following incarceration in Paraguay: a retrospective cohort study.

Sequera, G., Estigarribia-Sanabria, G., Aguirre, S., Piñanez, C., Martinez, L., Lopez-Olarte, R., Andrews, J. R., Walter, K. S., Croda, J., & Garcia-Basteiro, A. L. (**2024**). *The Lancet Regional Health - Americas*, 100668.

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Specific Objectives

3.- Identify risk factors associated with the development of TB disease in prisons.

4.- Identify the proportion of prison-associated TB cases in the community.

Excess tuberculosis risk during and following incarceration in Paraguay: a retrospective cohort study

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Summary

Background The increased risk of tuberculosis (TB) among people deprived of liberty (PDL) is due to individual and institution-level factors. We followed a cohort of PDL from 5 prisons in Paraguay to describe the risk of TB during incarceration and after they were released.

Methods We linked a 2013 national census of prisons with TB records from the TB Program from 2010 to 2021 to identify TB notifications among incarcerated and formerly incarcerated individuals. We used multivariable Cox regression models to quantify the risk of TB during and following incarceration and to identify risk factors associated with TB.

Findings Among 2996 individuals incarcerated, 451 (15.1%) were diagnosed with TB. Of these, 262 (58.1%) cases occurred during incarceration and 189 (41.9%) occurred in the community after release. In prison, the hazard ratio of developing TB was 1.97 (95% CI: 1.52–2.61) after six months of incarceration and increased to 2.78 (95% CI: 1.82–4.24) after 36 months compared with the first six months. The overall TB notification rate was 2940 per 100,000 person-years. This rate increased with the duration of incarceration from 1335 per 100,000 person-years in the first year to 8455 per 100,000 person-years after 8 years. Among former prisoners, the rate of TB decreased from 1717 in the first year after release to 593 per 100 000 person-years after 8 years of follow up.

Interpretation Our study shows the alarming risk of TB associated with prison environments in Paraguay, and how this risk persists for years following incarceration. Effective TB control measures to protect the health of people during and following incarceration are urgently needed.

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Keywords: Tuberculosis; Transmission; Prisons; Epidemiology; Paraguay

Introduction

Tuberculosis (TB) in prisons is an important public health concern, as the increasing burden in various regions of the world is hindering overall TB control. Recent estimates for the South American region show that the contribution of TB among people deprived of

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Research in context

Evidence before this study

It is well known that prisons are high-risk environments for tuberculosis (TB) transmission. TB prevalence within prisons far exceeds community rates, and with regular movement of incarcerated individuals between prisons and communities, this excess TB often spreads beyond prison walls. However, there is limited data on the incidence risk patterns following incarceration and release. We searched PubMed with the key terms: "tuberculosis", "incidence", "prison", and "release" or "community" for articles published in English before October, 2023. This search identified 254 articles. We identified only one article from Brazil that quantified the risk of TB during and after incarceration, showing that the risk of TB was 30 times higher in prisons than in the community and persisted several years after release among those who were previously deprived of liberty.

Added value of this study

This study, conducted from an initial cohort of 2996 people deprived of liberty from 5 prisons in Paraguay, allowed us to retrospectively observe how TB cases progressively developed over time during imprisonment and after release. We also measured how some factors such as overcrowding, time spent in prison, and re-incarceration affect the risk of developing TB within prison or after release. This study shows that the rate of developing TB increases considerably after each year spent in prison, from 1335 per 100,000 person-years in the first year to 8455 per 100,000 person-years after 8 years. Upon release, the incidence of TB decreases. However, even after 8 years of follow up in this group, their TB rates remain 10 times higher than community rates. This study also quantifies the relationship between a higher density of people deprived of liberty living per prison cells and an increased risk of developing TB.

Implications of all the available evidence

Our study shows the alarming risk of TB associated with prison environments in Paraguay, and how this risk persists for years following incarceration and release. These results call for urgent and effective TB control measures to protect the health of people during and following incarceration. Given the high contribution of TB in prisons to the overall TB burden in Paraguay, successful TB control interventions (including structural changes that reduce overcrowding) are likely to have a considerable impact on national TB burden indicators.

liberty (PDL) to the general TB burden more than doubled between 2011 and 2017.[1] This upward trend of TB in prisons, the increase in its population attributable fraction, and its role as an amplifier of TB epidemics in the community call for renewed attention to this public health problem[2,3]

The spread of TB in prisons presents a complex health problem that is magnified in the context of mass incarceration. In this setting, a continuous and dynamic process of social interaction between the prison population and the community exists. TB outbreaks in prisons have been associated with higher TB incidence in the community and vice versa. Another contributing factor to poor TB control in countries with high incarceration rates is the inconsistent follow-up of individuals infected with TB in prison who are released before completing treatment.[4,5] Limited data is available on the spillover effect and risk of TB among PDL once they are released from prison.

In 2020, the TB incidence rate in Paraguay was 48 cases per 100,000 inhabitants, but in prison settings it climbed higher than 3000 cases per 100,000 inhabitants.[6] In the last 10 years, the incarceration rate in Paraguay has doubled, reaching 200 PDL/100,000 population in 2020. Consequently, the increase in incarceration has been accompanied by a rapid increase in the proportion of TB cases nationally that occur in prison (7.1% in 2009 compared to 14.5% in 2018).[7] This has forced the National TB Control Program (NTP) to redirect its limited health resources on integral control strategies with an increasing focus on prison health.[1,8]

In order to better understand the impact of incarceration on the global TB burden, we must first characterise the excess TB burden among incarcerated and previously incarcerated individuals.[4,9] Therefore, we conducted a study to estimate the risk of TB in a cohort of PDL who were registered in a national prison census of Paraguay in 2013 and followed up until December 2021. In addition, we examined risk factors and the time course of TB during incarceration and following release from prison.

Methods

Study design

This was a retrospective longitudinal study following a cohort of PDL for the development of TB during and after incarceration. We linked three national data sources, including incarceration census data (2013), digital records of five prisons (2010–2021), both from the Ministry of Justice (MoJ) and TB case notification data from Paraguay's NTP (2010–2021) in the Ministry of Health (MoH).

Study setting

Our study focusses on five of Paraguay's 18 prisons: the National Penitentiary Tacumbú, the National Penitentiary Emboscada, the Regional Prison of Ciudad del Este (CDE), the Padre Juan Antonio de la Vega Penitentiary,

and the Esperanza Penitentiary. These prisons represent 30% of the overall prison population in the country and located near the country's most populated cities: Asunción (Paraguay's capital city) and CDE. All five prisons house only men. Further, these prisons register the movement of PDL within the national prison system including their entry, relocation, release, and re-incarceration data. Movement between prisons other than those included in the study is rare, especially due to their geographic location. In these cases, the PDL were censored at the time of relocation.

Data sources and linkage

Our study cohort included all PDL registered in the National Prison Census conducted from March 4 to March 15, 2013 (data provided by MoJ). The prison census provided the following variables for this study: name, surname, sex, date of birth, literacy, occupation, income level prior to entering the prison, cigarette or drug use, type of prison, reason for incarceration (with or without a sentence) and pavilion or prison sector.

The National TB registry (provided by MoH) is a compulsory report of all TB confirmed cases. For this study, data from 2010 to 2021 were analysed, and the variables collected were: names, surnames, national identity number, date of birth, date of diagnosis, date of death, and sociodemographic and clinical data. For all individuals diagnosed with TB in Paraguay, the national TB registry included information on patient incarceration status and the institution in which TB diagnosis occurred.

The five prisons selected for this study are the largest and oldest ones, which also have digital records with high quality data such as: dates of prison entry, relocation, release dates and re-incarceration through December 2021.

We conducted two analyses. First, we matched the 2013 prison census to the NTP registry. This enabled us to measure the percentage of TB cases among individuals included in the prison census up to 8 years after the census. However, the prison census was cross-sectional and did not include information on prison transfer or releases. Second, we conducted a sub-analysis in 5 prisons for which prison entry and release dates were available. We matched this dataset with the TB registry to identify individuals who were diagnosed with TB during and after incarceration. We used the second matched dataset to estimate hazard rates (Fig. 1).

We used a multi-stage approach to match these registries. First, our matching algorithm used individual identity numbers provided in both databases; however, some identity numbers were incomplete. Secondly, we used initials of the first name and first surname, date of birth, and sex. We confirmed all matches manually. The sensitivity of the analysis was measured by comparing people with TB registered in the NTP, according to the evaluation of "*incarceration*" variable (which provides the information on whether the TB patient was incarcerated at the time of diagnosis).

TB cases notified three years prior to the prison census (2010–2012) were excluded in order to appraise the effect of recent past TB episodes on the subsequent risk of TB. Individuals incarcerated prior to 2010 were excluded because of incomplete registration data.

Statistical analysis

We performed a survival analysis among PDL using notified TB (all forms) as the primary endpoint. Subsequently, we conducted survival analyses among two sub-populations: 1) PDL who remained incarcerated after 2013; 2) PDL who were released (and who had no TB episodes recorded while in prison), commencing observation-time at release. In the first group, the follow up period for each individual was determined from the date of their admission to prison until their release, death, or the end of the follow-up period (Dec 31, 2021). For the second group, follow-up time began at the time of their release until re-entry to prison or the end of the follow-up period. We performed all statistical analyses in R, version 3.5.0. We made right-censored survival analyses for the cohort of PDL and ex-PDL using the "survival" package.[10]

We calculated the TB notification rate and 95% confidence intervals for both PDL and former PDL over the study period as the number of new cases each year divided by the number of people at risk at the beginning of that year, repeating this model for each study year. We had mortality dates when death occurred in prison, but not if it happened after the release period if it was not associated with a TB episode.

We used multivariate Cox regression to measure risk factors associated with TB. We included a set of pre-specified sociodemographic and behavioural variables from the prison census and those related to the prison environment, such as readmissions, type of prison, level of overcrowding in the cell room or total time in prison. We performed two main models: 1) considering only the initial incarceration (Fig. S1) and 2) considering all the episodes of incarceration, which is the one ultimately presented in the main text (Table 1)). The life table showing cumulative risk for TB notification among PDL by months periods, and the Hazard Function of cumulative risk for TB notification is showed in supplemental material (Fig. S4). Furthermore, we obtained the number of people living in each cell or pavilion from the prison census, and the area of each cell or pavilion from an architectural study of the prisons carried out in 2018.[11] Therefore, overcrowding was defined as a density measure based on the number of occupants in a prison pavilion or cell and its area in square meters, divided in three categories: level 1 = Low: >7 m^2 per person; level 2 = High: 3.5–7 m^2 per person and level 3 = Very High: <3.5 m^2 per person. Total time in prison

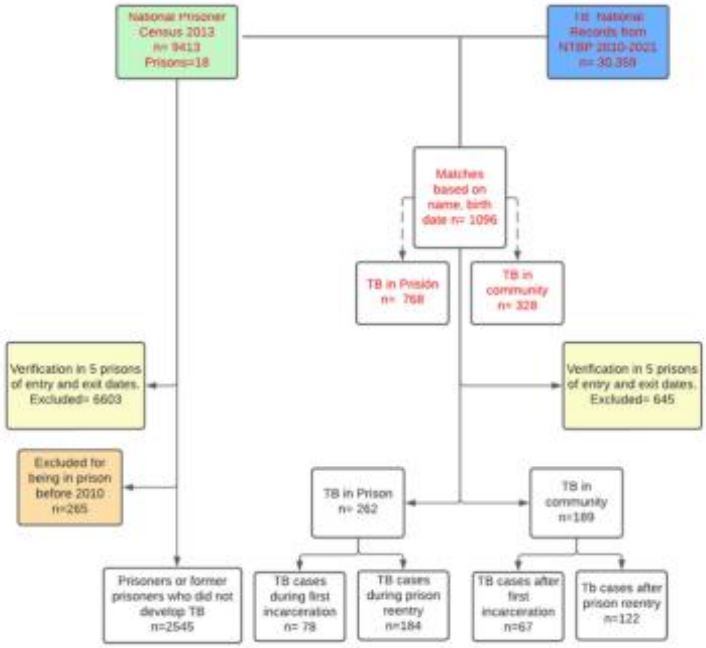

Fig. 1: Flow diagram illustrating the process of linking databases and selecting individuals for survival analysis. The 2013 National Census of Penitentiary Centers (green) was matched with the National Registry of TB Cases of the NTP, period 2010-2021 (blue). This analysis revealed 1096 cases of TB. PDL from prisons without documented records of entry and exit dates were subsequently excluded (yellow). These cases represent a total of 2996 PDL in five prisons. Out of these, 451 have developed TB and 2545 prisoners did not develop TB. PDL that had a prolonged duration of incarceration were excluded (orange).

is the sum of the time periods in all recorded admissions, is a calculated variable that we named duration of incarceration. Additionally, we compared the TB risk within prisons that house <200 prisoners (National Penitentiary Emboscada, Juan Antonio de la Vega Penitentiary and Esperanza Penitentiary) with larger prisons (Tacumbú and CDE), which host >1000 PDL.

Missing data was found in various behavioural variables of the census, but in all cases, it was less than 10% and its value has been inputted using a predictive mean matching algorithm that utilizes 3 nearest knots for continuous predictors (aregImpute function, Hmisc package in R). Overcrowding variable was the exception, as this analysis only considered data from the largest prisons and did not account for missing values in the other prisons.

Ethics statement

The study was approved by the ethics committee of the Central Laboratory of Public Health of the MoH of Paraguay (International Certification FWA N° FWA0000020088) with code CEI-LCSP 91/010217. It was also authorised by the MoJ of Paraguay (reference number, MJ/GM/N°929/2021). Databases needed for matching were kept in password protected files. Once matching was completed, the resulting database was anonymised and data analysis conducted.

Role of the funding source

The funder had no role in data collection, analysis, interpretation, writing of the manuscript, or decision to submit the manuscript for publication.

Results

Sociodemographic characteristics of incarcerated individuals

In 2013, 9413 people were deprived of liberty in the Paraguayan penitentiary system, of which the five prisons included in this cohort study accounted for 2996 (31.8%) PDL. All were men and the mean age was 31.1 (SD 9.6) years. Of these, 417 (13.9%) were illiterate (four-fold more than the general population).[13] The most frequent crime reported was theft or

Variable	Categories	Total	TB	%	HR	95% CI, p-value
Age	More than 45	286	23	7.9	1	
	31–45	1070	131	12.2	1.59	(0.94–2.69, p = 0.086)
	18–30	1640	297	18.1	2.53	(1.51–4.23, p < 0.0001)
Illiterate	Yes	417	61	14.5	1	
	No	2579	390	15.1	1.11	(0.82–1.49, p = 0.50)
Worked	No	428	61	14.1	1	
	Yes	2568	390	15.2	1.12	(0.85–1.49, p = 0.41)
Wage	Minimun	717	105	14.6	1	
	More than Minimun	944	143	15.1	0.96	(0.73–1.27, p = 0.80)
	Less than Minimun	1335	203	15.2	1.00	(0.78–1.30, p = 0.97)
Addiction to substances	Yes	1203	169	14.1	1	
	No	1793	282	15.7	1.12	(0.91–1.38, p = 0.29)
Tobacco smoking	Yes	1410	199	14.1	1	
	No	1586	252	15.9	1.15	(0.94–1.41, p = 0.18)
Prision entries	1	1347	145	10.9	1	
	2	677	109	16.2	1.99	(1.52–2.61, p < 0.0001)
	3	405	79	19.4	2.58	(1.89–3.51, p < 0.0001)
	4 and more	567	118	20.6	3.36	(2.50–4.50, p < 0.0001)
Duration of incarceration	Less than 6 months	400	25	6.3	1	
	6–18 months	657	90	13.8	1.97	(1.26–3.08, p = 0.0034)
	19–36 months	503	100	19.9	2.81	(1.80–4.38, p < 0.0001)
	More than 36 months	1436	235	16.4	2.78	(1.82–4.24, p < 0.0001)
Prisons	Tacumbú	2209	345	15.6	1	
	Ciudad del Este	418	96	22.9	2.04	(1.50–2.76, p < 0.0001)
	Others[a]	369	10	2.7	–	
Crime	Others Crimes	274	26	9.6	1	
	Drug trafficking	372	43	11.7	1.42	(0.85–2.37, p = 0.19)
	Against persons	940	117	12.4	1.54	(0.98–2.41, p = 0.060)
	Against property	1410	265	18.8	1.87	(1.22–2.84, p = 0.0044)
Legal situation	Await sentencing	1940	275	14.2	–	
	Sentenced	1056	176	16.7	1.05	(0.84–1.31, p = 0.69)
Overcrowding[c]	Level 1	214	21	9.8	1	
	Level 2	367	41	11.2	1.48	(0.85–2.59, p = 0.18)
	Level 3	2046	379	18.5	2.06	(1.28–3.32, p = 0.0027)
	N/D[b]	369	10	2.7	–	

The variables used in this model were a-priori specified. [a]Other prisons: National Penitentiary Emboscada, Padre Juan Antonio de la Vega Penitentiary, and Esperanza Penitentiary. [b]Missing value from other prisons. [c]Overcrowding levels were defined as follows: 1 = Low:>7 m^2 per person; level 2 = High: 3.5–7 m^2 per person and level 3 = Very High: <3.5 m^2 per person.

Table 1: **Multivariable Cox regression of factors associated with developing TB among Paraguayan PDL included in the 2013 census of 5 selected prisons.**

robbery (47.1%; 1410/2996) and 51.3% (1539/2996) of PDL had a prior history of incarceration. Around two thirds of PDL (64.8%, 1940/2996) were pre-trial detainees who were awaiting their sentencing process in prison. All other individuals had a confirmed and definitive sentence.

TB hazard during and following incarceration

First, we matched the 2013 prison census with the 2010–2021 TB registry to identify TB cases among people incarcerated at the time of the census. During the 8 years following the census, 11.6% (1096/9413) developed TB. Of these, 70.1% (768/1096) were diagnosed with TB in prison and 29.9% (328/1096) were diagnosed in the community, according to the national TB registry.

To estimate the hazard of TB during and following incarceration, we focussed on five study prisons, for which we obtained information on timing of prison entry, release, and transfer. Within the 5 study prisons, the total follow-up time of the cohort was 15,341 person-years, with an overall median length of 8.2 years (IQR: 5.63–9.01). The median time of incarceration was 2.25 years (IQR: 0.75–4.58). Almost a third (31.1%); of the prison population is renewed each year. Once released from these prisons, the median out-of-prison cumulative follow-up was 4.30 years (IQR: 0.92–7.08). The overall notification rate was 2940 TB cases per 100,000

person-years. We observed that 15.1% (451/2996) developed TB during the total years of follow-up. Of these TB episodes, 58.1% (262/451) occurred while incarcerated and 29.7% (78/262) of them during the initial incarceration. Over two fifths (41.9%, 189/451) of the TB cases registered occurred outside the prison, with 35.4% (67/189) of them happening after their first incarceration.

The overall TB notification rate in prison increased with duration of incarceration, from 1335 per 100,000 person-years (95% CI: 1073–1664) in the first year to 8455 per 100,000 person-years (95% CI: 6651–10,752) after 8 years in prison. During the first incarceration, the notification rate in the first incarceration year was 549 per 100,000 person-years (95% CI: 351–865) and increased to 6459 per 100,000 person-years (95% CI: 4556–9160) after 8 years in prison. The TB notification rate among released PDL decreased immediately and steadily through time, from 1717 (95% CI: 1396–2115) in the first year to 593 per 100,000 person-years after (95% CI: 267–1326) 8 years of follow up. Among those who were incarcerated only once, the notification rate after release decreased immediately from 1196 (95% CI 848–1692) in the first year to 439 per 100,000 person-years (95% CI 153–1268) after 8 years of release (Fig. 2).

Factors associated with TB risk

The risk of TB during incarceration was greater among younger PDL, as those aged 18–30 years old had a higher risk of TB (HR: 2.53; 95% CI: 1.51–4.23, $p < 0.0001$) compared to incarcerated individuals older than 45 years. TB rate was also greater for subsequent incarcerations. Individuals in their second incarceration had almost a two-fold risk of TB (HR: 1.99 (95% CI: 1.52–2.61, $p < 0.0001$), as compared to those during their first incarceration. The risk increased with each new admission and reached a HR of 3.36 (95% CI: 2.50–4.50, $p < 0.0001$) after the fourth readmission, compared to the initial one (Table 1). However, the shape of the time course of risk during incarceration did not differ substantially when considering only the first incarceration. In this group, there was a 2.36 (95% CI: 0.89–6.31, $p = 0.086$) increased hazard for developing TB after 6 months of entry, reaching 4.55 (95% CI 1.82–11.35, $p < 0.0001$) after 36 months in prison (Fig. S1).

The two largest prisons reported 97.8% (441/451) of the TB cases in this study. Individuals incarcerated in the prison of CDE had the highest rate of TB–twice that of the Tacumbú prison (HR: 2.04; 95% CI: 1.50–2.76, $p < 0.0001$). Individuals charged with or convicted of property crimes, mainly due to theft and robbery, had a higher risk of TB (1.87, 95% CI 1.22–2.84, $p = 0.0044$) compared to drug trafficking or crimes against person. This was independent of the type of prison, but poorly associated with the time of the sentence. The rate of TB also increased with the density of cell. When compared to individuals residing in cells with >7 m^2 per person, those residing cells 3.5–7 m^2/person had a HR of 1.48 (95% CI 0.85–2.59, $p = 0.17$) and those with <3.5 m^2 per person had HR of 2.06 (95% CI 1.28–3.32, $p = 0.0027$). Fig. 3 illustrates how almost all of the TB prison population of the larger prisons (CDE and Tacumbú) were living in the overcrowding level 3 (87.0% and 81.5%, respectively).

Discussion

By linking Paraguay's prison census with the National TB registry, we report a previously undocumented burden of TB among currently and formerly incarcerated individuals. We found that a substantial proportion (15.1%) of those incarcerated or who had been recently incarcerated experienced TB. This study shows that the risk of TB in PDL remains elevated several years after incarceration. The TB rates among PDL seen in this study are amongst the highest for any at-risk population

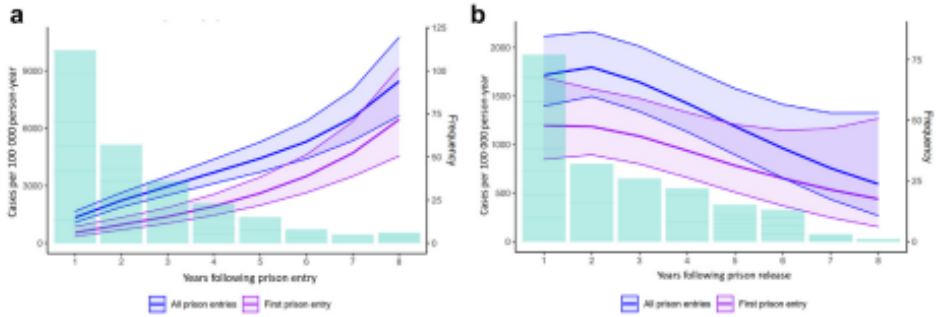

Fig. 2: Modeled TB notification rate among a) PDL and b) people formerly deprived of liberty. Blue lines indicate notification risk among all incarcerated individuals, purple lines indicate notification risk among individuals during their first incarceration. Blue bars indicate the number of notified TB cases by year.

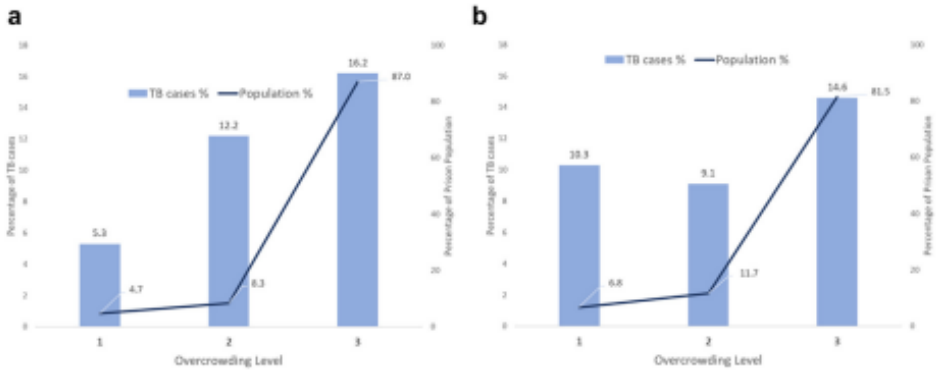

***Fig. 3*: Percentage of TB cases according to the level of overcrowding and the distribution of prison population inside the two largest prisons in the study: a) CDE prison and b) Tacumbú prison.** Bars correspond to the percentage of TB cases among PDL according to the rate of overcrowding of their cell rooms. Blue line corresponds to percentage of TB prison population in each level. A) Regional Prison of Ciudad del Este and b) National Penitentiary of Tacumbú. Overcrowding level are 1 = Low: >7 m^2 per prisoner; 2 = High: 3.5-7 m^2 per prisoner; 3 = Very High: <3.5 m^2 per person.

recorded. The detailed data on incarceration entries and exits provided us a unique opportunity to follow this population that is notoriously difficult to track after leaving prison. Our findings highlight a previously undocumented population health consequence of incarceration in Paraguay and underscores the urgency of new programs to protect the health of incarcerated and formerly incarcerated individuals.

In this study, we found that the risk of developing TB increases rapidly following prison admission; this risk was more than 100 times the TB risk in the community. The high TB rates found after eight years following incarceration indicate that when the conditions of incarceration remain unchanged (overcrowding, smoking, malnutrition, etc.) the risk of developing TB accumulates over time. This high risk can be attributable to the gradual progression of new TB infections acquired in prisons and/or of pre-existing infections. In both of these cases, risk is likely to be exacerbated by the presence of risk factors favouring TB progression. Similarly, when PDL are released, the risk of developing TB is much higher than in the general population at more than 30 times in the first two years after release. Although this risk decreases gradually, it remains 10 times higher than the risk of TB in the general population over 8 years of follow-up. This expected increase in the TB risk occurring while deprived of liberty and the decrease in risk after release confirms that prisons are environments which contribute to the propagation of TB. In this context, well known individual determinants of TB, such as smoking, drug abuse or poverty, are insignificant factors in enhancing the TB risk in the already high-risk, high transmission environment of prisons. The poor conditions of Paraguayan prisons, mostly driven by overcrowding, poor ventilation, limited access to healthcare and high exposure periods, may overshadow the progress that may be made in TB control in the community.[7,13]

The elevated risk of TB that is associated with incarceration also undermines TB control more broadly, as individuals infected with *M. tuberculosis* within prisons may transmit the bacteria beyond prison walls. We have found genomic evidence that *M. tuberculosis* genomic clusters span prisons and into the general population in Paraguay's major urban centres, indicating close transmission linkages between prisons and the community, with similar evidence of infection spillover in Central West Brazil[14,15]

Environmental and structural changes in these institutions may have a considerable impact on TB control. However, reducing overcrowding will not be sufficient to substantially reduce the TB burden in Paraguay's prisons (in the least crowded prison cells, 5.3–10.3% of individuals developed TB). Our findings are in line with those from Brazil, where the risk factors associated with prison environments, increase TB incidence more than the risk factors associated with the individual factors of this high risk population. Thus, targeted structural interventions within prisons could have a substantial effect on the broader TB epidemic. More importantly, reducing our reliance on incarceration and significantly reducing prison populations will decrease the population put at increased risk of TB.[16]

Although the overall incidence rate of TB in prisons is not dramatically increasing, the recent upward trends in general rates of incarceration will result in a considerable number of TB cases in prisons that would otherwise not exist. This observation correlates with the

increase in overall TB incidence rate reported for Paraguay. The proportion of PDL with a definitive sentence for a crime was only 26.2% at the time of the census, the remaining ones were pre-trial detainees, awaiting the judicial process for a trial. In Latin America, Paraguay has one of the highest proportions of pre-trial detainees among all PDL.[14,17] Reforms in the penal system must include decarceration as well as expediting a definite sentence. Although this problem seems to be primarily judicial, the impact on the public health is considerable.

We found that almost a third (31.1%) of the population in prisons is renewed each year. This population cycles between prison and the general community leading to substantial transmission spillover. We found that 3/4 of TB cases among former PDL occurred in the first 3 years after being released, suggesting that screening at the time of prison exit (and periodically after that) may be efficient targeted interventions.[16,18] When former PDL are reintegrated within a community with lower TB risk, exit screening may improve the identification of TB cases to allow for preventive interventions among persons at high-risk of subsequently developing TB, and thus reducing the spread of the disease. Furthermore, due to the high risk of TB among former PDL, primary care screening algorithms may consider persons with a recent history of incarceration.

We found that re-admission into prison is related to the type of crime (robbery/theft) with a short sentence (less than 3 years on average) that mostly occurs to younger prisoners. This is the most frequent profile of PDL readmitted into prison. A key question is whether the TB risk in this population is higher in the community before first entering prison. This could be done by comparing pre-existing high-risk attributes – such as poverty, illiteracy, drug abuse, HIV and others–among persons first entering prison and persons that have not been incarcerated. The evident increase in TB risk occurs upon incarceration. This phenomenon has already been described before for several contagious diseases.[16,19,20]

In Latin America the collaboration between authorities that are responsible for public health and those that govern prisons is insufficient. This is also why it is hard to find documented evidence of TB history or incarceration history among TB cases that occur in the community, which makes this analysis unique in the region.[16] Renewed joint efforts between these two sectors are needed to improve overall TB control, but particularly to address TB in prisons.

Our study has some limitations. First, utilizing cross-sectional data has certain limitations. The sociodemographic characteristics of the PDL were collected through census questionnaires and might not be accurately captured by the discrimination and stigma that the PDL population is subjected to. Second, our study cohort includes five of the 18 prisons in the Paraguayan penal system, including two of the largest prisons with the highest TB rates in the country. Larger prisons hold more prisoners with higher overcrowding, and these large-prisons were overrepresented in this study. Additionally, if any of the prisoners included in this study had readmissions to prisons other than those selected, this event could not be recorded for analysis, potentially underestimating the total time a PDL was in prison. Third, the TB episodes are based on TB notification from the NTP, and it is well known that the surveillance system misses a proportion of the real TB cases. Thus, although this study finds very high TB rates, the real prison attributable rate may be even higher. Lastly, deaths from non-TB related causes that occurred after release could not be registered, leading to an overestimation of the total follow up time and an underestimation of the TB burden in this subset of individuals.

Our data show that prisons are a high risk setting for the development of TB, and that the risk continues after release. There is a critical need to develop new TB control strategies to achieve the WHO global targets for 2035 and we believe that implementing and strengthening TB control interventions in prisons cannot be neglected to achieve these goals. Specific interventions within prisons are likely to have a valuable impact on reducing the tuberculosis epidemic. However, these interventions should not solely focus on biomedical approaches such as active diagnosis, mass screening, and secondary IPT.[16,18] In addition, it is important to consider architectural reforms aimed at improving the living conditions within penitentiary centres and legal reforms including decarceration, reducing reliance on incarceration, and preventing the imprisonment of individuals who have not been sentenced. These measures should be implemented with a strong focus on upholding human rights principles.[21]

Contributors

GS and AGB conceived the study and contributed to interpretation of the results. GS did statistical analyses and drafted the first manuscript. GES, SA, CP, LM, JRA, KSW, JC, AGB, and GS contributed to critical revision of the manuscript. GS attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. GS and AGB have accessed and verified the underlying data. All authors had access to the data and accepted responsibility for the decision to submit for publication.

Data sharing statement

Certain restrictions apply regarding the accessibility of these data. As such, the data are not publicly available. However, data may be obtained by reasonable request to the corresponding author, subject to institutional permission.

Declaration of interests

All authors report no potential conflicts.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2023.100668.

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Supplementary Information

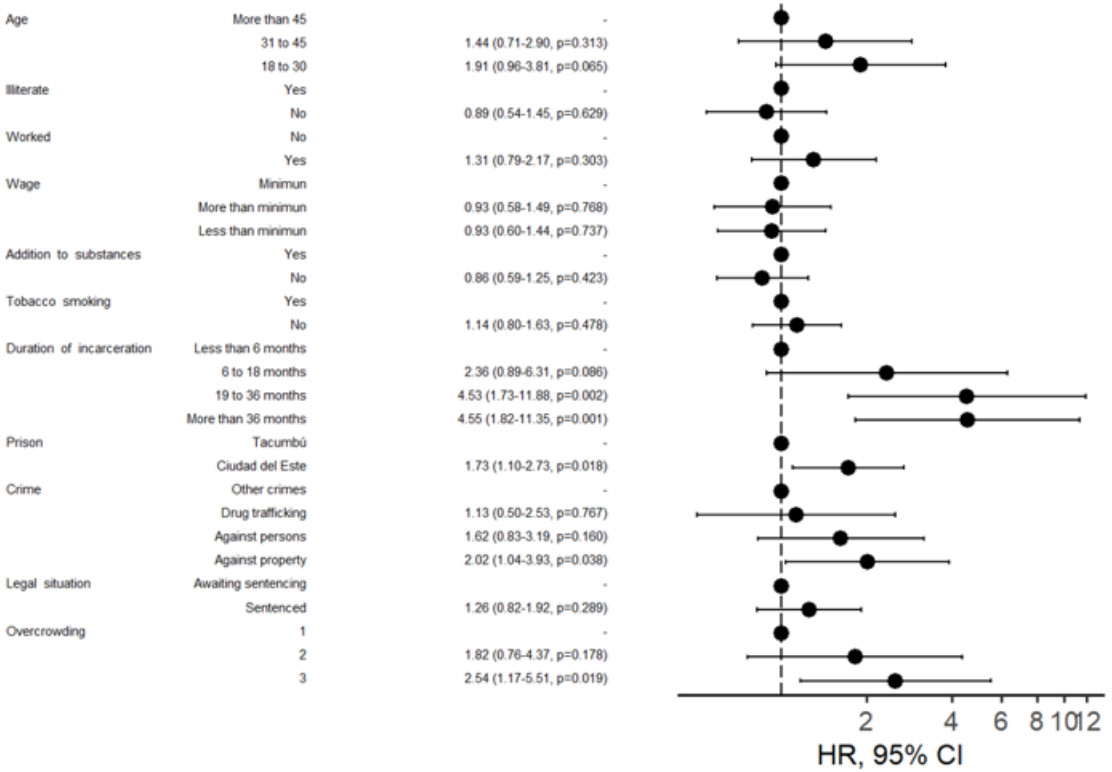


Figure S1. *Hazard Ratio and 95%CI. Multivariable Cox Regression of factors influencing develop of TB among Paraguayan prisoners showing survival analysis only in its first incarceration.*

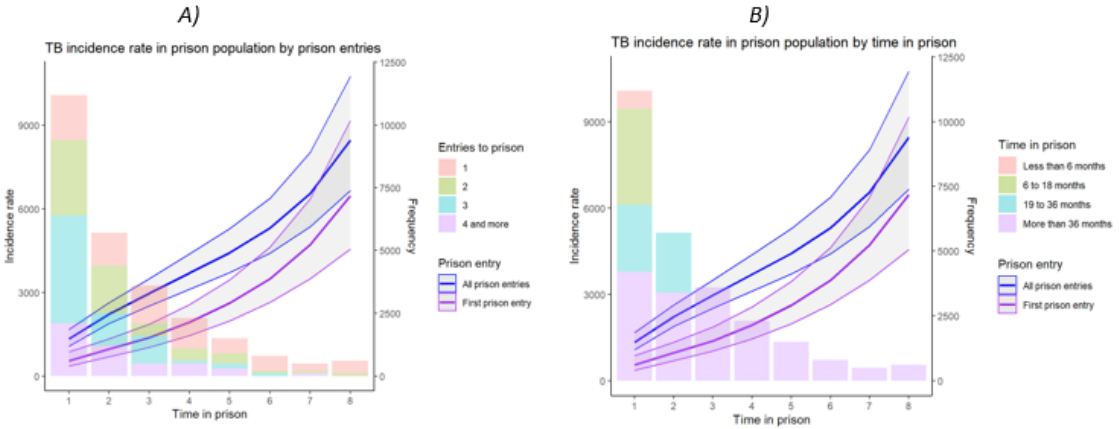


Figure S2. *Incidence risk of TB among incarcerated individuals by history of previous incarceration or not (1 to 4 or more re-entries) and length of time in prison. The incidence rate includes 95% confidence intervals, on blue and purple shading. Histograms are TB cases by each year of follow-up, and the colors are in A) number of prisons entries and B) time in prison.*

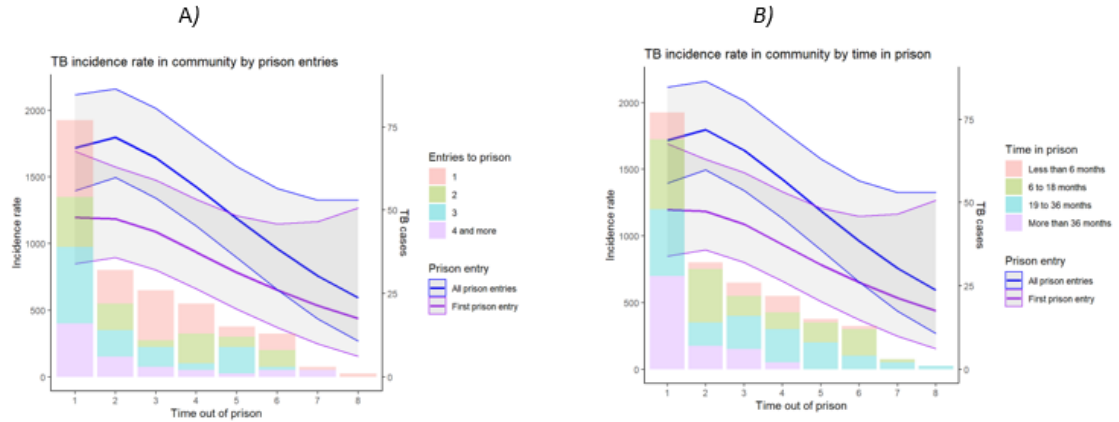


Figure S3. *Incidence risk of TB among released individuals by history of previous incarceration (1 to 4 or more re-entries) and length of time in prison. The incidence rates include 95% confidence intervals, on blue and purple shading. Histograms are TB cases by each year of follow-up once released, and the colors are in A) number of prisons entries and B) time in prison.*

Table S1. Life table showing cumulative risk for TB notification among PDL by months periods. The table is divided into two scenarios: A) PDL during incarceration, and B) former PDL who were released from prison.

A) Life Table during Time in Prison

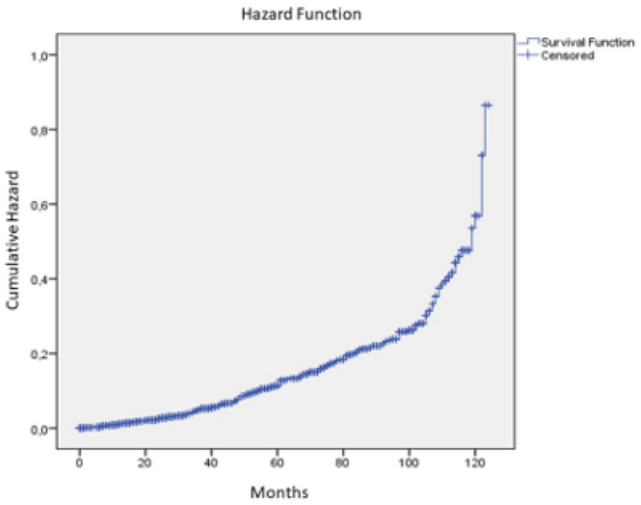
Interval Start Time by months	Number Entering Interval	Number Withdrawing during Interval	Number Exposed to Risk	Number of Terminal Events	Proportion Terminating	Proportion Surviving	Cum. Proportion Surviving at End of Interval	Probability Density	Std. Error of Probability Density	Hazard Rate
0	2996	604	2694.000	20	.01	.99	.99	.001	.000	.01
10	2372	413	2165.500	24	.01	.99	.98	.001	.000	.01
20	1935	294	1788.000	24	.01	.99	.97	.001	.000	.01
30	1617	271	1481.500	31	.02	.98	.95	.002	.000	.02
40	1315	260	1185.000	33	.03	.97	.92	.003	.000	.03
50	1018	143	946.500	26	.03	.97	.89	.003	.000	.03
60	849	190	754.000	22	.03	.97	.86	.003	.001	.03
70	634	113	577.500	21	.04	.96	.83	.003	.001	.04
80	499	119	439.500	16	.04	.96	.80	.003	.001	.04
90	364	97	315.500	12	.04	.96	.77	.003	.001	.04
100	255	116	197.000	18	.09	.91	.69	.008	.002	.10
110	119	104	67.000	15	.22	.78	.54	0.000	0.000	.29

B) Life Table during Time out of Prison

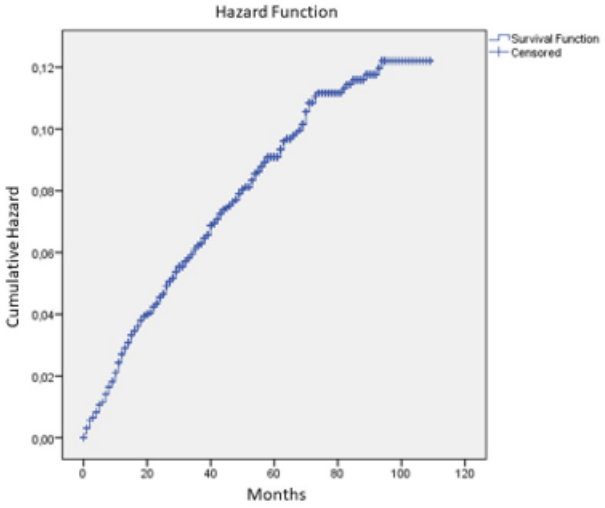
Interval Start Time by months	Number Entering Interval	Number Withdrawing during Interval	Number Exposed to Risk	Number of Terminal Events	Proportion Terminating	Proportion Surviving	Cum. Proportion Surviving at End of Interval	Probability Density	Std. Error of Probability Density	Hazard Rate
0	2298	80	2258.000	8	0.00	1.00	1.00	0.000	0.000	.00
10	2218	93	2171.500	8	0.00	1.00	1.00	0.000	0.000	.00
20	2125	155	2047.500	10	0.00	1.00	1.00	.000	.000	.00
30	1965	183	1873.500	8	0.00	1.00	.99	.001	.000	.00
40	1772	170	1687.000	18	0.01	0.99	.98	.001	.000	.01
50	1580	206	1477.000	12	0.01	0.99	.97	.001	.000	.01
60	1365	215	1257.500	15	0.01	0.99	.96	.001	.000	.01
70	1131	228	1017.000	30	0.03	0.97	.93	.003	.001	.03
80	873	208	769.000	26	0.03	0.97	.90	.003	.001	.03
90	637	482	396.000	38	0.10	0.90	.77	.013	.002	.11
100	99	78	60.000	16	0.27	0.73	.50	.027	.005	.36

Figure S4. Hazard Function of cumulative risk for TB notification among PDL by months in two scenarios: A) PDL during incarceration, and B) after release from prison (former PDL).

A)



B)



7.3- Article 3

Phylogeography and transmission of Mycobacterium tuberculosis spanning prisons and surrounding communities in Paraguay.

Sanabria, G. E*****., **Sequera, G*.,** Aguirre, S., Méndez, J., dos Santos, P. C. P., Gustafson, N. W., Godoy, M., Ortiz, A., Cespedes, C., Martínez, G., García-Basteiro, A. L., Andrews, J. R., Croda, J., & Walter, K. S. (**2023**). *Nature Communications*, *14*(1), 303.

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*These authors contributed equally: Gladys Estigarribia Sanabria & Guillermo Sequera

Impact Factor: 5.116 (SJR)

Q1 (Biochemistry, Genetics and Molecular Biology)

Specific Objectives

5.- Determine the lineages of *Mycobacterium tuberculosis* existing in prisons and their surrounding communities.

6.- Identify clusters and recent transmission of *Mycobacterium Tuberculosis* through whole genome sequencing in both prison and community cases.

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Article

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Phylogeography and transmission of *Mycobacterium tuberculosis* spanning prisons and surrounding communities in Paraguay

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Check for updates

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Recent rises in incident tuberculosis (TB) cases in Paraguay and the increasing concentration of TB within prisons highlight the urgency of targeting strategies to interrupt transmission and prevent new infections. However, whether specific cities or carceral institutions play a disproportionate role in transmission remains unknown. We conducted prospective genomic surveillance, sequencing 471 *Mycobacterium tuberculosis* complex genomes, from inside and outside prisons in Paraguay's two largest urban areas, Asunción and Ciudad del Este, from 2016 to 2021. We found genomic evidence of frequent recent transmission within prisons and transmission linkages spanning prisons and surrounding populations. We identified a signal of frequent *M. tuberculosis* spread between urban areas and marked recent population size expansion of the three largest genomic transmission clusters. Together, our findings highlight the urgency of strengthening TB control programs to reduce transmission risk within prisons in Paraguay, where incidence was 70 times that outside prisons in 2021.

Despite significant tuberculosis (TB) control efforts, the incidence rate of TB has declined only slowly in the World Health Organization Region of the Americas, and, alarmingly, has stagnated since 2014[1]. The COVID-19 pandemic disrupted access to healthcare—including critical TB diagnostic and treatment programs—compounding the burden of TB and reversing decades of progress in TB control[1].

New approaches to limit transmission are urgently needed in Paraguay, where TB control is chronically underfunded[2] and where TB

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incidence was 48 (41–56) per 100,000 people in 2020, higher than the mean incidence rate across the region[1]. More than a quarter of the country's population lives below the national poverty line[2] and are at heightened risk of TB infection and mortality. Further, recent dramatic increases in incarceration[3,4] put a rapidly growing population at high risk of infection and disease[5–7]. To guide interventions in Paraguay, there is a critical need to identify the populations at greatest risk of infection and locations and institutions where transmission most frequently occurs[8].

Whole genome sequencing of the *Mycobacterium tuberculosis* complex has been powerfully applied to characterize recent transmission dynamics. Genomic approaches have dated introductions of *M. tuberculosis* and reconstructed patterns of historic spread across Central and South America[9,10], estimated the contribution of recent transmission to incident TB cases[11], reconstructed the emergence of resistance-associated mutations[12], and inferred likely individual-level transmission events[13]. In Brazil[14] and Georgia[15], for example, genomic approaches identified frequent transmission within prisons as well as evidence of spillover from prisons to surrounding communities. A single *M. tuberculosis* molecular study from Paraguay[15] on strains collected in 2003 reported that *M. tuberculosis* families found across South America, including the Latin-American (LAM; sub-lineage 4.3) and Haarlem (4.1.2.1) were also common in Paraguay[15,16].

Genomic approaches have not been applied to address major gaps in our understanding of TB transmission in Paraguay. First, the conditions of incarceration put people at high risk of many infectious diseases, and globally, over the past twenty years, the incarcerated population in Central and South America has grown by 206%, the greatest increase in the world[4]. Escalating incarceration rates have been paralleled by an increasing concentration of notified TB among incarcerated individuals[6]. Yet the role of prison environments on TB transmission both inside and beyond prisons, as sources of broader infection, has not yet been described in Paraguay. Second, while incidence of TB is heterogeneous across the country[15], it remains unknown whether specific cities or regions function as hotspots, fueling transmission elsewhere. Finally, due to limited surveillance infrastructure, the prevalence of drug-resistance and multi-drug-resistance has not yet been systematically measured[17–19]. Only 56% of bacteriologically confirmed new cases of pulmonary TB were tested for rifampicin resistance in 2020[1].

To characterize transmission dynamics and circulating diversity of *M. tuberculosis* complex strains in Paraguay, we conducted prospective genomic surveillance across the country from 2016 to 2021, including surveillance within and outside prisons, generating a genomic resource for continued surveillance in Paraguay. We estimated the role of likely recent *M. tuberculosis* transmission within prisons, the relatedness of prison and community transmission, and the frequent movement of *M. tuberculosis* between Paraguay's urban centers.

Results

Population-based genomic surveillance

From 2016 to 2021, 16,734 TB cases were notified in Paraguay, with the majority of cases (60%; 10,095/16,734) occurring in the urban departments Central and Distrito Capital (which together comprise Asunción) and Alto Paraná (Ciudad del Este), where we conducted prospective genomic surveillance (Fig. 1a, Fig. S1). In 2021, the TB notification rate was 70 times higher in prisons than outside (3378 cases per 100,000 in prisons/49 cases per 100,000 in the general population) (Fig. 1b). Therefore, we focused genomic surveillance in the two largest prisons in the country, Tacumbú Prison and the Prison of Ciudad del Este, which together hold 36% (4950/13,821) of Paraguay's incarcerated population, notification rates are 2000 and 3500 per 100,000 people, respectively.

Of the 7780 TB cases notified in Asunción during the study period, 781 (10%) occurred among incarcerated individuals (Fig. S1). 64% (503/781) of these were culture-positive and, of these, we sequenced 21% (107/503). 33% (2306/6999) of non-incarcerated individuals with TB in Asunción were culture-positive and of these, we sequenced 7% (172/2,306). Of the 2,315 TB cases notified in Ciudad del Este during the study period, 422 (18%) occurred among incarcerated individuals (Fig. S1). 64% (269/422) of these were culture-positive and, of these, we sequenced 20% (55/269). 31% (578/1893) of non-incarcerated individuals with TB in Ciudad del Este were culture-positive and of these, we sequenced 27% (158/578) (Fig. S1).

Whole genome sequences (WGS) for a total of 532 isolates met our coverage and quality criteria (Methods), including 488 from unique TB notifications. Of the samples passing filters, 158 were from individuals diagnosed with TB while in prison and 330 were from people diagnosed in the community. TB isolates were collected in Asunción (274/488) and in Ciudad del Este (214/488). We excluded 17 isolates with evidence of mixed infection with more than one sub-lineage detected, resulting in 471 *M. tuberculosis* isolates for following analyses.

Genotypic resistance

The majority, 96% (454/471) of sampled *M. tuberculosis*, were drug-sensitive; 3% (15/471) were resistant to at least one drug; and 0.42% (2/471) were multi-drug resistant, resistant to both isoniazid and rifampin. Resistance was not associated with sub-lineage (X^2 (11) = 7.7, p = 0.74). We identified three unique isoniazid resistance-conferring mutations on the genes *fabG1*, *katG*, or both among the 10 isolates with any isoniazid resistance; the three rifampicin-conferring mutations in *rpoB* (two on multi-drug resistant isolates) were unique (Fig. 2).

Stable genomic diversity of *M. tuberculosis* in Paraguay

After excluding mixed infections, all *M. tuberculosis* isolates were strains from *M. tuberculosis* lineage 4. A single mixed lineage infection was co-infected with strains from both lineages 1 and 4. Samples predominantly fell into four sub-lineages: 4.3.3/LAM (42.5%; 200/471), 4.1.2 /Haarlem (38.2%; 180/471), 4.4.1/S (12.3%; 58/471), and 4.3.4/LAM (3.2%; 15/471) (Fig. 2). The distribution of strains representing these

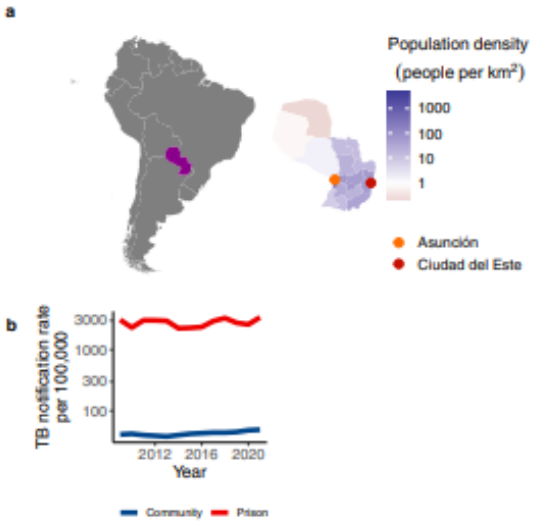

Fig. 1 | Genomic surveillance within and outside prisons in Paraguay's urban centers. a Map of South America, with Paraguay highlighted, and as an inset. Paraguay's departments are colored by population size density and points indicate the two largest urban centers in Paraguay, where we conducted focused genomic surveillance. **b** Notification rate of TB per 100,000 people in prisons (red) and in the general population (blue) from 2009 to 2020. Source data are provided as a Source Data file.

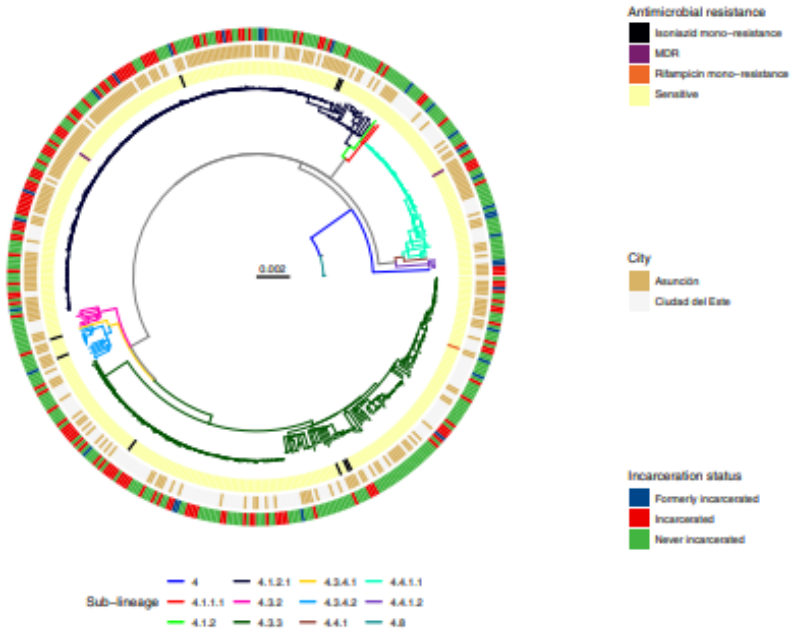

Fig. 2 | *M. tuberculosis* isolates from incarcerated and non-incarcerated people are closely related across Paraguay. A maximum likelihood phylogeny of 471 tuberculosis isolates from Lineage 4 inferred from a multiple sequence alignment of 9966 SNPs and rooted on three sub-lineage 4.8 samples from this study. Branch lengths are in units of substitutions per site. Branches are colored by sub-lineage. From the inside, rings are colored by antimicrobial resistance; city of sampling; and incarceration status at the time of TB notification. Other isoniazid includes isoniazid mono-resistant isolates without an *ahpC* promoter mutation. Rifampicin indicates rifampicin mono-resistant isolates. Other resistance includes isolates with mutations associated with resistance to pyrazinamide, streptomycin, or fluoroquinolones and not isoniazid or rifampicin. Source data are provided as a Source Data file.

sublineages was stable and did not change significantly from a collection of 173 *M. tuberculosis* isolates collected in 2003[15] (Fig. S1).

Recent expansion of *M. tuberculosis* transmission clusters

We next explored evidence of recent *M. tuberculosis* transmission in Paraguay. As seen in a maximum likelihood phylogeny (Fig. 2), sampled *M. tuberculosis* diversity was dominated by several highly related clones. Seventy-eight percent (369/471) of all isolates fell within 26 genomic clusters (each including 2 to 159 isolates) defined by a 12-SNP threshold[20], suggesting TB notifications were often attributable to recent transmission.

We reconstructed population size dynamics of the three largest genomic clusters—which comprised 56% (264/471) of our sample—with a Bayesian coalescent population size model. The three largest genomic clusters (including 159, 91, and 15 samples) increased in effective population size by 200, 90, and 40-fold, respectively. Cluster growth was relatively recent, with cluster most recent common ancestors (MRCA) occurring in 1998 (95% HDI: 1994–2001), 1996 (95% HDI: 1991–2000), and 1998 (95% HDI: 1992–2003) respectively, to 2021, when the most recent samples were collected (Fig. 3). All three clusters included isolates from individuals notified with TB during incarceration and individuals with no incarceration history.

We found no evidence that genomic loci were associated with successful genomic clusters, which we defined as the clusters containing more than 15 *M. tuberculosis* isolates, using a bacterial GWAS approach which controls for clonality and strong population structure[28]. We similarly found no association between genomic loci and clustered phenotype when we examined membership in a cluster with 10 or more isolates or 2 or more isolates.

M. tuberculosis genomic clusters span prisons and the general population

In a maximum likelihood phylogeny (Fig. 2), M. *tuberculosis* isolates sampled from incarcerated and non-incarcerated people are distributed across the tree and did not form distinct clades, indicating a recent shared evolutionary history of isolates sampled from prisons and the community. However, sub-lineage was associated with incarceration status ($\chi^2(22) = 52.3$, $p < 0.001$), with strains from sub-lineage 4.1.2.1 more frequently infecting people with a history of incarceration (46.1%; 83/180) compared to individuals with no incarceration history (33.0%; 96/291; $p = 0.006$).

Isolates from incarcerated people were more frequently clustered (92.6%, 138/149), than those from formerly incarcerated (71.0%, 22/31, $\chi^2(1) = 10.1$, $p = 0.001$) or never incarcerated people (71.8%, 209/291; $\chi^2(1) = 24.3$, $p < 0.001$), likely reflecting more recent transmission within prisons. With a stricter threshold of 5 SNPs, 45.4% (214/471) of all isolates in genomic transmission clusters. With this threshold, isolates from incarcerated individuals were again more frequently clustered (58.3%; 87/149) than those from those formerly incarcerated (45.2%; 14/31), though not significantly so ($\chi^2(1) = 1.3$, $p = 0.25$), and isolates from incarcerated individuals were more frequently clustered than those from never incarcerated individuals (38.8%; 113/291; $\chi^2(1) = 24.3$, $p < 0.001$).

We predicted that if prison and community-associated epidemics were distinct, isolates from the community would be most closely related to and cluster with other isolates from the community and vice versa. Approximately half (48.0%; 12/25) of genomic clusters, including people with no incarceration history also included individuals with a recent history of incarceration. The consequence is that 85.2% (178/209) of individuals with evidence of recent transmission and no recent

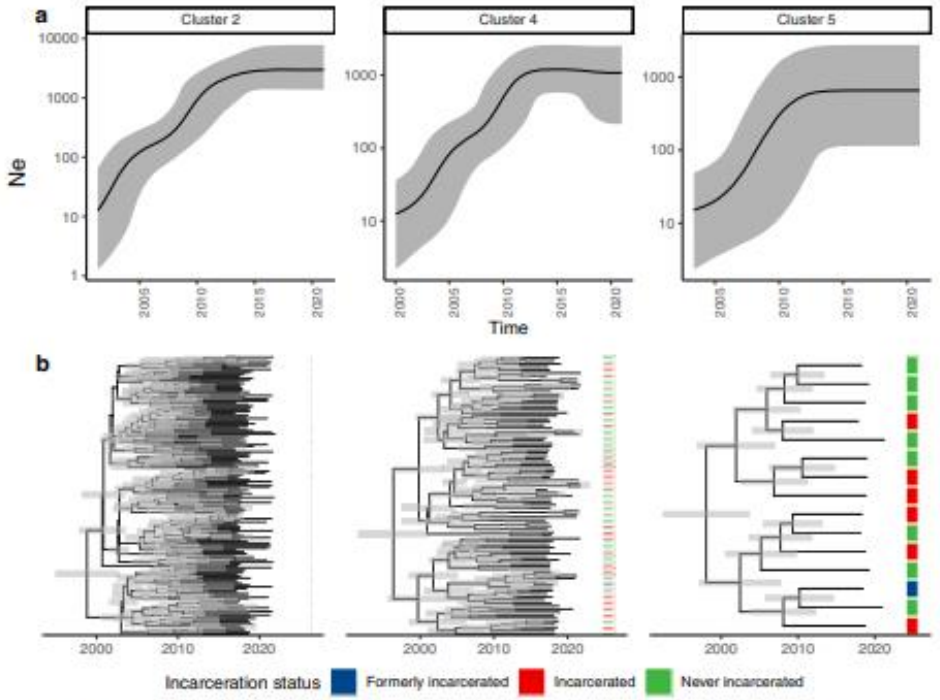

Fig. 3 | Genomic transmission clusters spanning prisons and neighboring communities have recently expanded. a Effective population size (*Ne*) estimates for the three largest genomic clusters in our sample over time. Black lines indicate *Ne* inferred in a Bayesian Skyline Coalescent model and grey shading indicates 95% high posterior density estimates. **b** Median clade credibility trees inferred from the Bayesian Skyline Coalescent model. Branch lengths are in years and grey bars indicate 95% high posterior density estimates of node date. The heatmap to the right of the phylogeny indicates patient incarceration status at the time of TB notification. Source data are provided as a Source Data file.

incarceration were within transmission clusters, including individuals with prior incarceration.

We additionally quantified *M. tuberculosis* recent transmission with time-scaled haplotype diversity, a measure of the centrality of a single tip isolate to all other isolates on the tree[22]. Individuals who were incarcerated at the time of TB notification had a higher time-scaled haplotype index for a short epidemic timescale (median: 0.59, IQR: 0.24–0.72) than did formerly (median: 0.18, IQR: −0.37–0.71; $t(36) = 1.7$, $p = 0.03$) or never incarcerated individuals (median: 0.20, IQR: −0.71–0.66; $t(360) = 5.9$, $p < 0.001$) (Fig. S3). This finding was consistent across epidemic timescales considered (Fig. S3). After adjusting for population structure, we found that incarceration status was significantly associated with time-scaled haplotype diversity (one-way ANOVA: $F(285) = 85$, $p < 0.001$), evidence that the association was independent of TB lineage.

Geographic structure despite frequent migration across *M. tuberculosis* sub-lineages

We found pattern of moderate geographic structure in sampled *M. tuberculosis* (Fig. 4), with strains from sub-lineage 4.1.2.1 dominant in Asunción (54.1%, 142/262 samples) and strains from sub-lineage 4.3.3 dominant in Ciudad del Este (60.8%, 127/209) ($\chi2(2) = 72$, $\rho < 0.001$) (Fig. 4). While we observed geographically distinct patterns of *M. tuberculosis* diversity in Asunción and Ciudad del Este, reconstruction of the ancestral locations for the three most prevalent sub-lineages revealed frequent movement of *M. tuberculosis* (Fig. 4).

To test whether Asunción and Ciudad del Este served as sources for *M. tuberculosis*, exporting infection elsewhere, we compared rates of arrival and export of each sub-lineage. Sub-lineage 4.1.2.1 moved more frequently Asunción to Ciudad del Este (mean: 75 transitions) compared to vice versa (mean: 70 transitions), and a model for asymmetric rates was supported ($\chi^2(2) = 4.1$, $\rho = 0.04$) (Fig. 4). Both sub-lineages 4.4.1.1 (with the prevalent *ahpC* mutation) ($\chi^2(2) = 0.16$, $\rho = 0.69$) and 4.3.3 ($\chi^2(2) = 0.56$, $\rho = 0.46$) had similar rates of migration to and from Ciudad del Este to Asunción. Despite the geographic structure observed, there was not a sufficient signal to infer a likely geographic source for any of the dominant sub-lineages.

Emergence of a putative resistance-associated *ahpC* promoter mutation

Eleven percent of samples (50/471) shared a mutation in *ahpC* promoter (G > A, 74 bases upstream of the 5' start codon), previously considered a location for compensatory mutations co-occurring with *katG* mutations in isoniazid-resistant isolates[23,24]. While *ahpC* promoter mutations are not included as an independent resistance-conferring mutation in the WHO resistance catalogue[21], in our collection, *ahpC* mutations occurred on otherwise susceptible genomic background within sub-lineage 4.4.1.1. The *ahpC* mutation occurred in a monophyletic clade of 49 samples in sublineage 4.4.1.1 (Fig. 5), which shared a most recent common ancestor in 1903 (95% HDI: 1888–1916), likely reflecting a single emergence event. Among the basal group of nine samples without a fixed *ahpC* promoter locus (*ahpC*−74) mutation, one sample was polymorphic, with 16% (13/79) of reads representing the *ahpC* mutation. Among the samples sharing the *ahpC* mutation, a single isolate had a co-occurring rifampicin resistance-conferring mutation in *rpoB* (His445Leu) (Fig. 5).

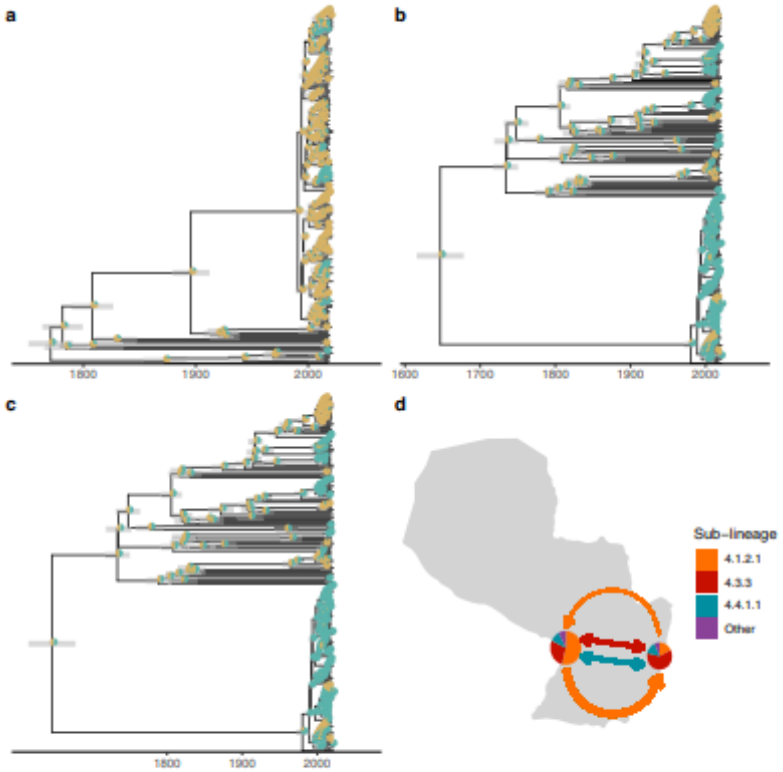

Fig. 4 | Frequent gene flow of *M. tuberculosis* connects Paraguay's major urban centers. a–c We used discrete ancestral state reconstruction to reconstruct migration between the two cities for the three dominant sub-lineages in our sample; lineages 4.1.2.1, 4.3.3, and 4.4.1.1. Bayesian maximum clade credibility trees of samples in the three dominant sub-lineages with tip points colored by city of sampling and pie charts at nodes indicating the inferred ancestral location. Branch lengths are in years and grey bars indicate 95% high posterior density estimates of node date. A model of asymmetric rates of movement between the two cities was supported for sub-lineages 4.1.2.1 a model of symmetric rates of movement was supported for 4.3.3 and 4.4.1.1. **d** Map of Paraguay with pie charts indicating the genomic diversity sampled in Asunción (at Paraguay's western border) and Ciudad del Este (eastern border). Arrows are colored by sub-lineage and are weighted by the relative rate of migration between cities. Bi-directional arrows indicate equal rates of migration in each direction. Source data are provided as a Source Data file.

We tested whether the success of the *ahpC* mutation in the absence of a *katG* mutation (i.e. outside of a compensatory context) we observed in Paraguay could be explained by an increase in associated transmissibility. The *ahpC* mutation was not associated with an increased time-scaled haplotype density (*ahpC* mutants, median 0.19, IQR: 0.09–0.22; *ahpC* non-mutants, median: 0.56, IQR: −0.44–0.71, $p = 0.93$). Further, individuals with an incarceration history (currently or formerly incarcerated) were no more likely to be infected with a *M. tuberculosis* isolate with the *ahpC* mutation than were individuals with no incarceration history ($\chi^2(1) = 0.25$, $p = 0.62$).

Discussion

We generated, to our knowledge, the first genomic portrait of circulating *M. tuberculosis* diversity and transmission dynamics to directly inform Paraguay's national TB control program priorities. We found the majority of TB cases included in our study were likely attributable to recent transmission and identified three dominant clones, which dramatically expanded over the past twenty years and spanned prisons and surrounding communities. Overall, we found a pattern of close genomic relatedness between *M. tuberculosis* sampled within and outside prisons. While *M. tuberculosis* is geographically structured in Paraguay, we identified a signal of continuous movement of *M. tuberculosis* between Paraguay's major urban centers.

We found that most sampled infections were likely attributable to recent transmission rather than long-distance migration or activation of latent disease, similar to what has been reported in other medium-incidence countries[14]. Consistent with expectations that clustering rates may correlate with incidence, when applying a 5-SNP threshold, we found that isolates from Paraguay were more frequently clustered (45%) than those from a low-incidence setting in Spain (23%) and less frequently clustered than in a high-incidence setting in Mozambique (58%). Interestingly, we found a higher rates of clustering compared to what was reported in Malawi (36%), a high-incidence setting[11]. This could reflect the shorter, one year sampling timeframe of the Malawi study[11], resulting in different genomic sampling rates, the use of different genomic sequencing pipelines, or true differences in transmission in the sampled population.

Paraguay's incarceration rate has dramatically increased, from 60 per 100,000 people in 2000 to 194 per 100,000 in 2020[3,4,6]. More than seventy percent of the incarcerated population are pre-trial detainees, the highest proportion in South America[3]. The unhealthy conditions of prison environments put people at heightened risk of disease and mortality; this risk translates into an increasing concentration of TB

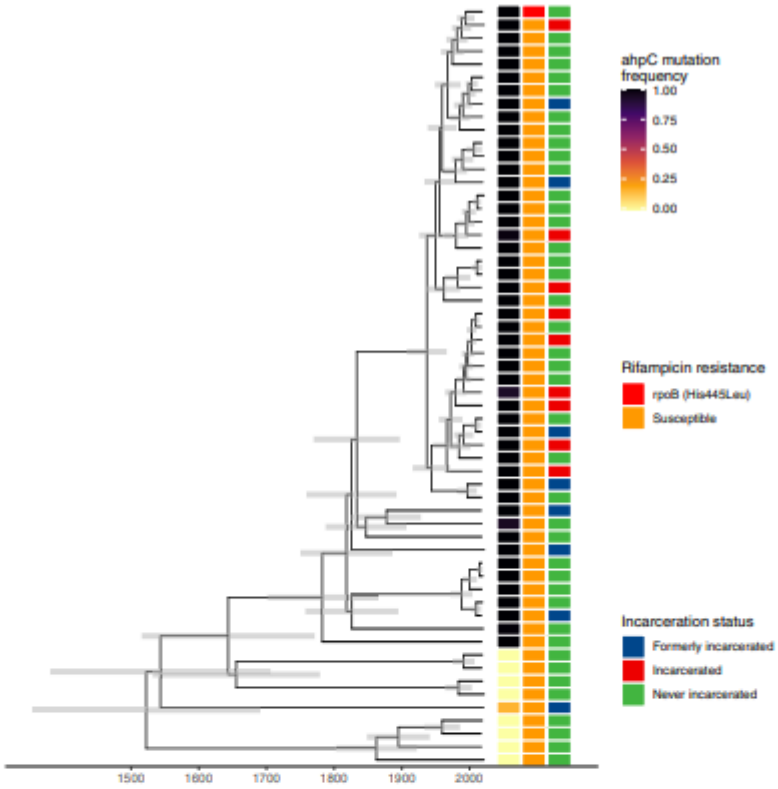

Fig. 5 | Emergence of a putative resistance-associated *ahpC* promoter mutation. Time-scaled Bayesian maximum clade credibility tree for 58 samples in sublineage 4.4.1.1. Branch lengths are in years and grey bars indicate 95% high posterior density estimates of node date. The heatmap to the right of the phylogeny indicates patient incarceration status at the time of TB notification, *ahpC* mutation frequency within an individual's infection, and the occurrence of a rifampicin resistance-conferring mutation (*rpoB* His445Leu). Source data are provided as a Source Data file.

within prisons, with 18% (537/2593) of notified TB cases in Paraguay occurring among incarcerated individuals in 2020[6]. Paraguay's TB Control Program has worked in prisons since 2004 to provide trainings for healthcare providers and all diagnostic and treatment supplies, including laboratory capacity for microbiological testing in four prisons.

Our findings highlight the critical need to expand and strengthen existing programs to detect and treat TB early and to expand awareness and knowledge of the risks associated with prison environments. Isolates sampled from prisons were more frequently found in genomic transmission clusters and had a higher time-scaled haplotype density than did isolates from outside prisons, phylogenetic evidence that recent transmission was more frequent in prisons than in communities outside prisons. Further, *M. tuberculosis* sampled from prisons and the community were closely evolutionarily related and the majority of putative transmission clusters, including individuals who were never incarcerated also included people who had a recent incarceration history, indicating that reducing transmission risk within prisons is an urgent public health priority with consequences both within and outside prisons.

While rates of drug resistance were relatively low, we found several phylogenetically unique mutations associated with both isoniazid and rifampicin resistance. These unique mutations could reflect either the de novo acquisition of a resistance mutation or the importation of a resistance mutation from outside Paraguay. Regardless, there is a critical need for expanding drug-susceptibility testing, including both rapid testing for rifampicin resistance in addition to isoniazid mono-resistance are critical to ensure patients are put on correct treatment courses and to reduce the risk of further resistance acquisition[18,19].

The emergence of an independent *ahpC* mutation within a single sublineage opens questions about its phenotypic consequences. Previous studies in laboratory strains have reported that *ahpC* mutations are compensatory in the context of *katG* isoniazid resistance-conferring mutations, by recovering the bacterium's ability to detoxify organic peroxides, but did not find measurable isoniazid resistance conferred by independent *ahpC* mutations[24]. Genome-wide association studies of clinical *M. tuberculosis* isolates confirmed the compensatory role of *ahpC* mutations[25]. *ahpC* mutations did not meet the criteria for being included in the 2021 WHO catalogue of resistance-conferring mutations because they were either too rare or had a low positive predictive value for isoniazid resistance as an independent mutation[23]. However, a study of isoniazid-resistant isolates from Brazil reported that while *ahpC* mutations often co-occurred with *katG* mutations, they were also found in the absence of known resistance mutations in *katG* or *inhA*[26].

A previous genome-wide survival analysis identified lineages and specific mutations associated with pre-resistance, genomic backgrounds that had a heightened likelihood of acquiring resistance-conferring mutations[27]. Whether *ahpC* acts as in a similar way, generating a "pre-compensated" genomic background, increasing the likelihood of future *katG* mutations, remains unknown.

Our study has several limitations. First, while we sequenced all available *M. tuberculosis* cultures, our final sample size of *M. tuberculosis* genomes was small relative to the number of notified TB cases in our study departments over the study period. Some locally circulating genotypes may, therefore, not be included in our sample and may lead to an underestimate of the contribution of recent transmission to incident TB. However, we sampled over a moderately long timeframe (five years) and included samples from high-incidence prisons and neighboring communities, providing greater opportunity to recover transmission events. Second, surveillance focused on Paraguay's urban centers, where the majority of TB notifications occur. Future *M. tuberculosis* genomic surveillance in the Chaco, western Paraguay, where incidence is three times higher than in eastern Paraguay[15], is needed. Additionally, further analysis at the regional-level will be critical for understanding transmission between Paraguay and neighboring countries. Third, we sampled TB infections from prisons at a higher rate than infections outside prisons, potentially biasing upwards estimates of the rate of genomic clustering within prisons compared to outside prisons. Further, we did not have access to more detailed epidemiological data, such as contact information. Future studies integrating genomic data with additional epidemiological data could be used to identify other locations potentially contributing disproportionately to *M. tuberculosis* transmission in Paraguay.

Finally, we sequenced isolates from cultured sputum, as is routinely done for *M. tuberculosis* genomic epidemiology, but which limits the within-host diversity recovered from an individual's infection. Future research is needed to develop sequencing approaches to recover within-host *M. tuberculosis* variation and incorporate this level of variation into the transmission and ancestral state reconstruction.

Together, our results underscore an urgent need for TB control measures to interrupt ongoing transmission in Paraguay, particularly in high-incidence prison settings, which have an outsized role in broader transmission. Further, the connectivity of Paraguay's urban centers indicates that TB control needs to be coordinated country-wide.

Methods

Study protocol

This research complies with all ethical regulations. The study was approved by the ethics committee of the Central Laboratory of Public Health of the Ministry of Health and Social Welfare of Paraguay (International Certification FWA N° FWA0002008B) with code CEI-LCSP 91/010217. Informed consent was obtained (Prospective population surveillance).

Inclusion and ethics

This study was designed and led by a team of researchers in Paraguay (GES, GS, SA) and Brazil (JC). The research seeks to characterize *M. tuberculosis* transmission in order to directly inform priorities of the National Program for Tuberculosis Control (NPTC) of Paraguay (SA, GS) and, more broadly, the Paraguayan Ministry of Health (GS). This work comprises part of the dissertation research of GES and GS. The research was approved by a local ethics review committee and involved minimal risk to study participants.

Prospective population surveillance

We conducted population-based genomic surveillance in three of Paraguay's departments: Central, Distrito Capital, and Alto Paraná, which together comprise approximately half (3,392,429 people) of the country's 2021 population of 7.4 million. Sputum samples are routinely collected from all individuals presenting with symptoms of TB at primary health clinics and sent to the National Program for Tuberculosis Control (NPTC) of Paraguay reference laboratory for microbial diagnostics including culture and smear microscopy. We sequenced all available culture-positive isolates from these three departments.

Study recruitment was done by study staff who visited patients at home and in prisons at the time patients began treatment (Directly Observed Therapy, DOT). At this time, the standard National TB Control Program questionnaire was conducted and patients who chose to enroll provided written consent for sequencing residual mycobacterial cultures for culture-positive samples. Study staff also collected additional demographic, clinical, residential, and epidemiolocal data, including information on history of prior or current incarceration with a structured questionnaire.

Sex was not considered in study design; it was determined through self-report. Of the 488 individual participants, 394 were men and 94 were women, reflecting that recruitment focused on prisons. In 2022, the incarcerated population in Paraguay was 95% male[3]. Median age of study participants was 31 (IQR: 24–44).

Laboratory and sequencing methods

Sputum samples were cultured in the Ogawa-Kudoh Method[28,29]. Cultures were incubated at 37 °C and observed for growth twice a week for 60 days. *M. tuberculosis* DNA was extracted using Cetyltrimethyl ammonium bromide (CTAB) method[30].

Sequencing was conducted at the Laboratorio Central de Salud Pública (LCSP), Paraguay Ministry of Health; Centro para el Desarrollo a la investigación Científica (CEDIC), Paraguay; and the Translational Genomics Research Institute (TGen), Arizona, US. DNA sequencing libraries were prepared with the Illumina DNA Prep library kit and sequenced on an Illumina MiSeq in Paraguay and an Illumina NextSeq (2 × 151-bp), at TGen. Raw sequence reads for samples passing filters are available at the Sequence Read Archive (PRJNA870648).

Variant identification

We identified *M. tuberculosis* genomic variation from whole genome sequence data with a pipeline available at https://github.com/ksw9/mtb_pipeline (v1)[32]. We previously conducted a variant identification experiment to compare commonly used mapping and variant calling algorithms in *M. tuberculosis* genomic epidemiology[32]. Briefly, we generated 20 independent Illumina readsets (2 × 151 bp) from the *M. tuberculosis* strain CDC1551 genome in silico, with the next-generation sequence-read simulator ART v. 2.5.8[33]. Measuring performance requires a truth VCF of true variant sites in the query genome with respect to a given reference genome. We generated a truth VCF for the strain CDC1551 query genome with respect to the H37Rv reference genome by pairwise aligning the query genome (strain CDC1551) to *H37Rv* with MUMmer 3.2.0[34] (*nucmer maxmatch -c 1500*). We identified SNP variants from the pairwise alignments using MUMmer *show-snps*, excluding SNPs with ambiguous mapping and indels (*show-snps -Clr*). Simulated *M. tuberculosis* genomic data and truth VCF files indicating variants with respect to the reference genome are available here: https://purl.stanford.edu/mr554nj9219. We compared variants identified with our pipeline with the "truth VCF" to determine sensitivity and precision of our pipeline (Table S1).

We previously found that the combination of the *bwa*[35] mapping algorithm and *GATK*[36,37] variant caller routinely minimizes false positive variant calls with minimal cost to sensitivity as compared to other tool combinations[32], in particular, when the PE/PPE genes are excluded. We, therefore, used this combination of tools in our pipeline. We report the performance of our computational pipeline in recovering true variants between the CDC1551 query genome and H37Rv reference genome in Table S1.

Briefly, we trimmed low-quality bases (Phred-scaled base quality <20) and removed adapters with Trim Galore v. 0.6.5 (stringency=3)[38]. We used CutAdapt v.4.2 to further filter reads (–nextseq-trim=20–minimum-length=20–pair-filter=any)[39].To exclude potential contamination, we used Kraken2 to taxonomically classify reads and removed reads that were not assigned to the *Mycobacterium* genus or that were assigned to a *Mycobacterium* species other than *M.*

tuberculosis[40]. We mapped reads with bwa v. 0.7.15 (*bwa* mem)[35] to the H37Rv reference genome (NCBI Accession: NC_000962.3) and removed duplicates with sambamba[41]. We called variants with GATK 4.1 HaplotypeCaller[36], setting sample ploidy to 1, and GenotypeGVCFs, including non-variant sites in output VCF files. We included variant sites with a minimum depth of 10X and a minimum variant quality score 40 and constructed consensus sequences with bcftools consensus[42], excluding indels. We excluded SNPs in previously defined repetitive regions (PPE and PE-PGRS genes, phages, insertion sequences and repeats longer than 50 bp)[43]. We identified sub-lineage and evidence of mixed infection with TBProfiler v.4.2.0[44,45], which is based on the identification of >1000 lineage-specific SNPs. We additionally used TBProfiler with the TBDB repository (https://github.com/jodyphelan/tbdb) which includes >2,000 resistance-associated mutations[44,45] compiled from several sources, including but not limited to the World Health Organization catalogue[25,44,45]

We do not categorize isolates harboring an independent (*ahpC*) mutation as drug-resistant in phylogenies because it is not considered independently associated with resistance in the World Health Organization Catalogue[23] or other references.

Phylogenetic and Bayesian evolutionary analysis

We constructed full-length consensus FASTA sequences from VCF files, setting missing genotypes to missing, and used SNP-sites to extract a multiple alignment of internal variant sites only[46]. We used the R package *ape* to measure pairwise differences between samples (pairwise.deletion=TRUE)[47]. We selected a best fit substitution model with ModelFinder[48], implemented in IQ-TREE multicore version 2.2.0[49], evaluating all models that included an ascertainment bias correction for the use of an alignment of SNPs only. The best fit model according to Bayesian Information Criterion was K3Pu + F + ASC + R5, a three substitution types model with unequal base frequencies, an ascertainment bias correction, and a FreeRate model of rate heterogeneity across sites, including four categories. We then fit a maximum likelihood tree with IQ-TREE, with 1000 ultrafast bootstrap replicates[49,50].

Genomic clustering is often used as a proxy measure of recent *M. tuberculosis* transmission; isolates that are more closely genetically related are hypothesized to be more likely linked through recent transmission rather than travel-associated importation or re-activation of genetically distinct latent infections.[20,51] We applied a commonly used genetic distance thresholds of 12- and 5- or fewer SNPs to identify genomic clusters[52–53].

To investigate transmission patterns in the three largest genomic clusters more closely, we fit timed Bayesian trees to multiple sequence alignments with BEAST 2.6.2[54], using TB notification dates to calibrate tips. Because of the short sampling timeframe of our data, we fixed the substitution rate to 1×10^{-7} mutations/site/year, as previously described[55], and consistent with previous estimates for the *M. tuberculosis* lineage 4 substitution rate[56]. To examine population dynamics in the three largest clusters, we used a Coalescent Bayesian Skyline model[57] with 5 dimensions, allowing the effective population size to change 4 times over the tree. We additionally fit a Bayesian tree to sublineage 4.2.1.1 samples using a constant population size, fixed substitution rate model. Markov chain Monte Carlo chains were run for 200 million iterations, or longer, if required for convergence, excluding 10% of samples as burn-in. We used *treeannotator* to produce maximum clade credibility trees. We used the R package *beautier* to construct XML files[58] and corrected XML files for the number of constant positions in SNP alignments. We visualized phylogenetic trees with the R package *ggtree*[59,60].

We calculated time-scaled haplotype density from a matrix of pairwise SNP distances with the R package *thd* as previously described[22] and compared time-scaled haplotype density between individuals who were never, formerly, or currently incarcerated with t-tests. We set the *M. tuberculosis* substitution rate to 1×10^{-7} substitutions per site per year and included an effective genome length of 3,916,441 basepairs (the length of the reference genome minus the PE/PPE regions excluded from variant calling) and used a short (20 year) and long (50 year) epidemic timescale. We compared time-scaled haplotype density by incarceration status with t-tests and used analysis of variance to test for the independent effect of incarceration status after controlling for *M. tuberculosis* population structure[22].

We tested for the association between sub-lineage and city with Chi-square tests. We included isolates within the three largest sublineages identified (4.1.2.1, 4.3.3, and 4.4.1.1) to avoid comparison of small sample sizes. We conducted discrete ancestral state reconstruction for sampling location with the R package ape for the three largest sub-lineages in our collection[47]. We restricted samples to those from Asunción and Ciudad del Este because of the small sample size outside those cities. We compared symmetric and asymmetric rates models fit with the R package *diversitree* (make.mk2) and compared model fits with analysis of variance[61]. We used stochastic character mapping[62] in the R package *diversitree*[61] to sample 500 location histories for each sublineage tree; we summarized these as the number of average movements between cities over the tree.

To test for genomic loci associated with transmissibility, we conducted a bacterial Genome-Wide Association Study implemented in the R package *treewas*[21]. This approach controls for bacterial clonality and population structure by simulating null genomic datasets, in which there is no genotype-phenotype association, to compare with the empirical dataset[21]. We tested if the binary phenotype of membership in a genomic cluster of size 15 or greater (including the three dominant clusters identified in our study) was associated with genotype. We additionally tested for a genotypic association with membership in a genomic cluster of size 10 or more, or any clustering (membership in a genomic cluster of size 2 or more).

Statistics & reproducibility

We included all *M. tuberculosis* genomes passing coverage and quality thresholds. We excluded isolates with evidence of mixed lineage infection from the analysis. No statistical method was used to predetermine the sample size.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Raw sequence data generated in this study have been deposited in the Sequence Read Archive under accession PRJNA870648. The H37Rv reference genome is available on NCBI under accession NC_000962.3) The phylogenetic trees and results of ancestral state reconstruction and all other information displayed in figures are provided in the Source Data files. Source data are provided with this paper.

Code availability

Our *M. tuberculosis* variant identification pipeline is available at https://github.com/ksw9/mtb_pipeline[31].

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Author contributions

All authors contributed extensively to work presented in this paper. G.E.S., G.S., S.A., and J.C. designed the study and sampling protocol and obtained ethical approval. J.M., P.C.P.S., N.W.G., M.G., A.O., C.C., and G.M. enrolled participants, and conducted laboratory work, and database management. A.L.G., J.R.A., and J.C. supervised analysis and study design. G.E.S., G.S., J.R.A., J.C., and K.S.W. analyzed the data and wrote the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages. J.C. and K.S.W. contributed equally to the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Supplementary Information

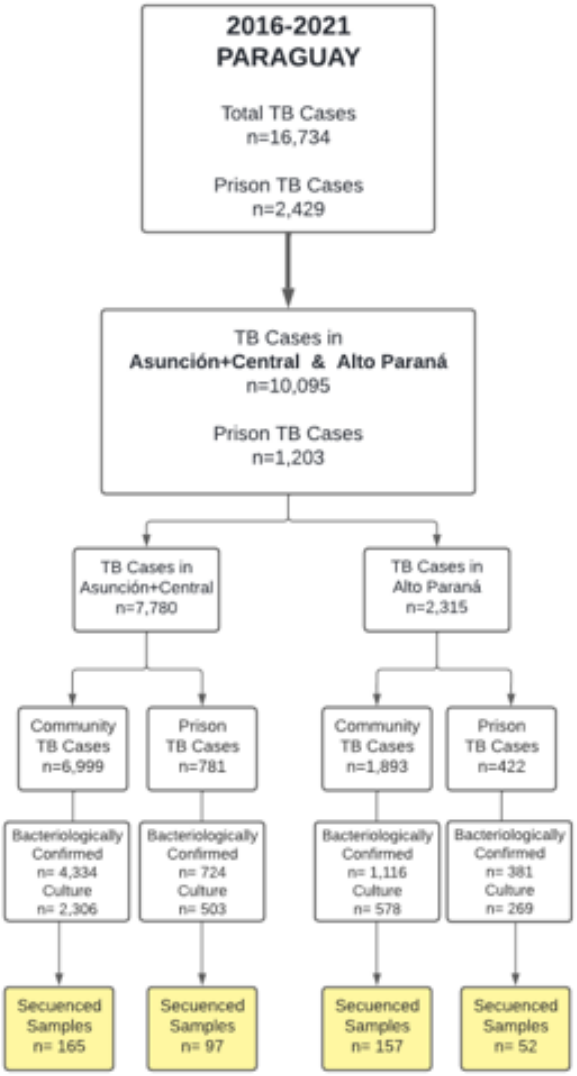

Figure S1. Genomic *M. tuberculosis* surveillance in Paraguay. Flowchart indicates the total number of notified cases of TB in Paraguay, from 2016 to 2021; TB cases in the three major urban departments of Paraguay; TB cases in urban departments stratified by incarceration status at the time of TB notification; bacteriologically and culture-confirmed TB cases; and number of cases for which sequenced *M. tuberculosis* passed all quality filters and represented single infections.

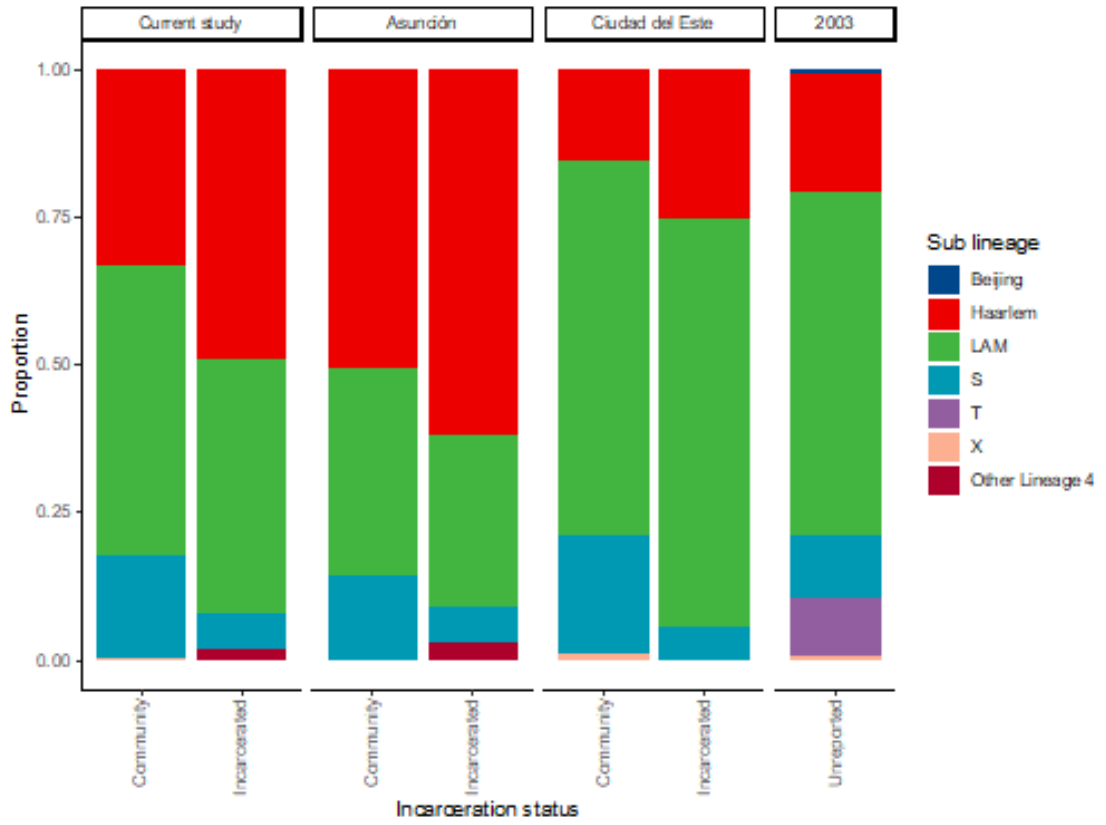

Figure S2. Longitudinal changes in sampled *M. tuberculosis* genomic diversity. We compared genomic diversity in our study (2016-2021) with that sampled in the only previous genetic study of *M. tuberculosis* in Paraguay[1]. Stacked bar plots indicate the proportion of samples falling in each clade. From left to right, panels indicate the total diversity sampled, samples from Asunción, Ciudad del Este, and from the 2003 study. Panels from the current study are stratified by incarceration status at the time of TB notification; the 2003 study did not present data stratified by incarceration status.

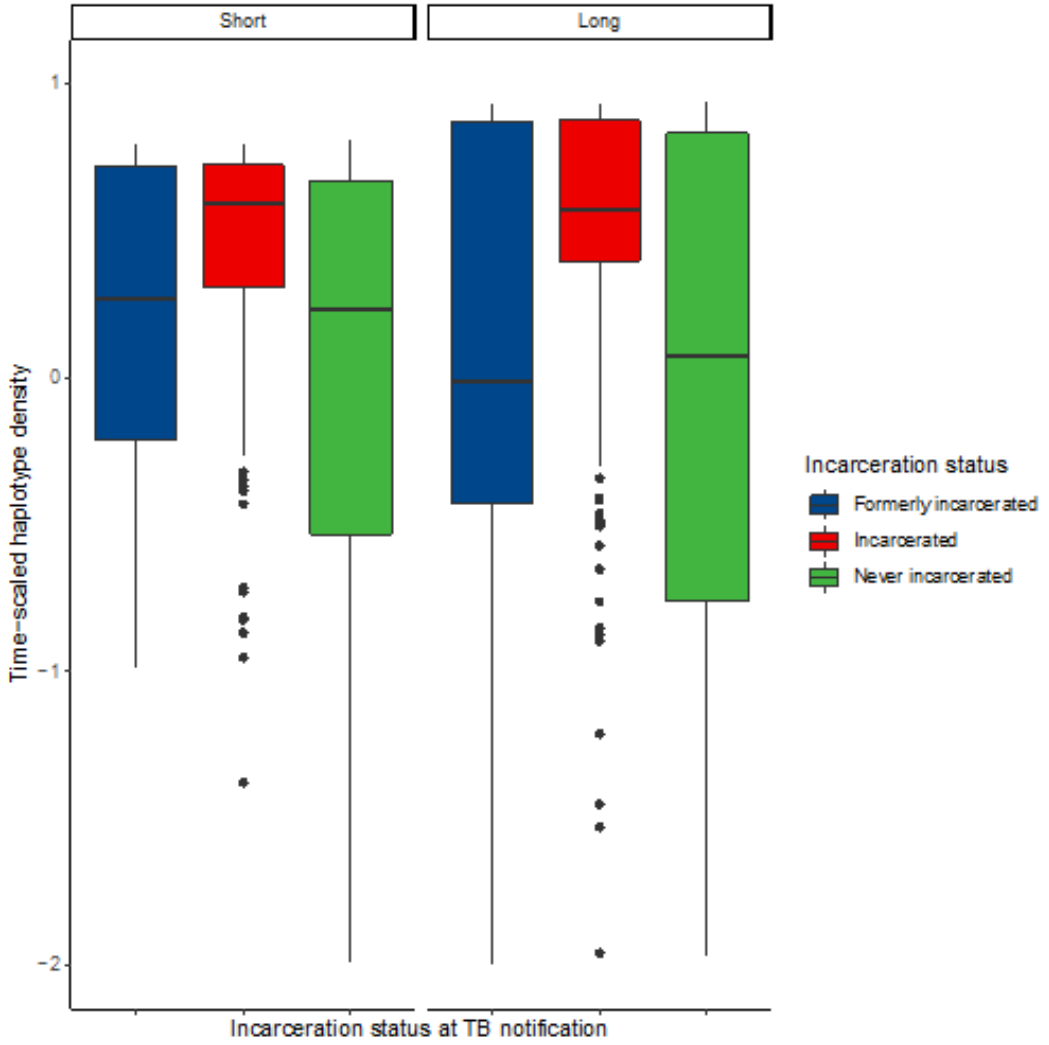

Figure S3. Time-scaled haplotype density by incarceration status at the time of TB notification. We measured time-scaled haplotype density, a measure of the centrality of a single tip isolate to all other isolates on the tree, a proxy for recent transmission that considers not only the nearest phylogenetic neighbor, but all tree trips. We calculated time-scaled haplotype density from a matrix of pairwise SNP distances with the R package *thd* as previously described[2]. We set the *M. tuberculosis* substitution rate to $1x10^{-7}$ substitutions per site per year and included an effective genome length of 3,916,441 basepairs (the length of the reference genome minus the PE/PPE regions excluded from variant calling) and used short (10 year) and long (20 year) epidemic timescales. Boxplots are colored by incarceration status at the time of TB notification and include isolates from n=31 formerly incarcerated individuals, n=149 currently incarcerated, and n=291 never incarcerated individuals. Boxes indicate the interquartile range, lines indicate median values, and whiskers indicate the range of the data. Points outside the whiskers indicate outliers.

Table S1. Performance metrics. Performance benchmarking of our pipeline in recovering single nucleotide polymorphisms (SNPs) in simulated Illumina sequence data. For each Metric, the number of simulated sequence sets (Replicates), the mean, median, and standard deviation. True variants indicates the number of "true" SNP variants identified through pairwise alignment of the query genome (strain CDC1551) to H37Rv with MUMmer[3] (*nucmer maxmatch -c 1500*). We identified SNP variants from the pairwise alignments using MUMmer *show-snps*, excluding SNPs with ambiguous mapping and indels (*show-snps -Clr*). True positives and false positives indicate the number of true or false positive SNPs, respectively, identified by our algorithm. Region indicates whether the PE/PPE genes were excluded from analysis (No PE/PPE) or whether the statistic refers to the full-length M. tuberculosis genome (Genome). In the analysis presented in this paper, we excluded the PE/PPE genes in order to maximize precision.

Metric	Replicates	Mean	Median	Std. Dev.	Region
True variants	20	924.00	924.00	0.00	No PE/PPE
True positives	20	845.40	845.00	1.14	No PE/PPE
Sensitivity	20	0.92	0.92	0.00	No PE/PPE
False positives	20	3.35	3.00	0.49	No PE/PPE
Precision	20	1.00	1.00	0.00	No PE/PPE
True variants	20	1501.00	1501.00	0.00	Genome
True positives	20	1148.80	1149.50	5.24	Genome
Sensitivity	20	0.77	0.77	0.00	Genome
False positives	20	70.75	70.00	8.91	Genome
Precision	20	0.94	0.94	0.01	Genome

8. Discussion

While the phenomenon of TB in prisons has been extensively described, this thesis delves deeper into the analysis of the characteristics of the TB epidemic in a country in which the burden is concentrated within this population. Its epidemiological characterization, including a genetic description component, as well as its interaction and spill-over effect into the broader community, is perhaps the most significant contribution of this entire body of work.

The overrepresentation of at-risk among people deprived of liberty (PDL) populations in prisons establishes them as a reservoir of TB for the broader community. The escalation of overcrowding over recent decades largely accounts for prisons not only serving as reservoirs but also as the driving force behind the epidemic in the country. As described in this study, these prisons overshadow the incremental successes achieved in combating TB within the general population.

Unveiling the impact of TB in prisons on the broader community offers a fresh perspective for shaping new TB control strategies within correctional facilities. Such an approach must transcend the realm of healthcare alone. On one hand, within the healthcare sector, efforts should focus on implementing more timely and accurate diagnostic strategies for this population, reducing treatment initiation times, enhancing treatment success rates, and proactively addressing potential challenges. Additionally, healthcare protocols should emphasize that individuals with a history of incarceration are at increased risk of developing TB outside prison settings.

However, a healthcare-focused approach alone will not suffice unless attention is directed towards the structural determinants of the problem: the building conditions of the prisons and overcrowding. As a result of the experiences and lessons learned during the pandemic, it was gained a deeper understanding of the importance of ventilation in shared spaces for controlling aerosol-transmitted diseases architecturally.

Overcrowding, stemming primarily from legal origins, due to an excess of individuals held in pretrial detention without a definitive sentence, must be addressed as part of TB control efforts. The integration of these approaches will not only reduce the burden of TB and other respiratory illnesses but will also improve the health and quality of life of incarcerated individuals. Consequently, this will lead to a reduction in the epidemic within the broader community. A healthy prison is also integral to the safety concerns of the wider community and public health.

This section will systematically present four key themes and research questions developed from the list of studies previously outlined in this thesis, along with the limitations inherent in each. This section concludes with future considerations on the topic and potential perspectives to implement that may help address this important public health challenge.

Concentration of the TB Epidemic in Prisons

These first findings highlight the importance of prioritizing national TB control efforts on prisoner populations. Although national TB incidence rates in Paraguay remained unchanged over the past decade, the number of TB cases in prisons doubled during this period. These increases among this high-risk population spoil gains from TB control efforts among the general population. This trend threatens achievement of the END TB and Sustainable Development Goals TB control targets(114).

The percentage of TB cases within Paraguayan prisons increased from 7.1% to 14.5% of the total TB cases nationwide. In line with studies in Brazil, the general TB incidence rate in Paraguay remained steady despite the escalating incarceration rate(41). These results differ from the findings of a project with data of 26 eastern European and central Asian countries, which showed that each percentage point of increase in incarceration rates relates to an increased TB incidence of 0.34% for the whole country(122). Several factors, such as different prisons infrastructure; HIV prevalence; or changes in surveillance, economic, demographic, and political indicators might explain this finding. Also, there could be a trend towards decline in Paraguay because the improvement in other health-related indicators, but incarceration offset is such that the national trend

was stable instead of decreasing. Thus, it is likely that national TB figures might not decline until a considerable reduction in the TB burden is prisons is achieved(123).

Among the high-risk groups for TB in Paraguay are inmates and indigenous populations. However, the extent to which TB cases among these groups contribute to the overall TB burden in the community remains poorly understood(3). The high PAF of prison's TB to overall TB burden (14.4%) indicates that a considerable weight of national TB burden occurs precisely in prisons. Although the PAF formulae has been used by landmark studies for global TB burden in prisons, such as a 2010 systematic review (33), it does not consider the cases of disease that arise in the community which are connected to transmission in prisons: in relatives following infections during prison visits or disease in ex-prisoner after released(124). A study from Brazil found that TB incidence among ex-prisoners is higher than that of the general population several years following their release, suggesting that including only cases arising duration incarceration results in an underestimate of the true effect of prisons on the TB epidemic (39). Nonetheless, a high PAF emphasizes the need for continuous efforts to prevent the spread of TB within prisoners to reduce the TB burden.

In prison, individuals that are diagnosed for TB are more likely to have bacteriological confirmation compared with cases occurring outside of prisons, as it has been observed in three studies from Brazil (41,124,125). Prison conditions in Brazil are presumed to resemble those in Paraguay, particularly in states or departments bordering each other. Hence, there is a necessity to collaborate on transborder TB strategies for inmates in both Brazil and Paraguay.(39)

Multi-Drug-Resistance TB (MDR-TB) incidence is low in Paraguay (0.99 per 100,000 population) and, although there is no data in prisons, it seems to be lower in this population group(3). Importantly, much of the TB burden in the penal system is concentrated in two prisons where more than one third of the national prison population resides. Thus, although the TB epidemic among prisoners in increasing in Paraguay as a whole, it was identified the prisons where enhanced control efforts should be concentrated, and resources should be allocated. Perhaps one positive aspect of the prison system in Paraguay is that it exhibits a higher rate of successful TB treatment

compared to other at-risk groups such as indigenous populations, individuals with HIV coinfection, or substance abusers, among others (120).

This thesis initially encountered certain limitations. Firstly, substantial heterogeneity was observed mainly in the level of overcrowding and diagnostic capacity in differing geographical regions and prisons. There is likely under-diagnosis of TB mainly in extra-pulmonary TB (three times less in prisons than outside), as well as variation in reporting over time, which influences notification trends. This degree of under-diagnosis is distinct inside and outside of prisons. Secondly, the case detection rate is likely much lower in prisons than the general population, which suggests that the contribution of TB from prisoners to the national TB epidemic that was reported in this study is likely an underestimate. Thirdly, TB cases occurring in recently released prisoners could not be identified, contributing to poor TB case ascertainment, especially because the NTP does not collect data concerning to prison records (37). Also, the PAF obtained through the Levin formula is an underestimation. Levin's formula is oriented towards non-communicable disease phenomena and this formula does not consider transmission that occurs when prisoners leave prison and infect people in the general population. More complex PAF modelling techniques were recently proposed which show higher PAFs for communicable disease than those obtained with traditional analyses, but still need to be validated(126).

Finally, these findings alert to the magnitude of TB crisis within the prison system in Paraguay. They are useful to guide the development of TB control policies and national strategic plans to improve TB programs in penal institutions. Prisons act as "institutional amplifiers" or "reservoirs" of the disease on community (37,40). Special attention needs to be paid about including physical prison infrastructure, early detection of cases, isolation rooms and timely treatment initiation. This was a first baseline epidemiological assessment for the country, and next studies pretend to assess whether measures addressing prison specific TB drivers (implementation of mass screening strategies, introduction of molecular diagnostic tools) are effective.

Tuberculosis, a sentence during and after incarceration.

This part of the thesis reveals that the risk of TB remains heightened in individuals who have been incarcerated for several years. By relating Paraguay's prison census data with the National TB registry, it was uncovered a previously unrecorded prevalence of TB among both current and former inmates. These findings indicate that a significant percentage (15.1%) of those currently incarcerated or recently released have been affected by TB. The TB rates among PDL seen in this study are amongst the highest for any at-risk population recorded. Mix Ministries of Health (MoH) and Ministry of Justice (MoJ) databases, and access detailed incarceration data provided us a unique opportunity to follow this population that is notoriously difficult to track after leaving prison. This thesis findings highlight a previously undocumented population health consequence of incarceration in Paraguay and underscores the urgency of new programs to protect the health of incarcerated and formerly incarcerated individuals.

This thesis showed that the risk of developing TB escalates sharply after entering prison, surpassing the TB risk in the community by more than 100 times. The high TB rates found after 8 years following incarceration indicate that when the conditions of incarceration remain unchanged (overcrowding, smoking, malnutrition, etc.) the risk of developing TB accumulates over time. This high risk can be attributable to the gradual progression of new TB infections acquired in prisons and/or of pre-existing infections. In both cases, risk is likely to be exacerbated by the presence of risk factors favouring TB progression. Similarly, when PDL are released, the risk of developing TB is much higher than in the general population at more than 30 times in the first two years after release. Although this risk decreases gradually, it remains 10 times higher than the risk of TB in the general population over 8 years of follow-up. This expected increase in the TB risk occurring while deprived of liberty and the decrease in risk after release confirms that prisons are environments which contribute to the propagation of TB. In this context, well known individual determinants of TB, such as smoking, drug abuse or poverty, are insignificant factors in enhancing the TB risk in the already high-risk, high transmission environment of prisons. The poor conditions of Paraguayan prisons, mostly driven by overcrowding, poor ventilation, limited access to healthcare and high exposure periods, may overshadow the progress that may be made in TB control in the community (4,127).

The increased risk of TB linked to incarceration also weakens broader TB control efforts, as individuals infected with *M. tuberculosis* within prisons may spread the bacteria outside prison confines.

Environmental and structural changes in these institutions may have a considerable impact on TB control. However, reducing overcrowding will not be sufficient to substantially reduce the TB burden in Paraguay's prisons (in the least crowded prison cells, 5.3–10.3% of individuals developed TB). These findings are in line with those from Brazil, where the risk factors associated with prison environments, increase TB incidence more than the risk factors associated with the individual factors of this high-risk population. Hence, implementing focused structural interventions within prisons could significantly impact the wider TB epidemic. Moreover, diminishing the reliance on imprisonment and notably decreasing the size of prison populations will reduce the number of individuals susceptible to heightened TB risk (39)

Although the overall incidence rate of TB in prisons is not dramatically increasing, the recent upward trends in general rates of incarceration will result in a considerable increased number of TB cases in prisons that would otherwise not exist. This observation could be correlated with the increase in overall TB incidence rate reported for Paraguay. The proportion of PDL with a definitive sentence for a crime was only 26.2% at the time of the census, the remaining ones were pre-trial detainees, awaiting the judicial process for a trial. In Latin America, Paraguay has one of the highest proportions of pre-trial detainees among all PDL (31).

It was found that re-admission into prison is related to the type of crime (robbery/theft) with a short sentence (less than 3 years on average) that mostly occurs to younger prisoners. This is the most frequent profile of PDL readmitted into prison. A key question is whether the TB risk in this population is higher in the community before first entering prison. This could be done by comparing pre-existing high-risk attributes -- such as poverty, illiteracy, drug abuse, HIV and others– among persons first entering prison and persons that have not been incarcerated. The evident increase in TB risk occurs upon incarceration. This phenomenon has already been described before for several contagious diseases (26,39,128).

In Latin America, the collaboration between authorities that are responsible for public health and those that govern prisons is insufficient. This is also why it is hard to find documented evidence of TB history or incarceration history among TB cases that occur in the community, which makes this analysis unique in the region(39). Renewed joint efforts between these two sectors are needed to improve overall TB control, but particularly to address TB in prisons.

This part of the thesis had also several limitations. Firstly, utilizing cross-sectional data for the collection of baseline variables has certain considerations. The sociodemographic characteristics of the PDL were collected through census questionnaires and might not be accurately captured by the discrimination and stigma that the PDL population is subjected to. Secondly, the study cohort includes five of the 18 prisons in the Paraguayan penal system, including two of the largest prisons with the highest TB rates in the country. Larger prisons hold more prisoners with higher overcrowding, and these large prisons were overrepresented in this study. Additionally, if any of the prisoners included in this study had readmissions to prisons other than those selected, this event could not be recorded for analysis, potentially underestimating the total time a PDL was in prison. Thirdly, the TB episodes are based on TB notification from the NTP, and it is well known that the surveillance system misses a proportion of the real TB cases. Thus, although this study finds very high TB rates, the real prison attributable rate may be even higher. Lastly, deaths from non-TB related causes that occurred after release could not be registered, leading to an overestimation of the total follow up time and an underestimation of the TB burden in this subset of individuals.

The results of this part of the thesis show that prisons are a high risk setting for the development of TB, and that the risk continues after release. There is a critical need to develop new TB control strategies to achieve the WHO global targets for 2035 and it is believed that implementing and strengthening TB control interventions in prisons cannot be neglected to achieve these goals. Specific interventions within prisons are likely to have a valuable impact on reducing the TB epidemic. However, these interventions should not solely focus on biomedical approaches such as active diagnosis, mass screening, and secondary IPT(39,75). In addition, it is important to consider architectural reforms aimed at improving the living conditions within penitentiary

centres and legal reforms including decarceration, reducing reliance on incarceration, and preventing the imprisonment of individuals who have not been sentenced. These measures should be implemented with a strong focus on upholding human rights principles.

Genetic Dynamics of Tuberculosis in Prisons and their Surrounding area.

This section of the thesis produced the initial genomic depiction of circulating *M. tuberculosis* diversity and transmission dynamics, directly informing the priorities of Paraguay's national TB control program. The thesis showed that most TB cases included were likely attributable to recent transmission and identified three dominant clones, which dramatically expanded over the past twenty years and spanned prisons and surrounding communities. Overall, a pattern of close genomic relatedness *between M. tuberculosis* sampled within and outside prisons was found. While *M. tuberculosis* is geographically structured in Paraguay, a signal of continuous movement of M. TB between Paraguay's major metropolitan areas was identified.

The thesis shows that most sampled infections were likely attributable to recent transmission rather than long-distance migration or activation of latent disease, like what has been reported in other medium-incidence countries(42). Consistent with expectations that clustering rates may correlate with incidence, when applying a 5-SNP threshold, it was found that isolates from Paraguay were more frequently clustered (45%) than those from a low-incidence setting in Spain (23%) and less frequently clustered than in a high-incidence setting in Mozambique (58%). Interestingly, a higher rate of clustering was shown, compared to what was reported in Malawi (36%), a high-incidence setting. This could reflect the shorter, one year sampling timeframe of the Malawi study(129), resulting in different genomic sampling rates, the use of different genomic sequencing pipelines, or true differences in transmission in the sampled population.

Isolates sampled from prisons were more frequently found in genomic transmission clusters and had a higher time-scaled haplotype density than did isolates from outside

prisons, phylogenetic evidence that recent transmission was more frequent in prisons than in communities outside prisons. Additionally, *M. tuberculosis* sampled from prisons and the community were closely evolutionarily related and most putative transmission clusters, including individuals who were never incarcerated also included people who had a recent incarceration history, indicating that reducing transmission risk within prisons is an urgent public health priority with consequences both within and outside prisons.

While rates of drug resistance were relatively low, several phylogenetically unique mutations associated with both isoniazid and rifampicin resistance were found. These unique mutations could reflect either the de novo acquisition of a resistance mutation or the importation of a resistance mutation from outside Paraguay. Regardless, there is a critical need for expanding drug-susceptibility testing, including both rapid testing for rifampicin resistance in addition to isoniazid monoresistance are critical to ensure patients are put on correct treatment courses and to reduce the risk of further resistance acquisition (130,131).

The emergence of an independent ahpC mutation within a single sublineage opens questions about its phenotypic consequences. Previous studies in laboratory strains have reported that ahpC mutations are compensatory in the context of katG isoniazid resistance-conferring mutations, by recovering the bacterium's ability to detoxify organic peroxides, but did not find measurable isoniazid resistance conferred by independent ahpC mutations(132). Genome-wide association studies of clinical M. TB isolates confirmed the compensatory role of ahpC mutations(133). ahpC mutations did not meet the criteria for being included in the 2021 WHO catalogue of resistance-conferring mutations because they were either too rare or had a low positive predictive value for isoniazid resistance as an independent mutation(134). However, a study of isoniazid-resistant isolates from Brazil reported that while ahpC mutations often co-occurred with katG mutations, they were also found in the absence of known resistance mutations in katG or inhA(135).

A previous genome-wide survival analysis identified lineages and specific mutations associated with pre-resistance, genomic backgrounds that had a heightened likelihood of acquiring resistance-conferring mutations(136). Whether ahpC acts as in a similar

way, generating a “pre-compensated” genomic background, increasing the likelihood of future katG mutations, remains unknown.

This part of the thesis had several limitations. Firstly, while all available *M. tuberculosis* cultures were sequenced, the final sample size of *M. tuberculosis* genomes was small relative to the number of notified TB cases in the study departments over the study period. Some locally circulating genotypes may, therefore, not be included in the sample and may lead to an underestimate of the contribution of recent transmission to incident TB. However, they were sampled over a moderately long timeframe (five years) and included samples from high-incidence prisons and neighbouring communities, providing greater opportunity to recover transmission events. Secondly, surveillance focused on Paraguay’s urban areas, where most TB notifications occur. Upcoming *M. tuberculosis* genomic surveillance in the Chaco, western Paraguay, where incidence is three times higher than in eastern Paraguay(137), is needed. Additionally, further analysis at the regional level will be critical for understanding transmission between Paraguay and neighbouring countries. Thirdly, TB infections were sampled from prisons at a higher rate than infections outside prisons, potentially biasing upwards estimates of the rate of genomic clustering within prisons compared to outside prisons. Moreover, access to more detailed epidemiological data, such as contact information, was not available. Coming studies integrating genomic data with additional epidemiological data could be used to identify other locations potentially contributing disproportionately to *M. tuberculosis* transmission in Paraguay.

Finally, isolates from cultured sputum were sequenced, as is routinely done for *Mtb* genomic epidemiology, but which limits the within-host diversity recovered from an individual’s infection. Future research is needed to develop sequencing approaches to recover within-host M. tuberculosis variation and incorporate this level of variation into the transmission and ancestral state reconstruction.

Together, these results provide evidence of transmission from prisons to the community and underscore an urgent need for TB control measures to interrupt ongoing transmission in Paraguay, particularly in high-incidence prison settings, which have an outsized role in broader transmission. Supplementary, the connectivity of Paraguay’s metropolitan areas indicates that TB control needs to be coordinated country wide.

Some proposals based on the knowledge gained.

Most NTP in the Latin American region define PDL as one of the high-risk populations (such as indigenous population, drug users, immigrants, among others). The proportion of TB cases occurring among PDL is commonly reported in the performance indicators of NTPs. However, this indicator underestimates the true fraction of all TB cases that are attributable to prisons. The significant turnover of the incarcerated population, combined with long and variable TB latency periods, results in a considerable segment of individuals (ranging from 23% to 42%) who acquire TB infection in prison but only progress to disease once they are released (37,39,138). Even those who develop TB disease in prison may not be diagnosed until after release from prison, due to under-detection in prisons. History of incarceration is typically not an element of notification databases, so cases occurring in the community among individuals with prior incarceration are not currently recognized by the NTPs as being related to prisons. Furthermore, this thesis provides evidence from molecular epidemiology, indicating that genomic clusters of TB present in the community are shared among individuals, regardless of their history of incarceration. This suggests the potential onward transmission of TB cases originating in prisons to the broader community (42,43).

It is also essential to focus efforts on identifying and treating latent TB infection more effectively. There are fewer data available concerning the incidence of TB infections, a recent meta-analysis documented a very high pooled incidence of 15 people acquiring infection per 100 person-years (27). These high rates of infection point towards an opportunity prevent TB disease and subsequent transmission using TPT.

A key question for programs providing testing and treatment of TB infection is the timing of screening. In high TB–burden communities, and among previously incarcerated individuals, the prevalence of TB infection is often high, supporting an approach of screening at entry or re-entry. By contrast, among individuals incarcerated for the first time in communities with low or moderate TB incidence, prevalence may be low at entry. For example, a study in Brazil found that <10% of individuals had positive tuberculin skin tests at the time of first incarceration but then incurred a >20% annual

risk of infection during incarceration (123). These data suggest that screening for TB infection should be performed at prison entry and periodically to assess for incident infections. In settings with high transmission rates, twice annual screening for incident infections may be needed, with the latter serving to identify recently infected individuals in time to initiate TPT and prevent disease.

Screening for TB infection may be performed by tuberculin skin tests (TSTs) or interferon gamma release assays (IGRAs); the latter are typically more expensive and require phlebotomy and laboratory equipment. Both approaches, however, confer different advantages with respect to specificity and sensitivity, as well as the need for follow-up. In some high TB–burden settings, including prisons, programs have elected to forego testing for TB infection and directly offer preventive treatment to all high-risk individuals (99). The rationale for this may be multifold: (1) in high transmission settings, the majority of individuals will harbor TB infection, and the risk of a false negative IGRA/TST is greater, particularly among those with HIV in whom TST has reduced sensitivity; (2) TPT may prevent infections while it is taken, as some clinical trial evidence suggests (96); (3) historical studies in high transmission settings suggested that individuals with negative TSTs were at highest risk of TB, presumably due to greater susceptibility to initial infection; and (4) the costs of TPT are lower than IGRA testing. Although there have been shortages in tuberculin for TST, new TB antigen-based skin tests may soon mitigate this problem. The innovation of providing “community-wide” preventive treatment in settings with very high transmission rates is not a new one, but such strategies need to be balanced against risks of adverse events, particularly in populations with medical comorbidities that elevate risk of drug toxicities. Testing for TB infection must be combined with screening for TB disease, ideally using both radiography and rapid molecular diagnostics, to identify individuals who require treatment. Many prisons, particularly in low and middle countries (LMIC), lack functioning equipment and personnel for performing chest radiography. New, portable chest radiography systems, paired with automated interpretation software, hold promise for making radiography-based screening for disease more accessible in prisons (78).

Regarding preventive treatment in carceral settings, historically, commonly utilized regimens included TPT, which consisted of daily isoniazid (INH) for 6 to 9 months. The effectiveness of this strategy in these settings has been limited, however, due to low treatment acceptance and completion rates. Equally efficacious short-course regimens, including a 3-month (12 weekly dose) regimen of high-dose isoniazid and rifapentine (3HP), have led to markedly improved completion rates in carceral settings compared with 6 or 9 months of INH (139,140). Two recent studies illustrate successful models for short-course–based TPT in high-burden LMIC prisons. In Malawi and Pakistan, 3HP acceptance in prisons exceeded 95% and completion rates were over 85%, with low rates of adverse events (99,102). The cost of rifapentine remains one of the major obstacles to adoption and scale-up of short-course TPT regimens in LMICs.

In another approach, a straightforward but crucial surveillance change is that NTP notification forms must include incarceration history, specifying facility, duration, and dates (141). This ensures more accurate documentation of the TB burden attributable to incarceration. Furthermore, considering that the risk of developing TB is high among former PDL, particularly during the first few years after release from prison, it is imperative to systematize this variable in the documentation of medical history or data collection tools (like questions about smoking or drug use). This approach enhances the likelihood of requesting a confirmatory laboratory test for TB in suspected cases and may also be relevant for contact tracing efforts in first-degree relatives or cohabitants. It is important to consider that a history of incarceration can be stigmatizing. Asking about it may cause tensions between patients and healthcare staff. Therefore, appropriate questions should be developed with relevant stakeholders and focus groups. This helps to craft questions that reduce barriers to healthcare access(142).

Another politically oriented element that needs profound rethinking is the governance of health care within prisons. On this topic, infectious disease surveillance experts and researchers actively working on TB control from 14 LA countries were consulted in May 2023. In most countries, except Ecuador and Panama, prison healthcare is not governed by the MoH. This responsibility is primarily vested in the MoJ. In all instances, MoH only intervenes in response to large-scale issues reported from prisons. This causes the entire hierarchical structure of the healthcare staff in prisons to have different rules, codes,

and work cultures than the rest of the healthcare system. This adds complexity to the management and communication with the country's healthcare service network. It may also pose challenges to continuity of care for individuals who are released from prison or become incarcerated during TB treatment.

There are experiences of good practices regarding governance of healthcare in prisons by MoH, mainly in Europe. In the 90s, the HIV epidemic and the increase in intravenous drug use exacerbated the health problem in European prisons and led to an increase in TB (13). This led to the creation of a network dedicated to improving prison health in the WHO European Region. The European Health in Prisons Program (HIPP) is the only WHO network addressing prison health, and it is not available in other regions. This network has greatly facilitated the exchange of experiences, the evaluation of the impact of various interventions, and the development of best health practices in prison settings. The transfer of governance in healthcare within prisons from the MoJ to the MoH is one of the initial and key recommendations of the HIPP (14,111). The implementation of this change not only affects TB indicators or the ability to effectively track cases once released from prison. It has a positive impact on the more comprehensive approach to the health of PDL, as demonstrated in several studies (46,143,144).

El Salvador and Brazil are countries that are in the process of transitioning the health governance in prisons. The former is making significant investments in improving the infrastructure of its prisons, amidst a crisis of excessive incarceration rates, but with an increasingly empowered support from the MoH. In the case of Brazil, the transfer of governance of health within prisons is being shifted from the MoJ to local governments, who oversee healthcare systems within their jurisdictions (145,146). These transitions are gradual and linked to the organizational health care system of each country.

It is worth acknowledging that Pan American Health Organization (PAHO) is one of only two WHO regional offices that does already report burden of TB in prisons (the other is the European WHO region)(147). Moreover, the WHO in its 2023 Global TB Report emphasized the importance of TB in prisons as a strong risk factor that deserves further attention and action towards (148). This shows recognition of the role of incarceration in driving TB transmission and the need for interventions in prisons. While these are important steps, it will be critical to ensure that TB surveillance includes information

regarding history of incarceration (either the person or close contacts), and to expand reporting of incarceration-related TB in other regions. This will improve the understanding of the TB burden attributable to incarceration, which will inform targeted prevention efforts to accelerate progress towards TB elimination targets.

To achieve a more holistic approach to addressing this crisis, it is needed to shift the focus from discussing TB in prisons to discussing incarceration-related TB. This would facilitate a more appropriate and comprehensive approach that would help understanding that this is a public health issue, centred in prisons, but extending beyond their boundaries and increasingly impacting community health in many countries.

9. Conclusions

1. Over the past decade, tuberculosis epidemic has been particularly concentrated in penitentiary centres, leading to a doubling in the proportion of cases within these establishments compared to the national tuberculosis case count. This trend is closely linked to the significant overcrowding observed in prisons across Paraguay and Latin America.

2. Tuberculosis becomes part of the sentence when one enters prison, a sentence that becomes even harsher during the incarceration period. Furthermore, upon release, individuals still exhibit a higher risk of falling ill compared to the population who has never been incarcerated.

3. Once incarcerated, individual determinants of tuberculosis development become insignificant compared to structural conditions such as ventilation and overcrowding in cellblocks and cells.

4. Nearly half of the tuberculosis cases among individuals with a history of incarceration develop after they have been released and are back in the community. This is not a typical prison-related tuberculosis burden.

5. Predominant tuberculosis lineages are evident in the epidemic, primarily concentrated within the oldest prison, spreading to other prisons through the PDL movement. Moreover, these lineages also extend into the broader community.

6. Clusters have been identified where prisoners, former prisoners, and individuals with no history of incarceration coexist, showing signs of recent transmission.

This underscores the significant interaction between the incarcerated population and the general population.

7. Key areas have been identified for proposing public health interventions, focusing primarily on biomedical strategies. These include implementing mass screenings for tuberculosis and tuberculosis infection, as well as advancing tuberculosis infection treatment. Secondly, enhancing surveillance and diagnostic criteria algorithms to investigate incarceration history. Thirdly, empowering Ministries of Public Health to address prison health issues. Lastly, facilitating the exchange of lessons learned in the different settings involved.

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