

Seminal *Mycobacterium Tuberculosis in vivo* Transmission Studies: Reanalysis Using Probabilistic Modelling

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Abstract

Much of our current knowledge of *Mycobacterium tuberculosis* (MTB) transmission originates from seminal human-to-guinea pig *in vivo* studies, carried out in the 1950s. Similar methodology has been used to investigate human immunodeficiency virus (HIV) co-infection and multidrug resistant TB. However, all these studies have had to reconcile the need to use high facility ventilation rates in order to decrease risks of human-to-human infection while demonstrating human-to-guinea pig transmission. While these studies demonstrate tuberculosis (TB) contagion can be airborne they also estimated extremely low infectivity of TB cases. However, calculated infectivity was based on a theoretical concept of quantal infection and assumed that the guinea pig model was 100% sensitivity for the remote detection of viable TB organisms in highly diluted air exhausted from the facility. High facility ventilation markedly decreases the probability of a successful guinea pig infection by both dilution of the exhaled breath and decreasing the proportion of air sampled by guinea pigs. In this study, we used a new mathematical model based on Poisson distribution and previous guinea pig experimental data to quantify a more realistic estimate of the number of infective organisms required to produce a successful infection for exposed guinea pigs in the *in vivo* studies. Furthermore, we explored the probability of exposed guinea pigs acquiring infection in these studies. We found that the *in vivo* studies to date were underestimated to demonstrate transmission derived from any but the most productive infectious cases. All four *in vivo* studies have remarkably low probability of infection of exposed guinea pigs due to either high ventilation rates or insensitive mathematical model used in these studies. Therefore, our analysis would suggest that the production of infective organisms by TB cases might have been markedly underestimated. This reassessment of the infectivity of guinea pigs is compatible with recent findings of very high numbers of TB genomes present in health care environments and the very diverse distribution of TB strains present in highly endemic settings which indicates a multiplicity of infective sources.

Keywords: Guinea pig; Tuberculosis; Probability; Threshold level; Infection

Introduction

Our current knowledge of the nature of *Mycobacterium tuberculosis* (MTB) transmission owes much to the *in vivo* guinea pig model of infection. In 1882 Koch, identified the causative agent responsible for consumption and demonstrated tuberculosis (TB) transmission to guinea pigs exposed to dried sputum from infected patients. Consequently, emphasis was placed on avoidance of desiccated secretions in dust and fomites, which was then considered the principal mode of transmission [1]. In the 1930's Wells, expanding on the earlier work of Flugge [2], proposed that TB transmission was the result of inhalation of infected droplet nuclei exhaled or coughed by a TB case [3]. In the 1950's, Riley and colleagues performed seminal experiments, which confirmed airborne transmission from TB cases to guinea pigs, supporting Wells theory [4]. Riley observed that guinea pig infections in his model occurred only as rare singleton lesions. He postulated that guinea pig TB infection was quantal, lesions followed a Poisson distribution ($1 - e^{-\lambda}$) and that TB case quanta production was as low as 1 - 2 per day. A Poisson distribution following a quantum exposure (expected value $\lambda=1$) would result in 36.8% with no lesions and 63.2% with lesions (36.8% with a single lesion and 26.4% with

multiple lesions). However, the Poisson distribution of lesions remained theoretical, as multiple lesions were not observed.

Riley et al. [5], Escombe et al. [6] and Dharmadhikari et al. [7] subsequently used similar guinea pig methodology to investigate differing infectiousness of human clinical populations. High facility ventilation makes the back calculation of TB case infectious quanta production very sensitive to sampling efficiency. However, quanta concentrations calculated from individual guinea pigs exposed to as little as 2 - 3 milliliters of patient-derived air per hour were used for cross-study quanta production comparisons [3,4].

All four guinea pig studies A [4], B [5], C [6] and D [7] identified only a minority of efficient transmitters or super-spreaders. While super-spreaders may be responsible for explosive outbreaks of TB in low prevalence settings [8], in endemic settings, molecular epidemiology indicates more generalized TB transmission [9]. Additionally, variable human [10] and guinea pig [7] immunological responses to TB infection that are predictive of subsequent reversion and TB progression risk may be more compatible with quantitative rather than quantal exposure. Recent advances in molecular technologies have enabled direct measurement of airborne TB patient derived particles that are several orders of magnitude greater than those estimated from guinea pig experiments [4,11,12]. However, protagonists of the *in vivo* guinea pig model have questioned the

clinical relevance of these large numbers of airborne TB genomes [13] and newer viability assays are awaited.

In this study we hypothesized that the guinea pig sampling system may lack sensitivity to identify any but the highest levels of infectivity. In order to re-explore the quantitative findings from these *in vivo* transmission studies, we developed a steady state mathematical model to estimate the likely source-patient airborne infectious particle (viable with potential for TB infection) production. The model incorporated relevant parameters that likely decrease guinea pig sampling efficiency including ventilation, sampling fraction and alveolar deposition proportion.

***In vivo* Study Methodology**

The data derived from four *in vivo* studies A [4], B [5], C [6] and D [7] is shown in Table 1. Patients (range 6-8) with untreated, treated, drug resistant TB disease or complications of treated TB disease were housed in facilities with closed circuit ventilation systems which were supplied with disease free ventilation at a rate Q (range 8.7×10^6 L/day to 57.6×10^6 L/day). Patient facilities were either communal wards, two bedded or single roomed facilities with all exhausted air piped to a guinea pig exposure chamber. Within each exposure chamber, S number of guinea pigs (range 120-362) were housed in communal cages through which the exhaled air from the patient facility was passed. The guinea pig cages were designed such that excreta fell through the floors in order to minimize chances of cross infection between animals by means of contaminated bedding. The temperature and relative humidity of the guinea pig cage environments were closely controlled. Each guinea pig was estimated to breathe approximately $p=10$ L/h. Regular tuberculosis skin testing identified G infections (range 63-213) in the guinea pigs during time t (range 112-730 days) with TB diagnosis confirmed by histology and or culture at subsequent autopsy. Uninfected substitute guinea pigs constantly replaced infected or euthanized animals in order to maintain an average number S of susceptible animals. Most newly infected animals had evidence of only one infectious focus in their lungs, supporting the study hypothesis that TB entered the body through a single invasive site in the respiratory system and the assumption that the number of infections reflected the number of airborne infectious particles inhaled.

Two studies A and B used proportionality to estimate the concentration of airborne infectious particles in the exhausted ward air from the number of infected guinea pigs G divided by the total air volume V (range 3.8×10^6 L to 27.3×10^6 L) breathed by all susceptible guinea pigs during the period of t days of the study. The total airborne infectious particles produced by TB patients were calculated from the total facility ventilation Q divided by calculated volume per infected guinea pig V/G containing a single airborne infectious particle. The calculated number of airborne infectious particles causing infection was considered to be identical to the number of airborne infectious particles inhaled by the guinea pigs and the concentration of such particles in the patient ward was identical to that of the inhaled guinea pig air.

One study, C, incorporated calculation of airborne infectious particles using a Poisson distribution (Wells-Riley model), but similar to studies A and B, assumed that the inhaled number of airborne infectious particles by guinea pigs was identical to the number of airborne infectious particles causing infection, and that there were no losses between exhalation from patients and inhalation by guinea pigs. The fourth study, D, focused predominantly on a new finding that

some guinea pigs were observed to revert their tuberculin reactions after infection with drug resistant TB. This observation led to a proposal that drug resistant strains were less virulent than the drug sensitive strains reported in the prior studies A, B and C. However, the number of susceptible guinea pigs (S) exposed and the number infected or dying of tuberculosis (G) after exposure to TB cases (I) was reported. Facility ventilation was reported only as 12 air changes per hour, which, in conjunction with published facility dimensions [14], allowed facility ventilation (Q) to be calculated.

For each of the studies A to D, we estimated the probability of finding the published infection rates using Poisson distribution approach with sensitivity analysis of TB infectious doses of between 10 to 50 colony forming units as reported in a review of the guinea pig as an infectious model [15]. We then went on to estimate the mean patient production rate of airborne infectious particles necessary to reproduce the reported guinea pig infection rates of each study as discussed below.

Mathematical Modelling and Simulation Results

Using guinea pig experimental data in the four *in vivo* studies A [4], B [5], C [6] and D [7] (Table 1), we explore the probability of exposed guinea pigs acquiring infection in these studies by application of the mathematical model developed in Issarow et al. [16] at steady state conditions. Furthermore, we quantify the number of surviving airborne infectious particles (infective organisms) required to reach the alveolar to induce infection for exposed guinea pigs in the *in vivo* studies. The mathematical model used in this study for the *in vivo* studies reanalysis is demonstrated as:

$$P(\psi | I, \beta, \theta, p, Q, t) = 1 - e^{-I\beta\theta pt/Q}$$

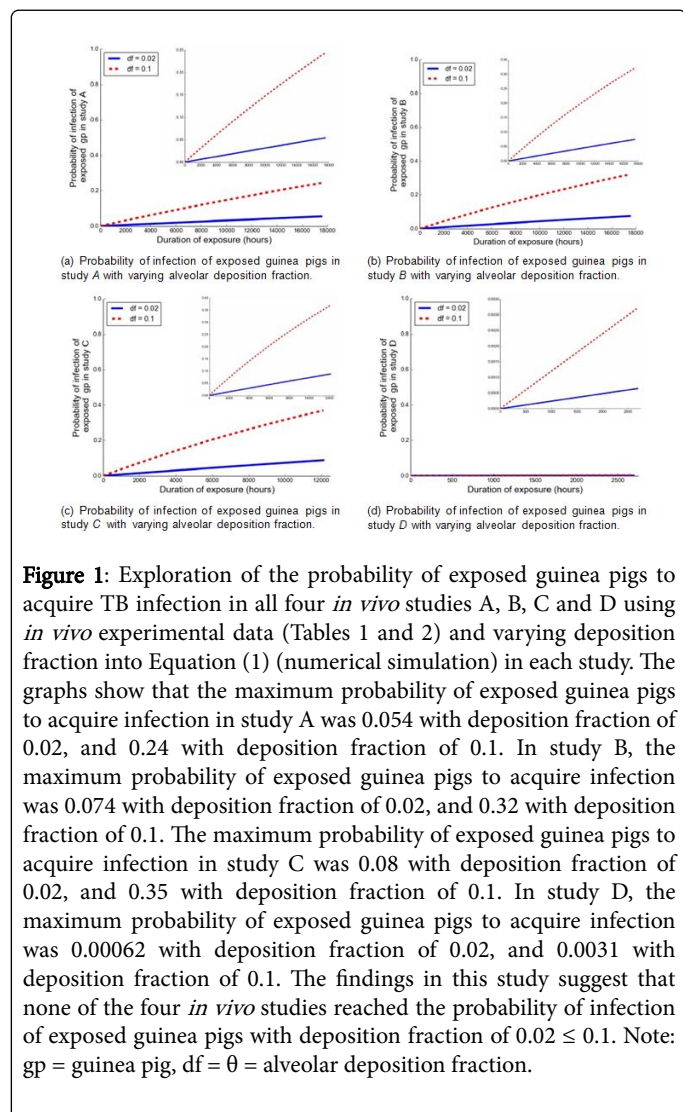
where $P(\psi | I, \beta, \theta, p, Q, t)$ denotes the probability of infection of exposed guinea pigs, ψ is whether or not infection occurred given that TB infection conditions are fulfilled, Q is the ventilation rate (L/h), p is the guinea pig breathing rate (L/h), I is the number of infectious individuals in the space, θ is the alveolar deposition fraction, t is the duration of exposure (h) and β ($\gamma_{total} - \mu_{loss}$) is the surviving airborne infectious particles per unit time (particles/h) that reach the alveolar to establish infection. γ_{total} is the total airborne infectious particle production rate and μ_{loss} is the mortality rate of airborne infectious particles before reaching the host infection site.

This mathematical model (Equation (1)) was preferred over others as it incorporates all significant parameters, including alveolar deposition fraction, bacterial mortality (loss) and survival rates, in the prediction of airborne infectious disease risks, without assumption of well mixed airspace and equilibrium conditions [16]. In reality, airspace can never be well mixed because regions of high and low contaminant concentrations exist simultaneously within the same airspace, with the highest concentrations usually occurring close to the contaminant source [16]. Furthermore, equilibrium conditions cannot be achieved consistently as the climate changes. From biological point of view, not all airborne infectious particles reach the target infection site of the host to establish infection, some deposit in the upper airways without inducing infection [17]. In our model, we take into consideration that alveolar deposition fraction determines the number and concentration of surviving airborne infectious particles that reach the alveolar to establish infection [16]. Therefore, β and θ in Equation (1) are considered as two different variable parameters. Separation of these parameters makes the model more flexible and sensitive than the

prior mathematical models in the prediction of airborne infectious disease risks, such as TB [16].

The *in vivo* studies claimed that they used Poisson distribution approach (Wells-Riley model) in their studies to predict the number of infective organisms required to induce infection and the probability of exposed guinea pigs acquiring infection. However, they didn't demonstrate mathematically or schematically how they used the Wells-Riley model in their studies to estimate the number of infective organisms and the probability of exposed guinea pigs acquiring infection.

In this study, using *in vivo* studies experimental data into Equation (1), we explore the probability of exposed guinea pigs acquiring infection and quantify the number of surviving airborne infectious particles required to reach the alveolar to induce infection in these studies. Since *in vivo* studies used a steady state mathematical model (Wells-Riley model) to predict the number of infective organisms required to establish infection and the probability of exposed guinea pigs to acquire TB infection, Equation (1) should prove a more accurate prediction of the probability of exposed guinea pigs acquiring infection in these studies.



Furthermore, this equation should estimate a more accurate number of surviving airborne infectious particles required to reach the alveolar to establish infection for exposed guinea pigs. In the application of Equation (1) to guinea pig experimental data, we made the following assumptions: first, there is no immune control of infection i.e., a single or more than one airborne infectious particle arriving at the alveolus will result in a guinea pig infection if the probability of exposed guinea pigs acquiring infection is attained; second, the experimental conditions were such that there was no cross infection between guinea pigs; and third, the infective TB dose required to establish infection in guinea pigs varies between 10 and 50 colony forming units resulting in alveolar deposition fractions between 0.02 and 0.1 [15].

Here, we explore the probability of exposed guinea pigs acquiring infection by using *in vivo* experimental data in studies A [4], B [5], C [6] and D [7] (Tables 1 for I, t, Q, p and 2 for β) into Equation (1), while varying alveolar deposition fraction ($\theta=0.02, \theta=0.1$). We use 0.9(0.8 - 1) as the probability of exposed guinea pigs acquiring TB infection, with the probability of 1 being the saturation point (threshold level) where infection will occur. Simulating Equation (1) numerically, we found that the maximum probability of exposed guinea pigs acquiring infection in the four *in vivo* studies A [4], B [5], C [6] and D [7] was 0.054, 0.074, 0.08 and 0.00062, respectively, with alveolar deposition fraction of 0.02 (Figures 1a-1d).

Furthermore, with alveolar deposition fraction of 0.1, the maximum probability of exposed guinea pigs to acquire infection in these studies was 0.24, 0.32, 0.35 and 0.0031, respectively (Figures 1a-1d). All four *in vivo* studies A [4], B [5], C [6] and D [7] seem to have remarkably low probability of infection of exposed guinea pigs due to either high ventilation rates, which dilute the concentration of airborne infectious particles or insensitive mathematical model used in these studies.

It shows that of all four *in vivo* studies, study D (Figure 1d) had the lowest infection probability of exposed guinea pigs, perhaps because of shorter duration of exposure and higher ventilation rate than the other studies (Table 1). The findings in this study show that the expected probability of exposed guinea pigs to acquire TB infection was not attained in all four *in vivo* studies, implying that their observations might be inaccurate.

The four *in vivo* studies seem to have several issues, including high ventilation rates, which led to remarkably low probability of exposed guinea pigs to acquire infection. Alveolar deposition fraction and surviving airborne infectious particles also seem to have a significant impact on the prediction of exposed guinea pigs acquiring TB infection.

Here, we quantify the number of surviving airborne infectious particles required to attain the threshold level to establish infection for exposed guinea pigs in the four *in vivo* studies by simulating Equation (1) numerically using *in vivo* experimental data (Table 1), varying airborne infectious particles ($\beta = 10, 100, 1000$ particles/h) and alveolar deposition fraction ($\theta = 0.02, 0.1$). We chose these values of airborne infectious particles because the number of surviving particles that reach in the host infection site to induce infection is an expected value and not an exact one [14].

Hence, by simulating Equation (1) numerically using *in vivo* experimental data, varying airborne infectious particles and alveolar

deposition fraction, we determined the threshold level of the surviving airborne infectious particles (β) for exposed guinea pigs to acquire TB infection in each *in vivo* study as follows: For study A, the surviving airborne infectious particles reached the threshold level when $\beta \geq 69$ particles/h with alveolar deposition fraction of 0.02 and $\beta \geq 14$ particles/h with alveolar deposition fraction of 0.1 (Figures 2a and 2b); for study B, the threshold level attained when $\beta \geq 75$ particles/h with alveolar deposition fraction of 0.02 and $\beta \geq 15$ particles/h with alveolar deposition fraction of 0.1 (Figures 2c and 2d); for study C, the threshold level attained when $\beta \geq 341$ particles/h with alveolar deposition fraction of 0.02 and $\beta \geq 69$ with alveolar deposition fraction of 0.1 (Figures 3a and 3b); for study D, the threshold level reached when $\beta > 1000$ particles/h with alveolar deposition fraction of 0.02 and $\beta \geq 596$ particles/h with alveolar deposition fraction of 0.1 (Figures 3c and 3d).

infection when β was greater than 10 particles/h, and none of the exposed guinea pigs infected in these studies when $\beta \leq 10$ particles/h (Figures 2 and 3).

Unfortunately, all four *in vivo* studies postulated that exposed guinea pigs were infected with less than 10 particles/h [4-7], and concluded that TB infection is induced by a single infective organism [4]. This implies that their observations might be inaccurate and they lacked sensitivity in their experimental studies, perhaps because of using a mathematical model with implausible assumptions and some limitations.

Because of shorter duration of exposure and higher ventilation rate than other *in vivo* studies, the surviving airborne infectious particles in study D failed to reach the threshold level at the maximum number of airborne infectious particles of 1000 particles/h with alveolar deposition fraction of 0.02 (Figure 3c).

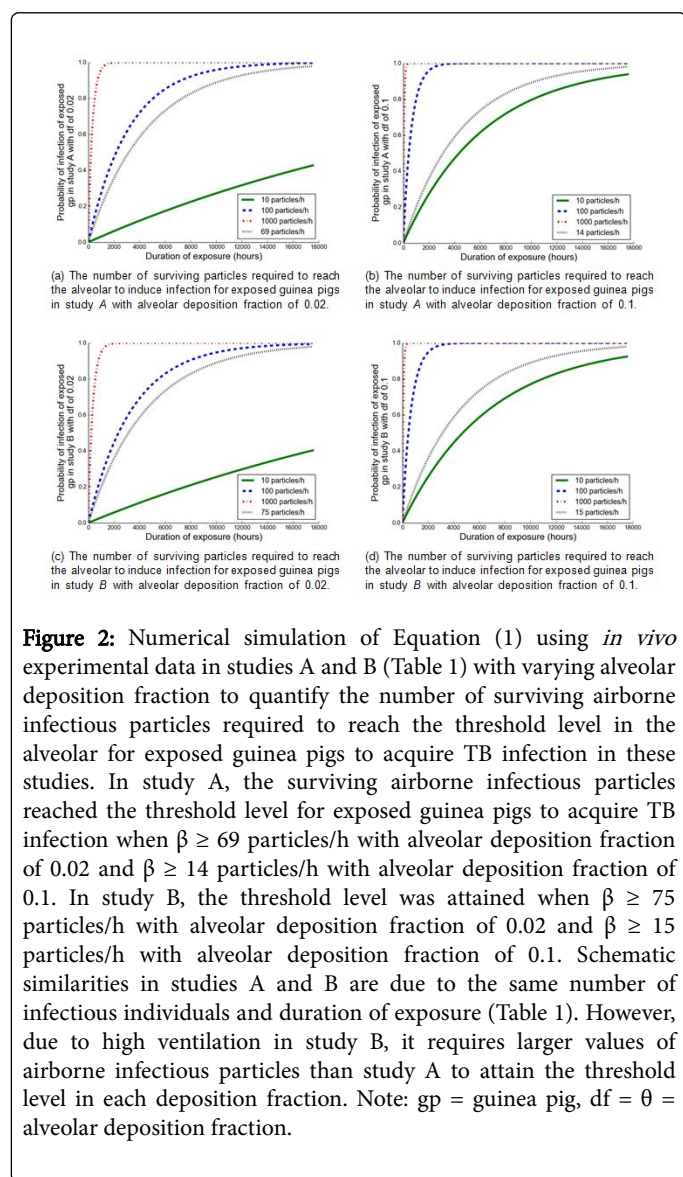


Figure 2: Numerical simulation of Equation (1) using *in vivo* experimental data in studies A and B (Table 1) with varying alveolar deposition fraction to quantify the number of surviving airborne infectious particles required to reach the threshold level in the alveolar for exposed guinea pigs to acquire TB infection in these studies. In study A, the surviving airborne infectious particles reached the threshold level for exposed guinea pigs to acquire TB infection when $\beta \geq 69$ particles/h with alveolar deposition fraction of 0.02 and $\beta \geq 14$ particles/h with alveolar deposition fraction of 0.1. In study B, the threshold level was attained when $\beta \geq 75$ particles/h with alveolar deposition fraction of 0.02 and $\beta \geq 15$ particles/h with alveolar deposition fraction of 0.1. Schematic similarities in studies A and B are due to the same number of infectious individuals and duration of exposure (Table 1). However, due to high ventilation in study B, it requires larger values of airborne infectious particles than study A to attain the threshold level in each deposition fraction. Note: gp = guinea pig, df = θ = alveolar deposition fraction.

The findings in this study show that the surviving airborne infectious particles in the four *in vivo* studies A [4], B [5], C [6] and D [7] reached the threshold level for exposed guinea pigs to acquire TB

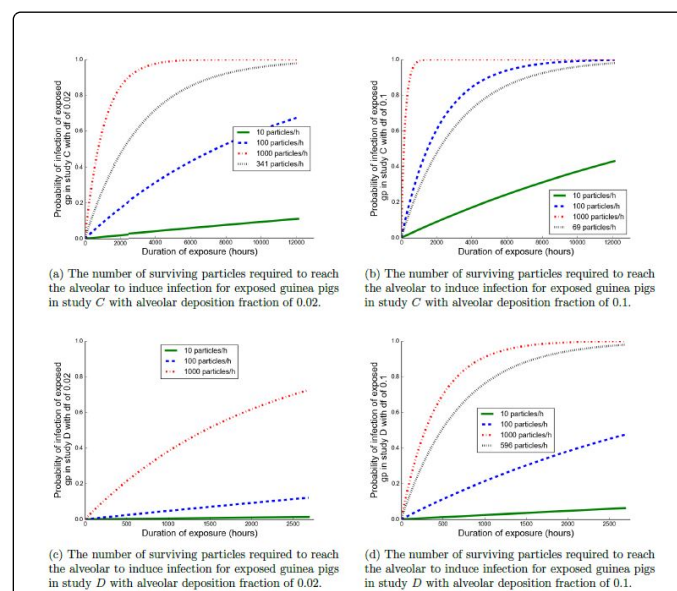


Figure 3: Numerical simulation of Equation (1) using *in vivo* experimental data in studies C and D (Table 1) with varying alveolar deposition fraction to quantify the number of surviving airborne infectious particles required to attain the threshold level in the alveolar for exposed guinea pigs to acquire TB infection in these studies. In study C, the surviving airborne infectious particles attained the threshold level for exposed guinea pigs to acquire infection when $\beta \geq 341$ particles/h with alveolar deposition fraction of 0.02 and $\beta \geq 69$ particles/h with alveolar deposition fraction of 0.1. In study D, the threshold level was reached when $\beta \geq 596$ particles/h with alveolar deposition fraction of 0.1 and $\beta > 1000$ particles/h with alveolar deposition fraction of 0.02. Note: gp = guinea pig, df = θ = alveolar deposition fraction.

Furthermore, we noted that studies C and D (Figure 3) seem to require larger numbers of surviving airborne infectious particles to reach the threshold level for exposed guinea pigs to acquire infection than studies A and B (Figure 2), perhaps because of high ventilation rates and short durations of exposure in the former studies (Table 1). Studies A and B are very similar schematically (Figure 2), probably because of the same number of infectious individuals and duration of exposure in the two *in vivo* experiments (Table 1).

| Study | Duration of exposure days = t | Patient=l(total) | Ventilation m ³ /day guinea =Q, | Susceptible guinea pigs | Guinea pig infected | Guinea pig inhaled =p, m ³ /day | Air sampled proportion |
|-------|-------------------------------|------------------|--|-------------------------|---------------------|--|------------------------|
| A [4] | 730 | 6(88) | 8, 685 | 156 | 71 | 35.3 | 0.0041 |
| B [5] | 730 | 6(130) | 9, 379 | 120 | 63 | 27.2 | 0.0029 |
| C [6] | 505 | 8(97†) | 39, 675 | 292 | 129 | 66.1 | 0.0017 |
| D [7] | 112 | 6(26‡) | 57, 600†† | 362 | 213 | 82 | 0.0014 |

l = maximum patient occupancy at any time, total = total number of patients in each study. Patient characteristics: treatment-naive and treatment failures, untreated, treatment-naive and drug resistant, † All HIV-infected, suspected tuberculosis, treatment-naive, and with treatment complication, ‡ multi-drug resistant tuberculosis and ††12 ACH estimated volume rate. Study A = Wells-Riley, study B = Riley et al., study C = Escombe et al., study D = Dharmadhikari et al.

Table 1: Guinea pig experimental data in studies A, B, C and D.

However, due to higher ventilation rate in study B than that of study A, we show that study B requires a larger number of surviving airborne infectious particles to attain the threshold level than study A. It shows that high ventilation rates in these studies is one the key factors which led to remarkably low probability of exposed guinea pigs to acquire infection. Since ventilation dilutes the concentration of airborne infectious particles, duration of exposure should be high in facilities with high ventilation rate for exposed guinea pigs to acquire infection.

As mentioned earlier, due to high ventilation rates in all four *in vivo* studies, the findings in this study suggest that the minimum number of surviving airborne infectious particles required to reach the threshold level for exposed guinea pigs to acquire infection in each study was greater than 11 particles/h per person with alveolar deposition fraction of 0.02 and greater than 2 particles/h per person with alveolar deposition fraction of 0.1 (Table 2).

| Study | Airborne infectious particles/person in the four <i>in vivo</i> studies | Airborne infectious particles/person required to attain the threshold level for exposed guinea pigs to acquire infection in our study | |
|-------|---|---|-------------------------------------|
| | | Alveola deposition fraction of 0.02 | Alveolar deposition fraction of 0.1 |
| A [4] | 0.167* | 11.5 | 2.333 |
| B [5] | 0.25* | 12.5 | 2.5 |
| C [6] | 1.0250* | 42.625 | 8.625 |
| D [7] | 0.083** | > 166.667 | 99.333 |

*Airborne infectious particles per person from *in vivo* studies, ** Calculated airborne infectious particles per person from *in vivo* study experimental data. Study A = Wells-Riley, study B =Riley et al., study C = Escombe et al., study D = Dharmadhikari et al.

Table 2: Comparison of estimated number of airborne infectious particles (particles/h) per person that required to reach the alveolar for exposed guinea pigs to acquire TB infection in the four *in vivo* studies and our study.

However, none of the four *in vivo* studies quantified these values, implying that their observation might be highly underestimated or false-negative. It shows that the likelihood of TB infection is directly correlated with alveolar deposition fraction, which determines the number of surviving airborne infectious particles that reach the alveolar to establish infection (Figures 2 and 3).

Table 2 compares the number of surviving particles/h per person in the four *in vivo* studies and our study that required to reach the alveolar to establish infection for exposed guinea pigs in the *in vivo* experiments.

The findings in this study suggest that TB infection commences when the surviving airborne infectious particles attain the threshold level in the host infection site, and not from a single infective organism as reported previously.

Discussion

Our reanalysis of *in vivo*experiments of human to guinea pig MTB transmission shows that they lacked sensitivity and indicates that the production of infectious particles by TB cases reported in these studies may have been greatly underestimated. The guinea pig facilities used in each study lack sensitivity due to the fundamental conflict between the need for enhancing transmission from human to animals while being constrained by the need to reduce human to human cross infection. The ventilation of the patient wards increased with each successive study, such that patient exhaled air was diluted by 125-fold, 135-fold, 530-fold and 833-fold in studies A[4], B[5], C[6] and D[7], respectively. Concurrently with the increasing dilution of the ward vented air the sampling proportion determined by the number of guinea pigs used in each study decreased progressively from 0.0041, 0.0029, 0.0017 and 0.0014 in studies A[4], B[5], C[6] and D[7], respectively. Furthermore, we have questioned the assumption that

because only a single bacterium was the source of a single granuloma at the site of infection within a guinea pig that the infective dose for a guinea pig is a single airborne infectious particle. The respiratory anatomy of mammals has evolved to filter environmental threats in order to limit access to the vulnerable alveolar bed. The infective TB dose required to establish infection in guinea pigs varies between 10 and 50 colony forming units resulting in deposition fractions between 0.02 and 0.1 [15]. The deposition of inhaled particles in the lung is determined by breathing patterns and lung geometry in conjunction with attributes of particles affecting physical processes, such as inertial impaction, gravitational sedimentation and Brownian diffusion [16-18]. The proportion of small-respired particles in the 1-2 microns range capable of transporting one or more MTB organisms capable of deposition in the human alveolar has been estimated at around 10% [19]. There are some important consequences of our findings. Cross study comparisons of the relative infectivity of different types of TB infected individuals may lead to false conclusions because the sensitivity of each study differed markedly, as did the methodology for calculation of airborne infectious particle production. For example, the conclusions that HIV infected patients in study C were more infectious than non HIV-infected patients in studies A and B should be viewed in light of the above limitations [6]. The use of calculated airborne infectious particle production from in vivo animal studies may also be inaccurate for mathematical modelling of human-to-human transmission [20-23]. If TB airborne infectious particles are much more frequent than previously estimated there should be a greater emphasis on environmental searching for airborne TB [24]. Finally our data may lead to a refocus of TB control on environmental factors to decrease re-breathed air volume (RBAV) [16] and a re-evaluation of strategies such as active case finding in high burden settings.

Competing Interests

The authors declare that they have no competing interests.

Author's contributions

CMI, RW and NM contributed in study design, data analysis, data interpretation and writing of the manuscript. All authors have revised the final manuscript critically and approved it.

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