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Detection, survival and infectious potential of *Mycobacterium tuberculosis* in the environment: a review of the evidence and epidemiological implications

Leonardo Martinez[1], **Renu Verma**[1], **Julio Croda**[2,3], **C. Robert Horsburgh Jr**[4,5], **Katharine S. Walter**[1], **Nicholas Degner**[1], **Keren Middelkoop**[6,7], **Anastasia Koch**[8], **Sabine Hermans**[6,9], **Digby F. Warner**[8,10], **Robin Wood**[6], **Frank Cobelens**[9], **Jason R. Andrews**[1]

[1]Division of Infectious Diseases and Geographic Medicine, School of Medicine, Stanford University, Stanford, CA, USA. [2]Oswaldo Cruz Foundation, Campo Grande and Salvador, Brazil. [3]School of Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Brazil. [4]Dept of Medicine, Boston University School of Medicine, Boston, MA, USA. [5]Dept of Epidemiology, Biostatistics and Global Health, Boston University School of Public Health, Boston, MA, USA. [6]The Desmond Tutu HIV Centre, Institute for Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa. [7]Dept of Medicine, University of Cape Town, Cape Town, South Africa. [8]SAMRC/NHLS/UCT Molecular Mycobacteriology Research Unit, Dept of Pathology and Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa. [9]Dept of Global Health, Amsterdam Institute for Global Health and Development, Academic Medical Center, Amsterdam, The Netherlands. [10]Wellcome Center for Infectious Diseases Research in Africa, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.

Abstract

Much remains unknown about *Mycobacterium tuberculosis* transmission. Seminal experimental studies from the 1950s demonstrated that airborne expulsion of droplet nuclei from an infectious tuberculosis (TB) patient is the primary route of transmission. However, these findings did not rule out other routes of *M. tuberculosis* transmission. We reviewed historical scientific evidence from the late 19th/early 20th century and contemporary studies investigating the presence, persistence and infectiousness of environmental *M. tuberculosis*. We found both experimental and epidemiological evidence supporting the presence and viability of *M. tuberculosis* in multiple natural and built environments for months to years, presumably following contamination by a human source. Furthermore, several studies confirm *M. tuberculosis* viability and virulence in the environment using guinea pig and mouse models. Most of this evidence was historical; however, several recent studies have reported consistent findings of *M. tuberculosis* detection and viability in the environment using modern methods. Whether *M. tuberculosis* in environments represents an infectious threat to humans requires further investigation; this may represent an untapped source of

Correspondence: Leonardo Martinez, Division of Infectious Diseases and Geographic Medicine, School of Medicine, Stanford University, 300 Pasteur Drive, Lane L141, Stanford, CA 94305, USA. leomarti@stanford.edu.

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data with which to further understand *M. tuberculosis* transmission. We discuss potential opportunities for harnessing these data to generate new insights into TB transmission in congregate settings.

Introduction

> Much of our current understanding of the transmissibility of tuberculosis derives from inference and accident rather than from intentional scientific study
>
> Kent A. Sepkowitz, 1996 [1]

Every year, more than 10 million new cases of tuberculosis (TB) occur globally [2] and ongoing *Mycobacterium tuberculosis* transmission is the primary driver of incident disease. Despite significant advances in TB diagnostics, immunology and genomic epidemiology [3], much remains unknown about individual- and population-level transmission dynamics. Our current tools allow for the study of *M. tuberculosis* transmission only after a TB case is diagnosed and seminal studies of TB infectiousness, including the landmark studies of Wells and Riley in the mid-20th century [4–6], were conducted in modified hospital wards following TB diagnosis. “Community-based” exposure studies focus predominantly on household contacts, which account for <20% of infections in high TB burden settings [7–12]. Due to the prolonged infectious period of TB [13–15] and the potential for transmission from brief, casual exposures, less than one-third of cases can be epidemiologically and genetically linked [7–12]. Fundamental questions about where TB transmission occurs in communities, relationships between exposure and infection or disease risk, and heterogeneity in transmission at the population level remain poorly understood [1, 16, 17]. Much of this is due to our limited tools for studying transmission in community settings.

Prior to the mid-19th century, several competing theories existed regarding transmission of *M. tuberculosis*. For example, Calmette and Guérin [18] proposed in 1905 that TB could be transmitted through contaminated food. In the 1950s, innovative and groundbreaking studies from Riley and Wells demonstrated that droplet aerosols from infectious TB patients resulted in substantial rates of tuberculin conversion in exposed guinea pigs [4–6]. These experiments were recently re-created in guinea pig air sampling facilities in Peru and South Africa, with consistent results [19–23]. These studies found that TB transmission to guinea pigs occurred from small droplet aerosols expulsed by patients and that the infectiousness of source cases was highly heterogeneous [6]. This work clarified the fundamentals of airborne transmission of pulmonary TB and is currently widely accepted as the primary mechanistic transmission route [6, 17]. These and other findings by Loudon investigating the generation of droplet nuclei through various airway activities, such as coughing and singing, were highly influential [24–27]. These studies paved the way for the study of TB transmission in contemporary studies, as well as the development and implementation of effective infection control strategies in healthcare facilities [17, 22].

While these critical studies shifted the focus of research to airborne transmission, their results did not rule out other routes of *M. tuberculosis* transmission. Earlier evidence for the detection of *M. tuberculosis* in, and potential transmission from, environmental settings may have been set aside with the discovery of the predominance of transmission through airborne

droplet nuclei. The study of *M. tuberculosis* in the environment could potentially address limitations in our understanding of transmission, which is currently achieved almost exclusively through studying patient-derived samples.

We review historical scientific evidence from the late 19th/early 20th century as well as contemporary studies investigating the presence, persistence and infectious potential of *M. tuberculosis* in environmental settings. We evaluate the strengths and limitations of this body of evidence, identify unresolved questions and areas requiring further research, and discuss how studies of *M. tuberculosis* in environmental samples could facilitate new approaches towards the study of TB transmission.

Historical evidence

Following Robert Koch's discovery of the *M. tuberculosis* bacillus in 1882, many scientists and researchers sought to determine how the bacterium was transmitted. Their studies focused on detecting *M. tuberculosis* in various environments, culturing it to demonstrate its viability and, in some cases, injecting it into animals to confirm that it caused TB disease. Much of this evidence was generated in the late 19th/early 20th centuries. Here, we review these historical studies concerning the detection, viability and infectiousness of *M. tuberculosis* in natural and built environments.

Certain caveats should be considered in the interpretation of historical studies. First, many of these studies were done before the diversity of mycobacteria was understood and it is possible that nontuberculous mycobacteria (or other members of the *M. tuberculosis* complex) were described in some of the studies. While many of the studies reported use of clinical *M. tuberculosis* isolates or sputum from TB patients, others reported direct detection in various environments such as soil or water, in which nontuberculous mycobacteria are commonly found. Studies lacking proper controls are susceptible to inaccurate inference about the role of *M. tuberculosis* in the environment. Given this uncertainty in the historical microbiology, we have attempted to emphasise studies in which controls were tested, clinical isolates from human cases were used or TB was induced in animal models for confirmation.

Natural environments

A number of studies investigated the survival of tubercle bacilli in natural environments, including soil and water (table 1). In 1888, Chantemese and Widal [28] performed several experiments on river water attempting to quantify the number of days that the tubercle bacilli remained active. They first collected several tubes of water from the Seine River in Paris, France, subsequently sterilising some tubes. They then inoculated all tubes (sterilised and not) with patient cultures of tubercle bacilli. The tubes were kept at different temperatures (8–12°C and 15–20°C). After 50 days, the tubercle bacilli stored at both temperatures could still be cultured. A year later, STRAUS and DUBARRY [29] inoculated 10 tubes of 10 cm^3 of distilled water and water from the Ourcq River, also in France. Culture from a TB patient was inoculated into tubes containing these different water samples. Samples maintained in river water were culturable for up to 30 days and those in distilled water were culturable for up to 115 days. Three guinea pigs were then inoculated with the river water into which *M. tuberculosis* had been inoculated. One guinea pig developed

abscesses from which *M. tuberculosis* was culturable, while the other two guinea pigs remained healthy. In addition to these tests of environmental persistence and viability, several studies detected *M. tuberculosis* in environmental water and sewage samples collected near TB sanatoria, suggesting contamination from human sources [28].

Other studies demonstrated prolonged viability of *M. tuberculosis* in soil. In 1887, Feltz [30] mixed soil and sputum rich in *M. tuberculosis* bacilli. He then exposed the soil to differing degrees of sunlight, periodically collected extracts of the soil and inoculated it into guinea pigs. Inoculated soil to which *M. tuberculosis* had been added 137 days prior produced TB lesions. A decade later, Mitchell and Couch [31] attempted to investigate the virulence of *M. tuberculosis* in soil after exposure to sunlight. They placed sputum from TB patients onto heat-sterilised soil, exposed the soil to sunlight for varying lengths of time and injected the soil into guinea pigs. When inoculated with soil exposed to sunlight for <35 h, TB manifested in guinea pigs; longer durations of sunlight exposure resulted in no infections in guinea pigs. *M. tuberculosis* was also culturable from soil exposed for <35 h. This study suggested that sunlight may have some influence on the presence of *M. tuberculosis* in the environment from some outdoor settings.

Built environments

In addition to natural environments, many studies investigated the viability of *M. tuberculosis* found in built environments and on fomites such as clothing and cooking utensils (table 1). In the late 1800s, several studies investigated dust collected from rooms of TB patients as a potential mode of transmission. In 1888, Cornet [32] collected dust from TB medical wards as well as from hospitals, asylums and apartments housing TB patients. After subcutaneous inoculation of the dust in 91 guinea pigs, 15 were later autopsied and found to have developed TB. This study also evaluated negative control groups with no TB exposure, using dust from surgical wards (none of eight inoculated guinea pigs developed TB) as well as from streets and public buildings (none of 41 inoculated guinea pigs developed TB). This study provided strong empiric evidence using guinea pig controls and several locations with a high TB risk that exposure to *M. tuberculosis* through dust may lead to disease in guinea pigs.

In 1920, Rogers [33] also conducted a series of experiments using dust. In all experiments, dust was treated with sterile 2% sodium hydroxide solution, and then centrifuged and injected into guinea pigs. In the first three experiments, he injected guinea pigs with dust and found evidence of disease in four out of 11 guinea pigs injected with dust from floors of TB wards, four out of four guinea pigs injected with dust from a morgue floor, and two out of five guinea pigs injected with dust from the windows and shelves of a morgue. A final experiment involved placing 10 guinea pigs in a sterilised wire cage kept about 3 feet (1 m) above the morgue floor for an average of 5 h per day. The floor was swept every morning. After 18 days, all guinea pigs were autopsied and seven were subsequently diagnosed with disseminated TB. These experiments suggested that exhaled TB, which had settled on surfaces, could be re-aerosolised and generate infections. Importantly, however, Rogers [33] did not have adequate controls.

Twitchell [34] placed dried sputum from a TB patient on a handkerchief, wood and a woollen blanket, and waited up to 70 days, subsequently subcutaneously inoculating the sputum into the groin of one guinea pig per object. After 4–6 weeks, each guinea pig was killed, autopsied and inspected for the presence of TB. TB lesions were detected in each guinea pig. Twitchell [34] also investigated the viability of *M. tuberculosis* on carpets. He placed dried sputum from a patient on a carpet for 39 days and then inoculated a guinea pig. The guinea pig subsequently developed disease. When the same experiment was conducted with the sputum exposed to sunlight for up to 7 h, the bacilli died in a short time span, suggesting that sunlight may affect *M. tuberculosis* on these objects.

In 1920, Cumming [35] conducted a series of experiments with the goal of testing whether common household objects could represent fomites for TB. In a first experiment, three spoons from separate smear-positive TB patients were washed after each meal with a cloth in hot water. This wash water was subsequently centrifuged and injected subcutaneously into guinea pigs. Of 31 guinea pigs injected and subsequently autopsied, 11 died from TB. In a separate experiment, the hands of TB patients were placed in warm water for several minutes after which epithelia from the hands were scraped with a scalpel and injected into seven guinea pigs. Three of these guinea pigs subsequently died due to TB.

Flies and M. tuberculosis

Several early studies examined the ability of flies to carry and spread *M. tuberculosis* [36–40]. In 1904, Lord [39] reported on several studies on TB in flies performed in the late 19th/early 20th century. In one experiment, he confined 30 flies in a glass jar and fed them sputum from TB patients over 4 days (figure 1). Two sets of controls were performed: 1) six flies were put into a jar and fed nontuberculous sputum, and 2) six flies were confined to a jar and fed only water, sugar and meat. Among the flies fed sputum from TB patients, *M. tuberculosis* was recovered in the excrement from the flies as well as the intestines of all 30 flies. No *M. tuberculosis* was found in excrement or intestines from control flies. The investigators then let the excrement from the flies who were fed TB patient sputum sit for differing periods of time (1, 8, 15, 28 and 55 days). Excrement from each duration of time was fed to guinea pigs. The guinea pigs who were fed excrement that sat for 1, 8 and 15 days all developed TB, while the two guinea pigs fed faeces older than 15 days remained healthy. Just a few years later, Buchanan [37] exposed flies to a Petri dish with *M. tuberculosis* and then transferred them to a sterile Petri dish with growth media, which resulted in growth in the second dish; he thereby demonstrated that flies could spread *M. tuberculosis* to other surfaces.

Other researchers used natural experiments to further study the viability of *M. tuberculosis* in flies. In 1887, Hoffman [40] found flies in the house of a recently deceased TB patient. He collected the flies, dissected them, and identified tubercle bacilli in their intestines and faeces by microscopy. In 1904, Hayward [38] caught several flies feeding on TB sputum in his laboratory and subsequently put them into clean cages. After several days he found TB bacilli in the faeces on 10 out of 16 cover-slips. Examination of unexposed, control flies resulted in no tubercle bacilli.

In the early 20th century, several cities (including Toronto and Montreal in Canada, London in the UK, and Detroit in the USA) launched large-scale campaigns to eradicate flies for the sake of public health [41–43]. During this time period, TB was a leading cause of paediatric mortality in the USA, Canada and much of the world, and flies were widely believed to be a vector of infectious diseases, including TB. “Fly Swatting” contests for children offered prizes for those that captured, killed and brought in the most flies (figure 1). These campaigns were promoted by physicians and the media (figure 1) who believed improved sanitation would have positive ramifications for paediatric health, including TB [41].

Contemporary evidence

Since the demonstration of transmission by airborne droplet nuclei, few studies have investigated *M. tuberculosis* in the environment (table 2) [44–47]. GHODBANE et al. [44] inoculated soil with *M. tuberculosis*, *Mycobacterium bovis* and *Mycobacterium canetti*, and assessed the survival of the distinct mycobacteria in the soil for 12 months. The soil was cultured monthly and all three types of mycobacteria were still present in the soil after 12 months. The soil contaminated with *M. tuberculosis* was then intermixed with the food for five healthy mice for 60 days. All five of these mice developed granulomas, whereas all of the control mice remained healthy. Similar results were found by KOZLOV and ROTOV [45], who seeded three strains of *M. tuberculosis* in a natural turf–podzol sandy soil and found that they were culturable for up to 3 months. These studies suggest that *M. tuberculosis* may retain viability and infectiousness after prolonged periods in soil. Natural sunlight may affect the persistence of *M. tuberculosis* in soil; however, this was not investigated. In addition, whether humans or other animals exposed to soil contaminated with *M. tuberculosis* could also become infected is unclear.

A recent study investigated re-aerosolisation of dust containing *Mycobacterium smegmatis*, a common surrogate marker for *M. tuberculosis*. TSHILOMBO et al. [46] conducted a prospective *in vitro* study whereby they mixed 20 mL of 10^6 CFU·mL^{-1} *M. smegmatis* with 125 mg of sterile dust. Air sampling was conducted pre- and post-re-aerosolisation and the number of CFUs was measured. The authors found that *M. smegmatis* survived in the dust for 19 days and could be successfully re-aerosolised, remaining viable. Although a limitation is the use of *M. smegmatis*, this study suggests the possibility that re-aerosolisation of *M. tuberculosis* in dust and other environments may be possible. Further research is needed to validate and extend the findings of this study [46]. Studies in real-world settings are also needed to assess the generalisability of these findings outside the laboratory. VELAYATI et al. [47] collected 1500 randomly selected soil and water samples in three counties in Tehran, Iran. *M. tuberculosis* was isolated by Löwenstein–Jensen culture media from 1% of soil samples and 10% of water samples, with confirmation by phenotypic and molecular tests. Soil and water samples were additionally stored and retested for culturability over time, which demonstrated that *M. tuberculosis* could be recovered from stored soil and water samples for 9 months after sample collection. MIRU-VNTR (mycobacterial interspersed repetitive unit-variable number tandem repeat) typing of environmental and clinical isolates revealed partial overlap in *M. tuberculosis* families present in the area.

Implications and future directions

Potential for TB transmission through environmental samples

It is not surprising that *M. tuberculosis*, an organism that originally evolved from a soil saprophyte and, by virtue of its capsule and mycolic acid and lipid-rich cell wall, is comparatively resilient against environmental stresses, would be capable of surviving for prolonged periods outside of humans [48, 49]. Historical and contemporary evidence for *M. tuberculosis* viability and infectiousness in environmental samples does not establish that environmental reservoirs are important routes of transmission. The evidence does, however, raise the possibility that transmission could occur through aerosolisation of environmental bacilli. Currently, little attention is given to environmental surfaces or fomites, including clothing and other objects, in the prevention of transmission. If environmental exposures do confer substantial risk, we will need to develop methods to prevent these transmission routes as global control efforts turn towards elimination. Areas with densely populated housing and hospital settings that have little sunlight may be particularly susceptible environments for the spread of *M. tuberculosis*.

To investigate this potential route of transmission, there is a need to test whether, and how readily, *M. tuberculosis* can be re-aerosolised from surfaces in a form that can generate infections in animals. There is evidence that re-aerosolisation causing human infection can occur through nosocomial transmission during surgical procedures from incision and irrigation of tuberculous abscesses [50, 51], and through autopsies [52].

Current animal models of TB typically involve bronchoscopic installation or aerosol exposure via nebulisation of freshly cultured *M. tuberculosis*. These models could be used to investigate the risk of infection from aerosolised *M. tuberculosis* on environmental surfaces (*e.g.* floors), as a function of the duration over which the bacteria are present prior to the exposure.

Methods for environmental detection of M. tuberculosis

There is a need for standardised, highly accurate methods for environmental detection of *M. tuberculosis*. *M. tuberculosis* can be cultured from soil and other materials, but sensitivity may be limited due to bacterial overgrowth and the presence of “differentially culturable” (or “viable but nonculturable”) organisms [53, 54]. A better understanding of *M. tuberculosis* viability in various environmental matrices will require methods for optimal promotion of their growth following recovery from the environment.

Molecular detection of *M. tuberculosis* has been demonstrated in filtered air samples [55–57], but to the best of our knowledge there are no studies investigating its detection on environmental surfaces. This is despite an increasingly robust literature on detection of various pathogens in natural and built environments [58–60]. While conventional molecular methods (*e.g.* PCR) do not distinguish viable from nonviable organisms, a number of molecular methods have been developed to do so, including detection of mRNA or selective detection of intracellular DNA [61–63].

Viability stains have recently been developed for *M. tuberculosis*, which could facilitate the investigation of its viability in natural and built environments [64]. Validating these methods for *M. tuberculosis* detection could enable higher throughput studies on *M. tuberculosis* in the environment.

In addition to the development of rigorous laboratory protocols for the detection and growth of *M. tuberculosis* from environmental sampling, there is a need for a better understanding of how to perform sampling in various environments. This includes questions pertaining to the frequency, location, sample collection methods and site selection, as well as understanding how disruptions (cleaning, airflow, water, *etc.*) might affect *M. tuberculosis* persistence in those environments.

Potential for enhancing our understanding of TB transmission through environmental detection of M. tuberculosis

A deeper understanding of *M. tuberculosis* transmission could improve TB prevention and control efforts [16, 17]. However, there are several fundamental challenges in studying TB transmission at present. First, individuals with TB are believed to be infectious for many months to years prior to their diagnosis [13–15]. During this time period, they may have numerous indoor contacts during which transmission could occur [11, 65, 66]. Because TB can be spread through airborne droplet nuclei between strangers sharing the same space and can even transmit to individuals without being in a room contemporaneously, contact-tracing investigations may have significant social network gaps. Efforts to link transmission based on contact investigations or social network analysis have found that only ~10–30% of genetically linked cases in endemic settings can be epidemiologically linked [12, 14, 16, 67]. An alternative goal has been to identify high-risk environments for transmission, which has implicated public transport, bars, churches, schools and workplaces based on where individuals spend time [11, 68]. However, given that individuals with active TB may have distinct social mixing patterns, or may alter these due to illness, this also represents an imperfect approach. Finally, most studies evaluating infectiousness of TB patients involve characterising their clinical features and examining their sputum (or, more recently, their cough or respiratory aerosol content) at the time of their diagnosis [69–72]. However, this approach ignores most of the TB disease spectrum prior to diagnosis and may not correlate well with the total TB exposure received by their household members or other contacts.

Environmental samples of *M. tuberculosis* may hold valuable epidemiological information, including where transmission occurs and where interventions should be targeted. In contrast to sampling in one location at the time of diagnosis, environmental sampling may provide a longer time and spatial window for TB. Given substantial interindividual variability in infectiousness [5, 6, 20], there may be substantial spatial heterogeneity in *M. tuberculosis* in the environment, influencing the chances of detection. If sensitive diagnostics become available for environmental sampling, they could theoretically be used to monitor longitudinal risk in community settings such as healthcare facilities, prisons and schools. If environmental *M. tuberculosis* can be genotyped, it could be possible to link environmental samples with clinical samples. Molecular epidemiology studies could then potentially

reconstruct not only transmission chains, but spatially explicit transmission chains: not only "who infected who", but where they were infected.

By utilising genotyping methods on environmental samples, it would be possible to determine when certain strains were detected in the community compared with when they were detected clinically, to understand the duration of undetected TB. Environmental sampling could also be used to quantify the sum of *M. tuberculosis* exposures over a time period. For example, in household contact investigations, a high degree of indoor contamination in the home may be a better indicator of exposure to household members than smear or cough aerosol status, collected at the time of diagnosis. Finally, environmental detection could direct case-finding efforts: mass screening could be targeted to sites where environmental samples are positive.

Critical knowledge gaps in TB epidemiology and the potential use of environmental sampling for *M. tuberculosis* are listed in table 3.

Conclusions

Research on TB transmission has focused almost entirely on studying clinical isolates from sputum or detection of airborne *M. tuberculosis* through biological sensors (*e.g.* guinea pig models) or, more recently, air samplers in controlled environments. However, there is a considerable historical evidence base suggesting the presence of *M. tuberculosis* in both natural and indoor environments. Animal models suggest that *M. tuberculosis* may remain infectious in some of these environments, raising the possibility that re-aerosolisation could represent an unappreciated route of transmission. Moreover, even if environmental sources do not represent important sources of transmission, sampling for *M. tuberculosis* in indoor environments could nevertheless provide temporal and spatial dimensions for the study of TB transmission in ways not achievable through conventional patient-focused investigations. A re-assessment of the abundance and viability of *M. tuberculosis* in the indoor environment is warranted and, if confirmed, may yield novel insights into the transmission and community distribution of this important pathogen.

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References

1. Sepkowitz KA. How contagious is tuberculosis? Clin Infect Dis 1996; 23: 954–962. [PubMed: 8922785]
2. Lönnroth K, Castro KG, Chakaya JM, et al. Tuberculosis control and elimination 2010–50: cure, care, and social development. Lancet 2010; 375: 1814–1829. [PubMed: 20488524]
3. Borgdorff M, Van Soolingen D. The re-emergence of tuberculosis: what have we learnt from molecular epidemiology? Clin Microbiol Infect 2013; 19: 889–901. [PubMed: 23731470]
4. Riley R, Mills C, Nyka W, et al. Aerial dissemination of pulmonary tuberculosis. A two-year study of contagion in a tuberculosis ward. Am J Hyg 1959; 70: 185–196.

5. Riley R, Mills C, O'Grady F, et al. Infectiousness of air from a tuberculosis ward: ultraviolet irradiation of infected air: comparative infectiousness of different patients. Am Rev Respir Dis 1962; 85: 511–525. [PubMed: 14492300]
6. Sultan L, Nyka W, Mills C, et al. Tuberculosis disseminators: a study of the variability of aerial infectivity of tuberculous patients. Am Rev Respir Dis 1960; 82: 358–369. [PubMed: 13835667]
7. Martinez L, Shen Y, Mupere E, et al. Transmission of Mycobacterium tuberculosis in households and the community: a systematic review and meta-analysis. Am J Epidemiol 2017; 185: 1327–1339. [PubMed: 28982226]
8. Middelkoop K, Mathema B, Myer L, et al. Transmission of tuberculosis in a South African community with a high prevalence of HIV infection. J Infect Dis 2014; 211: 53–61. [PubMed: 25053739]
9. Crampin AC, Glynn JR, Traore H, et al. Tuberculosis transmission attributable to close contacts and HIV status, Malawi. Emerging Infect Dis 2006; 12: 729–735. [PubMed: 16704828]
10. Verver S, Warren RM, Munch Z, et al. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. Lancet 2004; 363: 212–214. [PubMed: 14738796]
11. Andrews JR, Morrow C, Walensky RP, et al. Integrating social contact and environmental data in evaluating tuberculosis transmission in a South African township. J Infect Dis 2014; 210: 597–603. [PubMed: 24610874]
12. Shah NS, Auld SC, Brust JC, et al. Transmission of extensively drug-resistant tuberculosis in South Africa. N Engl J Med 2017; 376: 243–253. [PubMed: 28099825]
13. Corbett EL, Bandason T, Cheung YB, et al. Prevalent infectious tuberculosis in Harare, Zimbabwe: burden, risk factors and implications for control. Int J Tuberc Lung Dis 2009; 13: 1231–1237. [PubMed: 19793427]
14. Horton KC, Sumner T, Houben RM, et al. A Bayesian approach to understanding gender differences in tuberculosis disease burden. Am J Epidemiol 2018; 187: 2431–2438. [PubMed: 29955827]
15. Wood R, Middelkoop K, Myer L, et al. Undiagnosed tuberculosis in a community with high HIV prevalence: implications for tuberculosis control. Am J Respir Crit Care Med 2007; 175: 87–93. [PubMed: 16973982]
16. Peters JS, Andrews JR, Hatherill M, et al. Advances in the understanding of Mycobacterium tuberculosis transmission in HIV-endemic settings. Lancet Infect Dis 2019; 19: e65–e76. [PubMed: 30554995]
17. Yates TA, Khan PY, Knight GM, et al. The transmission of Mycobacterium tuberculosis in high burden settings. Lancet Infect Dis 2016; 16: 227–238. [PubMed: 26867464]
18. Calmette A, Guérin C. Origine intestinale de la tuberculose pulmonaire. [Intestinal origin of pulmonary tuberculosis.] Ann Inst Pasteur 1905; 19: 601–618.
19. Escombe AR, Moore DA, Gilman RH, et al. Upper-room ultraviolet light and negative air ionization to prevent tuberculosis transmission. PLoS Med 2009; 6: e1000043.
20. Escombe AR, Moore DA, Gilman RH, et al. The infectiousness of tuberculosis patients coinfected with HIV. PLoS Med 2008; 5: e188. [PubMed: 18798687]
21. Escombe AR, Oeser C, Gilman RH, et al. The detection of airborne transmission of tuberculosis from HIV-infected patients, using an in vivo air sampling model. Clin Infect Dis 2007; 44: 1349–1357. [PubMed: 17443474]
22. Dharmadhikari AS, Mphahlele M, Stoltz A, et al. Surgical face masks worn by patients with multidrug-resistant tuberculosis: impact on infectivity of air on a hospital ward. Am J Respir Crit Care Med 2012; 185: 1104–1109. [PubMed: 22323300]
23. Dharmadhikari AS, Mphahlele M, Venter K, et al. Rapid impact of effective treatment on transmission of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2014; 18: 1019–1025. [PubMed: 25189547]
24. Loudon RG, Bumgarner LR, Lacy J, et al. Aerial transmission of mycobacteria. Am Rev Respir Dis 1969; 100: 165–171. [PubMed: 4979742]
25. Loudon RG, Roberts RM. Droplet expulsion from the respiratory tract. Am Rev Respir Dis 1967; 95: 435–442. [PubMed: 6018703]

26. Loudon RG, Roberts RM. Singing and the dissemination of tuberculosis. Am Rev Respir Dis 1968; 98: 297–300. [PubMed: 5667756]
27. Loudon RG, Spohn SK. Cough frequency and infectivity in patients with pulmonary tuberculosis. Am Rev Respir Dis 1969; 99: 109–111. [PubMed: 5762102]
28. Chantemesse A, Widal G-F. Resistance of the tubercle bacilli in the river water Congrès pour l'Etude de la Tuberculose, Paris, 1888; p. 317.
29. Straus I, Dubarry A. Recherches sur la duree de la vie des microbes pathogenes dans I'eau. [Research on the duration of life of pathogenic microbes in water.] Arch Med Exp Anat Pathol 1889; 1: 5.
30. Feltz. Essai expérimental et clinique sur le rôle des poussières bacillaires dans la contagion de la tuberculose et sur la durée de la virulence de ces poussières. [Experimental and clinical trial on the role of bacillary dust in the contagion of tuberculosis and on the duration of the virulence of these dusts.] Publisher undetermined, 1890.
31. Mitchell WC, Crouch H. The influence of sunlight on tuberculous sputum in Denver: a study as to the cause of the great degree of immunity against tuberculosis enjoyed by those living in high altitudes. J Pathol Bacteriol 1900; 6: 14–31.
32. Cornet G Die Verbreitung der Tuberkelbacillen ausserhalb des Körpers. [The spread of tubercle bacilli outside the body.] Z Hyg 1889; 5: 191–331.
33. Rogers J Studies on the viability of the tubercle bacillus. Am J Public Health 1920; 10: 345–347.
34. Twitchell DC. The vitality of tubercle bacilli in sputum Paper presented at the National Association for the Study and Prevention of Tuberculosis Annual Meeting, Washington, DC, 1905.
35. Cumming JG. Can the tuberculosis transmission rate be reduced? JAMA 1920; 74: 1072–1074.
36. Andre C Flies as agents in the dissemination of Koch's bacillus Paper presented at the Anti-Tuberculosis Congress, Washington, DC, 1908.
37. Buchanan R The carriage of infection by flies. Lancet 1907; 170: 216–218.
38. Hayward E The fly as a carrier of tuberculosis infection. NY Med J 1904; 80: 643–644.
39. Lord FT. Flies and tuberculosis. Boston Med Surg J 1904; 151: 651–654.
40. Hoffman E Die Gefahr der Weiterverbreitung der Tuberculose durch unsere Hausfliege. [The risk of tuberculosis spreading through our house fly.] Jähret Gesellsch Nat Heil Dresden 1887; 8: 145–147.
41. Minnett V, Poutanen M-A. Swatting flies for health: children and tuberculosis in early twentieth-century Montreal. Urban Hist Rev 2007; 36: 32–44.
42. Crumbine SJ. Men of medicine: swat the fly! Postgrad Med 1949; 5: 57–67.
43. Rogers N Germs with legs: flies, disease, and the new public health. Bull Hist Med 1989; 63: 599–617. [PubMed: 2696572]
44. Ghodbane R, Medie FM, Lepidi H, et al. Long-term survival of tuberculosis complex mycobacteria in soil. Microbiology 2014; 160: 496–501. [PubMed: 24425768]
45. Kozlov V, Rotov V. [Isolation of Mycobacterium tuberculosis cultures from soil]. Probl Tuberk 1977; 9: 78–80.
46. Tshilombo KV, Mehtar S, Sampson S, et al. Survival and re-aerosolization in dust of Mycobacterium smegmatis, a surrogate marker for Mycobacterium tuberculosis. Antimicrob Resist Infect Control 2015; 4: P100.
47. Velayati AA, Farnia P, Mozafari M, et al. Identification and genotyping of Mycobacterium tuberculosis isolated from water and soil samples of a metropolitan city. Chest 2015; 147: 1094–1102. [PubMed: 25340935]
48. Rogall T, Wolters J, Flohr T, et al. Towards a phylogeny and definition of species at the molecular level within the genus Mycobacterium. Int J Syst Evol Microbiol 1990; 40: 323–330.
49. Smith NH, Hewinson RG, Kremer K, et al. Myths and misconceptions: the origin and evolution of Mycobacterium tuberculosis. Nat Rev Microbiol 2009; 7: 537–544. [PubMed: 19483712]
50. Hutton MD, Stead WW, Cauthen GM, et al. Nosocomial transmission of tuberculosis associated with a draining abscess. J Infect Dis 1990; 161: 286–295. [PubMed: 2105362]
51. Keijman J, Tjhie J, Damink SO, et al. Unusual nosocomial transmission of Mycobacterium tuberculosis. Eur J Clin Microbiol Infect Dis 2001; 20: 808–809. [PubMed: 11783698]

52. Templeton GL, Illing LA, Young L, et al. The risk for transmission of Mycobacterium tuberculosis at the bedside and during autopsy. Ann Intern Med 1995; 122: 922–925. [PubMed: 7755227]
53. Chengalroyen MD, Beukes GM, Gordhan BG, et al. Detection and quantification of differentially culturable tubercle bacteria in sputum from patients with tuberculosis. Am J Respir Crit Care Med 2016; 194: 1532–1540. [PubMed: 27387272]
54. Mukamolova GV, Turapov O, Malkin J, et al. Resuscitation-promoting factors reveal an occult population of tubercle bacilli in sputum. Am J Respir Crit Care Med 2010; 181: 174–180. [PubMed: 19875686]
55. Mastorides SM, Oehler RL, Greene JN, et al. The detection of airborne Mycobacterium tuberculosis using micropore membrane air sampling and polymerase chain reaction. Chest 1999; 115: 19–25. [PubMed: 9925058]
56. Chen P-S, Li C-S. Quantification of airborne Mycobacterium tuberculosis in health care setting using real-time qPCR coupled to an air-sampling filter method. Aerosol Sci Technol 2005; 39: 371–376.
57. Wood R, Morrow C, Ginsberg S, et al. Quantification of shared air: a social and environmental determinant of airborne disease transmission. PLoS One 2014; 9: e106622. [PubMed: 25181526]
58. Karkey A, Jombart T, Walker AW, et al. The ecological dynamics of fecal contamination and Salmonella Typhi and Salmonella Paratyphi A in municipal Kathmandu drinking water. PLoS Negl Trop Dis 2016; 10: e0004346. [PubMed: 26735696]
59. Afshinnekoo E, Meydan C, Chowdhury S, et al. Geospatial resolution of human and bacterial diversity with city-scale metagenomics. Cell Syst 2015; 1: 72–87. [PubMed: 26594662]
60. Bischoff WE, McNall RJ, Blevins MW, et al. Detection of measles virus RNA in air and surface specimens in a hospital setting. J Infect Dis 2015; 213: 600–603. [PubMed: 26386428]
61. Li L, Mahan CS, Palaci M, et al. Sputum Mycobacterium tuberculosis mRNA as a marker of bacteriologic clearance in response to antituberculosis therapy. J Clin Microbiol 2010; 48: 46–51. [PubMed: 19923475]
62. Weigel KM, Jones KL, Do JS, et al. Molecular viability testing of bacterial pathogens from a complex human sample matrix. PLoS One 2013; 8: e54886. [PubMed: 23365682]
63. Cangelosi GA, Meschke JS. Dead or alive: molecular assessment of microbial viability. Appl Environ Microbiol 2014; 80: 5884–5891. [PubMed: 25038100]
64. Kamariza M, Shieh P, Ealand CS, et al. Rapid detection of Mycobacterium tuberculosis in sputum with a solvatochromic trehalose probe. Sci Transl Med 2018; 10: eaam6310.
65. Dodd PJ, Looker C, Plumb ID, et al. Age- and sex-specific social contact patterns and incidence of Mycobacterium tuberculosis infection. Am J Epidemiol 2015; 183: 156–166. [PubMed: 26646292]
66. Johnstone-Robertson SP, Mark D, Morrow C, et al. Social mixing patterns within a South African township community: implications for respiratory disease transmission and control. Am J Epidemiol 2011; 174: 1246–1255. [PubMed: 22071585]
67. Glynn JR, Guerra-Assunção JA, Houben RM, et al. Whole genome sequencing shows a low proportion of tuberculosis disease is attributable to known close contacts in rural Malawi. PLoS One 2015; 10: e0132840. [PubMed: 26181760]
68. Chamie G, Wandera B, Marquez C, et al. Identifying locations of recent TB transmission in rural Uganda: a multidisciplinary approach. Trop Med Int Health 2015; 20: 537–545. [PubMed: 25583212]
69. Fennelly KP, Martyny JW, Fulton KE, et al. Cough-generated aerosols of Mycobacterium tuberculosis: a new method to study infectiousness. Am J Respir Crit Care Med 2004; 169: 604–609. [PubMed: 14656754]
70. Martinez L, Sekandi JN, Castellanos ME, et al. Infectiousness of HIV-seropositive patients with tuberculosis in a high-burden African setting. Am J Respir Crit Care Med 2016; 194: 1152–1163. [PubMed: 27181053]
71. Shaw J, Wynn-Williams N. Infectivity of pulmonary tuberculosis in relation to sputum status. Am Rev Tuberc Pulm Dis 1954; 69: 724–732.
72. Behr M, Warren S, Salamon H, et al. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. Lancet 1999; 353: 444–449. [PubMed: 9989714]

FIGURE 1.

Flies and tuberculosis (TB): 20th century experimentation and fly extermination campaigns. Experiments in August 1904 on *Mycobacterium tuberculosis* and flies by Frederick Lord [39]: flies trapped in a jar for feeding of sputum from a TB patient in a hospital ward (top left). Advertisement for eradication of flies for paediatric health in the *Toronto Daily Star* in 1912 (right). "Fly Swatting" contest from *The Montreal Star* in 1912: buckets of flies brought in to collect prizes (bottom left).

TABLE 1

Historical studies investigating *Mycobacterium tuberculosis* in the environment (pre-1960)

First author, year [ref.]	Medium	Method of *M. tuberculosis* detection
Outdoor, natural environments		
FELTZ, 1887 [30]	Soil	Guinea pig model
CHANTEMESE, 1888 [28]	River water	Culture of samples; guinea pig model
STRAUS, 1889 [29]	River water and distilled water	Culture of samples; guinea pig model
MITCHELL, 1900 [31]	Soil exposed to sunlight	Guinea pig model
Indoor, built environments		
CORNET, 1889 [32]	Dust from tuberculosis medical ward and walls of rooms of TB patients; dust from streets and surgical wards [controls] with no TB patients	Guinea pig model
MITCHELL, 1900 [31]	Soil	Guinea pig model
TWITCHELL, 1905 [34]	Handkerchief, woollen blanket, wood	Culture of samples; guinea pig model
ROGERS, 1920 [33]	Dust from floors in open TB wards, from a morgue floor, and windows and shelves of a morgue; lastly, a morgue floor was swept every morning	Guinea pig model
CUMMING, 1920 [35]	Utensils of a TB patient; hands of a TB patient	Guinea pig model

TB: tuberculosis.

TABLE 2

Contemporary evidence for environmental contamination of *Mycobacterium tuberculosis* (post-1960)

First author, year [ref.]	Methodology and medium	Description of results
KOZLOV, 1977 [45]	Three strains of *M. tuberculosis* cultured for several months in a natural turf-podzol sandy soil	Samples culturable for up to 3 months after seeding
GHODBANE, 2014 [44]	Inoculated soil with *M. tuberculosis, M. bovis* and *M. canetti*; survival of the distinct mycobacteria in the soil for 12 months	Soil samples culturable; all types of mycobacteria found in soil after 12 months; mice inoculated with contaminated soil all developed granulomas; control mice did not grow *M. tuberculosis*
TSHILOMBO, 2015 [46]	Prospective *in vitro* study; mixed 20 mL of 10^6 CFU·mL^{-1} *M. smegmatis* with 125 mg of sterile dust; air sampling was conducted pre- and post-re-aerosolisation, and the number of CFUs was measured based on plate count	*M. smegmatis* survived in dust for 19 days and could be successfully re-aerosolised, remaining viable
VELAYATI, 2015 [47]	1500 random samples of soil and water	M. *tuberculosis* isolated from 1% of soil samples and 10% of water samples; persisted for 9 months

M. bovis: Mycobacterium bovis; M. canetti: Mycobacterium canetti; M. smegmatis: Mycobacterium smegmatis.

TABLE 3

Critical knowledge gaps in tuberculosis (TB) epidemiology and the potential use of environmental sampling for *Mycobacterium tuberculosis*

Epidemiological areas related to TB	Potential use of environmental sampling and ideas for further investigation
Where does TB transmission occur?	Characterising *M. tuberculosis* abundance in various congregate settings, including public transit, schools, churches, restaurants, bars and workplaces
How much interindividual heterogeneity is there in community transmission?	Compare abundance of *M. tuberculosis* genotypes across settings
How does dose of exposure influence risk of TB progression?	Compare abundance of *M. tuberculosis* in household with risk of progression among household contacts
How long are individuals infectious prior to detection?	Whole genome sequencing of environmental samples and comparing dates of detection in the environment with date of diagnosis
How does an individual's infectiousness change over time?	Assess rates of environmental contamination with specific isolates, identified by whole genome sequencing, over time
Was an intervention successful at reducing transmission?	Assess changes in *M. tuberculosis* abundance in congregate settings (*e.g.* hospitals, prisons, mines, schools] over time
Was an individual infectious when a potential exposure occurred?	Conduct environmental sampling at sites of potential exposure (*e.g.* hospital rooms]