important limitation of this study that the authors had little control over is the heterogeneity of the study population. Fifty-four percent of subjects had diaphragm pacing, 81% had a tracheostomy, and 48% were mechanically ventilated during HUT testing. It is likely that the interactions between breathing and the autonomic control of the cardiovascular system in individuals who are breathing spontaneously differ from those requiring ventilatory assistance. A standardized clinical management of individuals with CCHS and data collection across centers may be the only approach to overcome the limitations imposed by retrospective single-center studies. ■

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Tuberculosis: First in Flight

The ongoing coronavirus disease (COVID-19) pandemic has focused global attention on the airborne spread of infection, but tuberculosis (TB) was the first disease in which airborne transmission was convincingly demonstrated after many decades of doubt. The first line of solid evidence was presented in a series of studies documenting remote transmission from humans to guinea pigs (1), followed by reports of human-to-human airborne transmission in closed environments, such as ships (2). More recently, household contact studies have provided valuable evidence regarding transmission dynamics (3–5). Nevertheless, there is now substantial evidence that most transmission occurs outside the household (6, 7).

South Africa faces a devastating TB epidemic that is driven by widespread community transmission of *Mycobacterium tuberculosis*. A recent national survey found a prevalence of 852 cases per 100,000 individuals (8). The annual risk of infection is highest among adolescents and young adults, who often travel and have extensive social networks with multifamily households (9, 10). Schools may represent venues of amplified *M. tuberculosis* transmission (11) and, if so, would provide a logical intervention point for mass screening.

In this issue of the *Journal*, Bunyasi and colleagues (pp. 350–356) provide a detailed look at school environments in Worcester, South Africa, combining novel genomic DNA sampling with ambient carbon dioxide (CO_2) concentration measurements (as a marker of ventilation) and TB symptom screening (12). High-volume air filtration was performed for a median of 40 minutes in 72 classrooms and assayed by droplet digital PCR (ddPCR) for *M. tuberculosis* DNA. Positive DNA samples were found in 18% and 10% of samples in classrooms and clinics, respectively.

The documentation of airborne *M. tuberculosis* in classrooms from this study is an important advance, especially considering the brief period of air sampling. However, the value of estimating an average risk of an occupant inhaling one *M. tuberculosis* DNA copy as an outcome measure is unclear. We know of no data suggesting that the inhalation of one *M. tuberculosis* DNA copy equates to a transmission event or that the process of acquiring

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infection is a cumulative event. We do not know if very limited exposure events occurring weeks or months apart can lead to infection compared with exposure to plumes of aerosol at higher concentrations. Animal experimental data suggest that the latter is more likely (13). The risk of infection (i.e., transmission) is not directly observed or measured in this study; therefore, extrapolation of risk is beyond the results.

Two studies have linked cough aerosol cultures with conversions of both tuberculin skin tests and IFN-γ release assays in exposed household contacts (3, 14). Although they provided valuable data on the magnitudes and particle sizes of the aerosols, the methods were laborious. Fortunately, there is emerging evidence that both subclinical disease and transmission are associated with *M. tuberculosis* in exhaled breath collected by face mask sampling and quantitative PCR (15–16). Although PCR methods can detect the presence of airborne genomic material of *M. tuberculosis,* we do not know if this genomic material is from viable or even whole bacilli. All populations of bacilli include a mix of viable, dead, and injured cells. Even though there may be phenotypes that are not culturable but can be resuscitated, culture or growth in a host currently remains the only certain way of demonstrating viability (17). Aerosolization places considerable stress on bacilli because of rapid fluxes in mechanical forces, temperature, and desiccation, and air sampling methods may also damage pathogens (18). The research challenge will be to find molecular markers of viability that can both withstand these stressors and provide links to transmission.

In this study, ambient CO_2 concentrations were almost double in classrooms compared with clinics (886 ppm vs. 490 ppm) (12). Notably, 40% of classrooms were classified as "high-risk spaces" signifying poor ventilation (defined as a peak $CO_2 \geqslant$1,000 ppm). Comparatively, 100% of clinics were classified as "low-risk." Poor ventilation in schools is not unique to South Africa. The median CO_2 concentration in 60 schools in Scotland was 1,086 ppm (19) and 1,286 ppm in 120 classrooms in Texas (20). Bunyasi and colleagues compared the ventilation in schools to the "WHO-recommended 12 air changes per hour" (12). However, this World Health Organization and CDC ventilation rate is recommended for TB infection control in hospitals (i.e., for airborne infection isolation rooms for TB suspects or cases). Although ventilation appears to be poor in schools worldwide, it would be unrealistic in terms of both financial and energy expenditure to achieve this target. However, innovative architects are now designing buildings that offer more natural ventilation (21).

The COVID-19 pandemic has highlighted the importance of asymptomatic transmission. Similarly, transmission of TB also likely occurs from asymptomatic persons. Recent TB prevalence surveys, from South Africa and elsewhere, indicate that more than half of TB cases are asymptomatic (8). Bunyasi and colleagues (12) found no association between *M. tuberculosis* DNA detected by ddPCR and TB-related symptoms or the presence of confirmed TB. This may be due to the scarcity of participants with symptoms (90/1,836, 4.9%) or diagnosed with TB (1/1,836, 0.05%). Importantly, TB in this study was reliant on triage screening of students experiencing cough or overt symptoms. The authors propose that asymptomatic cases who were not detected as part of this screening algorithm may have contributed to the levels of *M. tuberculosis* DNA copies found in these school settings.

Although this study suggests that schools may be foci of transmission, it is not immediately clear how ddPCR air screening could "increase the efficiency of mass TB screening among adolescents," as the authors suggest. Although a "pooled aerosol sample" could theoretically identify a need to screen students for symptoms, this approach may be futile if air sampling detects asymptomatic disease. Furthermore, sputa specimens are unlikely produced at such an early stage of disease. However, exhaled breath could be captured via face mask sampling, as large numbers of students could wear masks while attending class (16). This strategy may have the potential to identify "superspreaders," as there is a 3–5 log range of pathogens in both exhaled breath and cough aerosols. Like all thought-provoking studies, this work raises more questions than answers.

This is an exciting time for research in infectious aerosols. COVID-19 has stimulated interest in infectious diseases and aerosol science (18), but it is unfortunate that the "slow pandemic of TB" has not received similar attention over past decades. Innovative approaches to the study of *M. tuberculosis* transmission dynamics, such as those in this study from the *Journal*, are urgently needed and have the potential to prevent airborne diseases, both old and new. ■

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