PNAS COMMENTARY OPEN ACCESS

The Titanic question in TB control: Should we worry about the bummock?

Sarah M. Fortune[a,1]

In the study of sea ice, the portion of an iceberg that sits below the water is known as the bummock, counterpart to the hummock, the visible crest of ice. As amateur oceanographers know, the bummock makes up ~90% of an iceberg's mass, lurking out of sight but capable of great impact—such as sinking the Titanic. The iceberg analogy is uncomfortably accurate in describing our current understanding of *Mycobacterium tuberculosis* (Mtb) infection, disease, and transmission risk. As the field develops higher-resolution tools to study tuberculosis (TB) in humans, we increasingly find what we don't know about TB's bummock—individuals infected with Mtb but who would not meet current clinical criteria for active TB disease but in whom there is emerging evidence of Mtb respiratory carriage, raising alarm bells for TB control.

The most recent challenge to domain dogma comes from Patterson et al. in the current issue of the *Proceedings* (1). These researchers used recently developed approaches to respiratory aerosol sampling to assess Mtb respiratory tract carriage in people presenting to TB clinics outside of Cape Town, South Africa, which has one of the highest prevalences of TB in the world. The research team found near ubiquitous carriage of viable, albeit metabolically quiescent, Mtb in the respiratory aerosols of these people. Surprisingly, they found high rates of carriage in people who were not ultimately diagnosed with TB using clinical diagnostics and persistent carriage after treatment. However, carriage did resolve with the resolution of symptoms, indicating biologic logic to the presence Mtb in the respiratory tract, but challenging our understanding of TB disease and transmission risk.

The definition of TB disease used to seem straightforward. TB disease or active TB, the hummock in our iceberg analogy, is diagnosed in individuals with signs of disease (objective findings such as cough, fever, weight loss, etc...) and diagnostic evidence of Mtb infection. These are the cases that we invoke when we say that in 2022, 10.6 million people had active TB (2). When health systems take on TB, the priority is finding and treating people with active disease. This makes sense; in the absence of treatment, ~50% of these patients will die (3). Moreover, rapid detection and treatment of active TB align with both intuitive and data-driven understandings of Mtb transmission in which the highest risk occurs where the patient is coughing has cavitary disease and heavily smear-positive sputum (4).

There is ambiguity in our understanding of transmission, however. Tracking Mtb transmission is complicated by the fact that most people infected with Mtb do not develop active disease and even if they do, it can take years for symptoms to emerge. Transmission may be tracked via incident disease or using guinea pig infection, but more often, it is inferred through the acquisition of an immune response to Mtb in close contact as measured by skin test tuberculin skin test (TST) or IFNγ release assay (IGRA) (4). However, new technologies are changing our understanding of these tests. For example, serologic screening was applied to people who had high Mtb exposure but did not seem to become Mtb infected as measured by TST or IGRA (5). A substantial minority of highly exposed, TST or IGRA-negative individuals have mounted an adaptive immune response to Mtb that is qualitatively distinct--not dominated by IFNγ production–and thus missed by conventional testing. Interestingly, it is possible that the intensity of exposure influences whether a contact develops TB disease and/or the quality of the contact's immune response (6). Thus, the use of TST/IGRA as a measure of transmission could systematically bias transmission studies toward over-estimating the importance of index cases expelling high numbers of bacteria (7).

Considering this type of bias is important because our understanding of TB disease is also evolving as better tools are applied in human populations. High-sensitivity diagnostics like GeneXpert are being applied in community and population-level screening studies (8). Stunningly, in some studies, up to 80% of people with evidence of TB in their sputum by GeneXpert have no reported clinical symptoms, meaning that they do not report being sick or even coughing (9). These people would not be identified as TB cases by most health systems. Indeed, it is unclear whether one should call them TB cases. The rates of asymptomatic sputum positivity in household contacts are higher than the number of people expected to progress to overt TB disease (8), suggesting that some asymptomatic infection could either be a stable state or individuals may move bidirectionally in and out of it.

Here, Patterson and colleagues define a different manifestation of Mtb infection, which forces us to further reconsider disease states and the implications for Mtb transmission. This team previously established the tools to collect respiratory aerosols from people (10). Respiratory aerosol is not sputum, but rather the little bursts of airway lining fluid exhaled upon cough but also less forceful ventilatory maneuvers like deep breathing (11). Aerosol collection was paired with air capture to enrich exhaled organisms and fluorescent

Author affiliations: [a]Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115

Author contributions: S.M.F. wrote the paper.

The author declares no competing interest.

Copyright © 2024 the Author(s). Published by PNAS. This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

See companion article, "Aerosolization of viable *Mycobacterium tuberculosis* bacilli by tuberculosis clinic attendees independent of sputum-Xpert Ultra status," 10.1073/pnas.2314813121.

[1]Email: sfortune@hsph.harvard.edu.

Published March 25, 2024.

trehalose-based labeling active bacterial cell wall synthesis to identify viable Mtb (12). For a visual of the complexity of the experimental setup, think Tardis not sputum cup. These tools were applied to people presenting with symptoms to TB clinics, some of whom were diagnosed with TB by conventional diagnostics (GeneXpert and/or culture) and some of whom were not (1). The team found near ubiquitous aerosol carriage of viable Mtb in both cases and putative controls. Importantly, aerosol positivity declined over time corresponding to a reduction in symptoms, but also very importantly it did not change with treatment. The prevalence of carriage was higher than that in studies of aerosol sampling in a similar population which used culture as a readout (13). However, the current study rigorously addresses reasonable alternative hypotheses including batch effect, contamination, and nontuberculous mycobacterial carriage.

The iceberg analogy is uncomfortably accurate in describing our current understanding of tuberculosis (TB) infection, disease and transmission risk.

Are these findings biological plausible? One of the challenging aspects of these data is that treatment did not clear carriage. Previous work has shown that appropriate antibiotic treatment of active TB disease leads to clearance of acid-fast bacteria from the sputum, resolution of clinical symptoms, and rapidly stops transmission in the experimental human to guinea pig model (14). However, the organisms that the team identified in aerosols were largely a stealth form of Mtb. They were detected through trehalose incorporation and thus were viable, but they were not acid-fast and so would be missed by smear microscopy and did not grow well enough to form a colony on a plate (nonculturable). Viable, nonculturable (VNC) bacteria are thought to be in a poorly understood but well-described stress resistant state, and in TB, VNC cells have been implicated in functional drug tolerance (15). These data are consistent with other recent data suggesting that antibiotic cure does not mean complete eradication of infection. For example, a study of patients after drug treatment using Positron emission tomography–computed tomography (PET-CT) imaging revealed persistent foci of inflammation in the lungs, and some patients had Mtb mRNA in the airways suggestive of persistent viable bacteria (16).

If plausible, we next must ask whether zombie Mtb in respiratory aerosols is important? Obviously, respiratory carriage suggests transmissibility, which poses a problem where current efforts to limit transmission focus on classic active TB (17). So is there unexplained Mtb transmission at a population level? Investigators have begun to address this question using bacterial population genomics. Genomic reconstruction of population level transmission chains can identify an index TB case in a minority of cases—fewer than 10% in some places (18). While there are many reasons index cases are missed, potential explanations include they don't come in (asymptomatic) or their sputum culture doesn't grow (nonculturable). Of course, sick people with large numbers of bacteria in their sputum might be the most effective transmitters but a huge number of individuals without classic evidence of active TB, but transmitting at low levels could be the Titanic-threatening bummock to TB control (19).

A changing understanding of TB disease and transmission risk raises new questions. If we believe transmission is driven primarily by the overtly unwell who meet conventional diagnostic standards, the goals of better treatment of the individual and limiting transmission are well-aligned—and make the case for faster diagnosis and faster implementation of the appropriate antibiotic regimen (17). But what should we do with a symptomatic person with VNC bacteria in their respiratory secretions? What about a clinically well person with culture or molecular evidence of Mtb in their sputum? Should we be exploring new drug regimens and if so, with what endpoints (20)?

There is reason for optimism in the iceberg analogy. Ships today are not sunk by icebergs thanks to better monitoring systems and ship design. We have the tools to make analogous advances in our understanding and control of TB transmission. However, this will require enough resources to free the TB field to think outside the box without compromising clinical care. The demise of the Titanic captured outsized attention because of the wealth and prominence of many of its victims. We must remember the equal value of investment into the lives of the global poor for whom TB is an ever-present threat.

1. B. Patterson *et al.*, Aerosolization of viable *Mycobacterium tuberculosis* bacilli by tuberculosis clinic attendees independent of sputum-Xpert Ultra status. *Proc. Natl. Acad. Sci. U.S.A.* **121**, e2314813121 (2024).
2. S. Bagcchi, WHO's global tuberculosis report 2022. *Lancet Microb.* **4**, e20 (2023).
3. E. W. Tiemersma, M. J. van der Werf, M. W. Borgdorff, B. G. Williams, N. J. Nagelkerke, Natural history of tuberculosis: Duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: A systematic review. *PLoS One* **6**, e17601 (2011).
4. G. B. Migliori *et al.*, Reducing tuberculosis transmission: A consensus document from the World Health Organization Regional Office for Europe. *Eur. Respir. J.* **53**, 1900391 (2019).
5. J. D. Simmons *et al.*, Immunological mechanisms of human resistance to persistent Mycobacterium tuberculosis infection. *Nat. Rev. Immunol.* **18**, 575–589 (2018).
6. E. C. Jones-Lopez *et al.*, Cough aerosol cultures of Mycobacterium tuberculosis: Insights on TST / IGRA discordance and transmission dynamics. *PLoS One* **10**, e0138358 (2015).
7. C. Acuna-Villaorduna *et al.*, Cough-aerosol cultures of Mycobacterium tuberculosis in the prediction of outcomes after exposure. A household contact study in Brazil. *PLoS One* **13**, e0206384 (2018).
8. B. Frascella *et al.*, Subclinical tuberculosis disease-A review and analysis of prevalence surveys to inform definitions, burden, associations, and screening methodology. *Clin. Infect. Dis.* **73**, e830–e841 (2021).
9. I. Govender *et al.*, Prevalence of Mycobacterium tuberculosis in sputum and reported symptoms among clinic attendees compared with a community survey in rural South Africa. *Clin. Infect. Dis.* **75**, 314–322 (2022).
10. R. Wood *et al.*, Real-time investigation of tuberculosis transmission: Developing the respiratory aerosol sampling chamber (RASC). *PLoS One* **11**, e0146658 (2016).
11. B. Patterson *et al.*, Cough-independent production of viable Mycobacterium tuberculosis in bioaerosol. *Tuberculosis (Edinb.)* **126**, 102038 (2021).
12. R. Dinkele *et al.*, Capture and visualization of live Mycobacterium tuberculosis bacilli from tuberculosis patient bioaerosols. *PLoS Pathog.* **17**, e1009262 (2021).
13. G. Theron *et al.*, Bacterial and host determinants of cough aerosol culture positivity in patients with drug-resistant versus drug-susceptible tuberculosis. *Nat. Med.* **26**, 1435–1443 (2020).
14. A. S. Dharmadhikari *et al.*, Rapid impact of effective treatment on transmission of multidrug-resistant tuberculosis. *Int. J. Tuberc. Lung. Dis.* **18**, 1019–1025 (2014).
15. L. A. Pazos-Rojas *et al.*, The viable but non-culturable (VBNC) state, a poorly explored aspect of beneficial bacteria. *Microorganisms* **12**, 39 (2023).
16. S. T. Malherbe *et al.*, Persisting positron emission tomography lesion activity and Mycobacterium tuberculosis mRNA after tuberculosis cure. *Nat. Med.* **22**, 1094–1100 (2016).
17. C. M. Yuen *et al.*, Turning off the tap: Stopping tuberculosis transmission through active case-finding and prompt effective treatment. *Lancet* **386**, 2334–2343 (2015).
18. L. Martinez *et al.*, Transmission of Mycobacterium Tuberculosis in households and the community: A systematic review and meta-analysis. *Am. J. Epidemiol.* **185**, 1327–1339 (2017).
19. E. A. Kendall, S. Shrestha, D. W. Dowdy, The epidemiological importance of subclinical tuberculosis. A critical reappraisal. *Am. J. Respir. Crit. Care Med.* **203**, 168–174 (2021).
20. H. Esmail, L. Macpherson, A. K. Coussens, R. Houben, Mind the gap–Managing tuberculosis across the disease spectrum. *EBioMedicine* **78**, 103928 (2022).