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# Reimagining the Transition of Mycobacterium Tuberculosis Research from Mammalian Models to Zebrafish

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## Abstract

Tuberculosis is a highly contagious and infectious disease caused by the microorganism *Mycobacterium tuberculosis* (Mtb). Tuberculosis remains a significant global health concern, affecting millions of people worldwide. This article reviews the transition of Mtb research with several animal models, focusing on zebrafish as a unique approach. Traditionally, guinea pigs, rabbits, mice, and monkeys have been used, but zebrafish models offer a new perspective on Mtb research due to their similarity to human symptoms and mechanisms. Zebrafish models have been crucial in understanding immune responses, drug efficacy, and initial pathogenicity to bridge the gap between small and large mammalian models. The zebrafish model has shown promising approach in understanding Mtb's intracellular pathogenicity, impaired lysosome functioning and macrophage survival strategies. This model has the potential to overcome the limitations of other animal models, providing an essential resource for tuberculosis research.

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## 1. Introduction

Tuberculosis (TB) is a life-threatening disease and has affected the global population. The landscape of *Mycobacterium tuberculosis* (Mtb) research is shifting. While the venerable lab mice have served as a workhorse for decades, their limitations in fully recapitulating human tuberculosis (TB) are becoming increasingly apparent (Singh & Gupta, 2018). This realization, coupled with the urgent need for new TB diagnostics, vaccines, and therapeutics, is driving a paradigm shift towards embracing diverse animal models that more accurately represent the complex interplay between Mtb and its host. Understanding the intricate mechanisms of Mtb pathogenicity is crucial for developing effective therapeutics (Yang et al., 2023). Animal models play a vital role in this endeavor, allowing researchers to observe the bacterium's behavior, immune responses, and disease progression in controlled settings (Park et al., 2021).

However, the traditional reliance on inbred laboratory mice has yielded models with limited immunological diversity and disease pathology compared to humans (Enriquez et al., 2020). This discrepancy can lead to misleading results and hinder the translation of promising discoveries from bench to bedside. The initial studies have compared several animal models to determine a model-specific approach for Mtb research (Dharmadhikari & Nardell, 2008). However, they often focus on describing individual model systems or specific aspects of TB pathogenesis, with less emphasis on the broader need for diversification. Additionally, they may inadequately address the limitations of traditional models or alternative species' emerging potential.

This review aims to bridge these gaps by providing a fresh perspective based on a succinct overview of transitions in Mtb research from small to large mammalian models and cumulatively propounding interest in invertebrates like Zebrafish as a current model of exploration. By fostering a deeper understanding of the strengths and weaknesses of various animal models, it can stimulate the development and utilization of more relevant and

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informative model systems. This may accelerate the progress toward conquering the global challenge of TB and improving the lives of millions affected by this devastating disease.

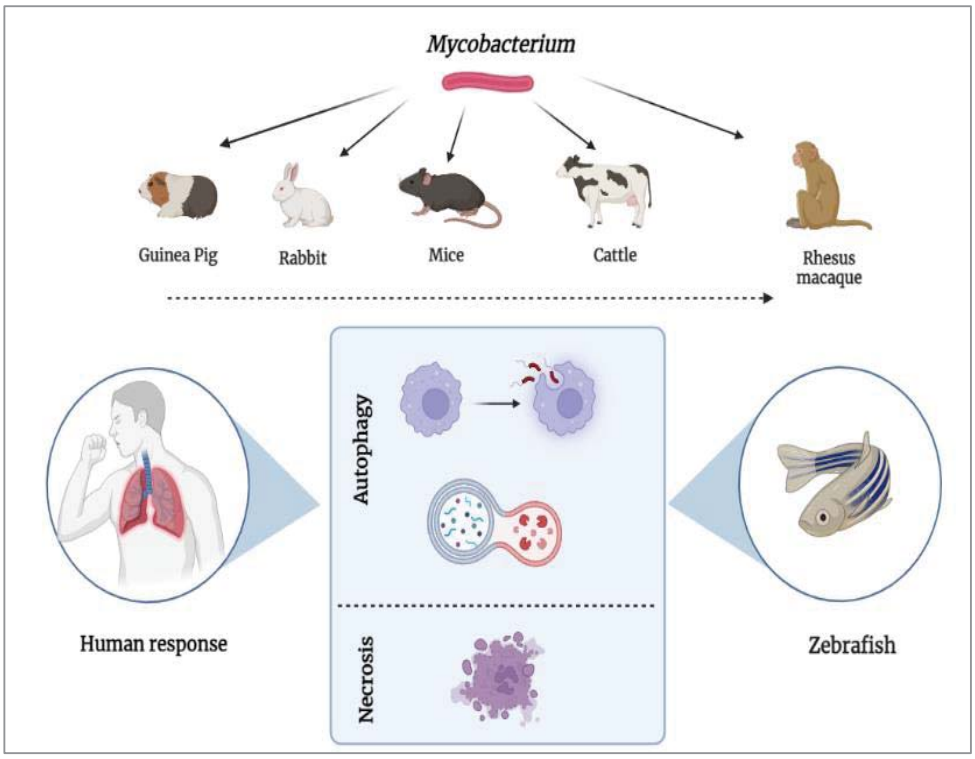


**Figure 1.** Depicts the transition of animal models for Mtb research from guinea pig to Zebrafish. Zebrafish mainly show symptoms and pathogenicity similar to human beings and help evaluate the efficacy of therapeutics.

## 2. Transition from small to large mammalian models

Various studies present strengths and limitations of mice, guinea pigs, and rabbits for TB research. Recent comparative studies have focused on comparing vaccine efficacy on animal models (Gong et al., 2020). As shown in Figure 1, guinea pigs were initially used to assess the pathogenicity of Mtb with the immune cells (lymphocytes). Mtb exhibits a high potential for its transmission to the airways of an organism, sometimes culminating in multidrug resistance (Seung et al., 2015). According to its pathological studies, spleen and lungs with lesions in infected guinea pigs helped researchers to elucidate the aspects of pulmonary TB

and site-specific infection, respectively (Gs et al., 2008; Myllymäki et al., 2015). Subsequently, several vaccine trials on guinea pigs also purveyed insightful pathogenicity of Mtb to develop novel therapeutics. However, limitations in the immunological reagents (like antibodies, etc.) for comparative studies led researchers to shift towards a better animal model that could overcome this burden.

Similar symptoms of lesions during infection by other species *of Mycobacterium bovis* have also been observed in rabbits (Dannenberg, 2001). Hence, by including similar other factors, rabbit models have also been utilized to investigate pathogenic aspects of TB with certain types of Mtb strains (Dannenberg, 2009). Lungs of the rabbit model also manifest some major symptomatic factors of TB, such as granuloma formation (a cluster of lymphocytes) and central necrosis with *the M. tuberculosis* HN878 strain. However, certain Mtb strains decide the severity of lung lesions based on their virulence (Mantilla Galindo et al., 2019). Overall, rabbit models are susceptible to the various strains but with certainty regarding their drawbacks.

Accordingly, the interest has been inclined toward the mice models, where it becomes easier to manipulate them genetically based on the desired criteria (Tran et al., 2016). Mice have become the prominent animal models of Mtb research, which can be understood based on the publication percentage shown in a comparative study (Li & Li, 2023) (figure 2). Mainly, mice strains such as mice C57BL/6 and BALB/c are used as suitable animal models in Mtb research because of their different susceptibility to *M. tuberculosis* H37Rv strain about several routes of infection. Variations in the routes of infection help to analyze several factors of immunity, such as the production levels of interleukins, interferons, antibodies, etc. This aspect has been successfully investigated in these models, where lower levels of interleukin-5 production were observed in intranasally immunized mice, and elevated levels of antibodies were determined in the intramuscular and subcutaneous immunization routes (Namvarpour et al., 2019). To analyze impact of Mycobacteria-induced immune responses with a symptomatic approach, gene knockout and immune-deficient mice models such as C3HeB/FeJ (symptomatic with necrotic granulomas and liquefatic necrosis), SCID mice, iNOS knockout mice (similarly symptomatic with granulomas of infected humans), and

CBA/JIL10 mice (with pulmonary granulomas) have been examined to speculate the possible pathogenicity of Mtb infection (Cooper, 2015). Humans can show the hematogenous spread of disease, central necrosis, cavity formation, and necrotic lesions in granuloma formation in tuberculosis.

On the contrary, most of the mice models do not exhibit such outcomes for the deep investigations, limiting their roles to understanding the mechanisms of immune responses and rapid evaluations for the development of crucial therapeutics (Gouveia et al., 2013; Leung-Theung-Long et al., 2015). Small mammalian models are helpful for preliminary studies for developing prospective therapeutics or vaccine candidates. However, they do not confirm a drug's or vaccine's efficacy for humans because of their different responses to protection against Mtb.

Therefore, the need for large mammalian models that could manifest the behavior of protection like human beings becomes evident. In this case, NHPs (non-human primates) are the best models for clinical studies among large mammalian models, like cattle and goats. Their close evolutionary relationship with humans has shored up clinical researchers to assess the similar efficacy of any drug candidate for its further development or validation. For instance, significant symptoms of Mtb infection in humans, i.e., liquefaction, granulomas, and caseous necrosis, are similarly manifested by monkeys infected with Mtb (Flynn et al., 2003). NHPs are very appropriate to distinguish different vaccine candidates in terms of their efficacy according to various routes of administration. Ultimately, after validation, NHPs highlight and support the data of preliminary studies conducted with small mammalian models. However, this chronological approach of evaluating Mtb pathology with protective action of therapeutics is very costly, rigorous, and daunting in terms of implementation.

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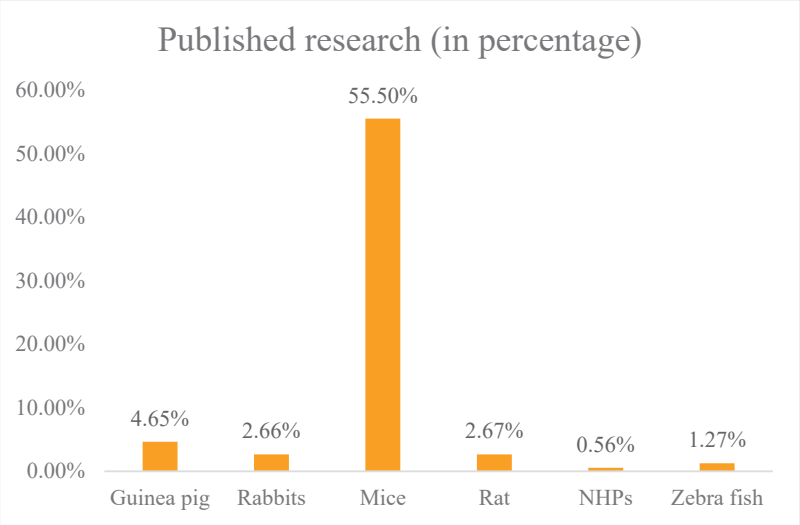


**Figure 2.** Publications (in percentage) based on the research conducted on *Mycobacterium tuberculosis* (Mtb) with animal models. The data has been considered from the comparative study by (Li & Li, 2023) and chronologically represented in the form of a bar graph by including the suitable subjects of study such as guinea pigs, rabbits, mice, rats, NHPs, and Zebrafish. Mice model has been used extensively in Mtb research (55.5%), followed by guinea pig (4.65%), Rat (2.67%), Rabbits (2.66%), Zebrafish (1.27%), and NHPs (0.56%).

### 3. Exploring new frontiers with Zebrafish

There is a void between small and large mammalian models for Mtb research, which demands time-intensive efforts to fill with limited resources. Consequently, a compiled approach has been channelized by a unique model of invertebrates, *viz.* Zebrafish (*Danio rerio*). Other invertebrates like fruit flies and amoeba are also used for Mtb research but are limited to studying innate immune responses and host-pathogen interaction, respectively (Gong et al., 2020). On the other hand, Zebrafish show similar symptoms of Mtb infection to human beings (Martinot, 2018; Myllymäki et al., 2017; Traver et al., 2003), evaluates the aspects of Mtb pathogenicity with mechanisms (Ramakrishnan, 2020), validates the efficacy of developed vaccines with preclinical screening (Oksanen et al., 2013, 2016), and henceforth fulfills the selection criteria of small and large mammalian models for Mtb research. Various advancements have been made in conducting Mtb research with the zebrafish model that

explores the pathogenicity of Mtb infection. Here, the lysosome is the critical intermediate of consideration to assess initial pathogenicity in Mtb infection, as it leads to the progression of necrosis (Alu et al., 2020).

Hereby, (Roca et al., 2019) found the inter-organelle signaling in the interaction between Zebrafish and *M. marinum* and *M. tuberculosis*. Findings stated that, during Mtb infection in the zebrafish model, immunological factors like TNF (tumor necrosis factor) induce the activation of mitochondrial ROS, further leading to macrophage necrosis. This research suggested the importance of $Ca^{2+}$ overload-preventing drugs culminating in the prevention of macrophage necrosis in Mtb infection by using Zebrafish as an animal model. The other study in this model showed the pathogenicity of *M. marinum* and *M. tuberculosis* by escaping the phagocytosis conducted by macrophages. These bacteria survive in the acidic environment to flinch from the host immune mechanism. Exquisitely, this research validates the importance of specific genes (MarP, ErP) possessed by *M. marinum* and *M. tuberculosis*, which leads to their survival mechanism inside the cells of innate immunity, *viz.* macrophages during infection by avoiding the interaction between phagosome and lysosome (Levitte et al., 2016). Some mycobacterial lipids like PDIMS (Phthiocerol dimycocerosates) help to disrupt the phagosome membrane to avoid phagocytosis (Osman et al., 2020). Such data indicates that the survival of Mycobacteria within macrophages is heavily reliant on avoiding the fusion of phagosomes with lysosomes. Another aspect of Mtb pathogenicity can be seen in tobacco smokers, who are most susceptible to Mtb infection due to the blocked migration of macrophages to the Mtb infection sites (Berg et al., 2016). However, these defects are related to impaired lysosome functioning inside the macrophages, suggesting the importance of the zebrafish model in understanding the intracellular pathogenicity of Mtb. By delving deeper into the subsequent strategies of Mtb species, the impaired intracellular response of macrophage is also related to the escape mechanism of Mycobacteria. Here, Mtb opts for a relocation strategy from macrophages to monocytes (permissive to mycobacterium) with the help of immunological factors like CCL2/CCR2 through a cell fusion event (Cambier et al., 2017). These findings interpret that *M. marinum* and *M. tuberculosis* are closely related to their amino acid homology and possess similar intracellular pathogenic

responses in Zebrafish. This contextual statement eradicates the limitations of zebrafish models recently claimed in the comparative review articles by Zhan et al. (2017) and Gong et al. (2020).

The zebrafish model has become a valuable tool for studying Mtb pathogenesis, drug screening, and host immune response. By investigating lysosome dysfunction in Mtb infection, this model has provided insights into the disease's development and its implications for humans. The zebrafish model's ability to observe host-pathogen interactions in real-time and its genetic tractability has led to the discovery of new immunological factors and therapeutic strategies. These findings support the zebrafish model's potential for translation and challenge the previously reported limitations of zebrafish models.

**4. Conclusion**

Using Zebrafish as an animal model for studying *M. tuberculosis* pathogenicity has shown promising results. Collaborative research using diverse models and innovative approaches is needed to advance our understanding of this disease. It is essential to balance scientific progress with ethical responsibility and integrate advances in genomics and personalized medicine into model development. The zebrafish model has advantages over other animal models, including its ability to mimic human symptoms of Mtb infection, evaluate pathogenicity with mechanisms, and validate the efficacy of developed vaccines with preclinical screening. The zebrafish model provides a unique resource for Mtb research and should be considered in future studies.

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