# Zebrafish as an infectious disease model: The *Mycobacterium marinum* case

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## Introduction

**Zebrafish** (*Danio rerio*) is a **multifaceted** animal modelthat is lately and increasingly being used to study human diseases such as **infections**[1,2]. One of those is **tuberculosis**, which is is particularly interesting, because in zebrafish it is studied using its natural pathogen, ***Mycobacterium marinum*** [3]. In order to become familiar with the zebrafish model and have a closer approach to the zebrafish model a few examples of its applications are shown next.

## Infecting Zebrafish

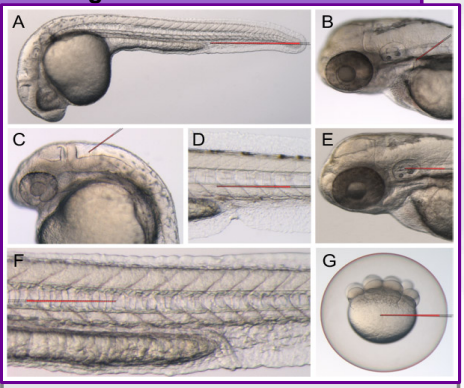


Figure 1. Overview of injection methods used to establish systemic or local infections in zebrafish embryos[4].

**Intravenous injections** to establish a rapid systemic infection are performed into:

- The **caudal vein** at posterior blood island at 1dpf (Figure 1A).
- The **Duct of Cuvier** at 2-3 dpf (Figure 1B).

**Local injections** to study macrophage and neutrophil chemotaxis are performed into:

- The **hindbrain ventricle** at 1dpf (Figure 1C).
- The **tail muscle** at 1-2 dpf (Figure 1D).
- The **otic vesicle** at 2-3 dpf (Figure 1E).
- The **notochord** at 1-2 dpf, which is inaccessible to phagocytes (Figure 1F).

To create an early systemic infection with slow growing bacteria can be performed into the **yolk** at 16-1000 cell stage (Figure 1G) [4]

## Granuloma formation in Zebrafish

UNDERSTAND GRANULOMA FORMATION (Importance of RD1 locus)

Volkman H.E. *et al.* (2004)

**Materials**
- Zebrafish embryos and larvae
- Wt and RD1 *M.marinum* strains

- Cellular basis of granuloma formation → Real-time monitoring (Figure 2 )
- Is aggregation and recruitment the only process mediated by RD1 locus? → Hindbrain ventricle infection
- Importance of RD1 locus in macrophage signal production and reception for mediate aggregation → Two superinfection experiments (Figure 3)
- Aggregate formation facilitate the bacteria dissemination to uninfected macrophage → Enumeration of bacteria and macrophage numbers during aggregate formation

Macrophage aggregation → Two steps affected → Intracellular bacterial spread via host cell death within aggregates

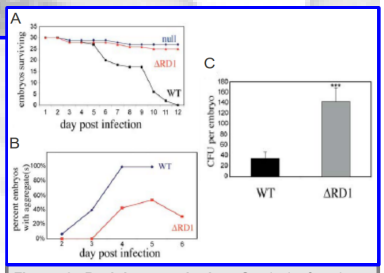


**Figure 2. Real-time monitoring.** Survival of embryos infected with ΔRD1 or wt or non infected (A). Whole embryo bacterial counts of wt and ΔRD1 infected embryos (B). Whole embryo bacterial counts of wt and ΔRD1 infected embryos on day of aggregate formation [5].

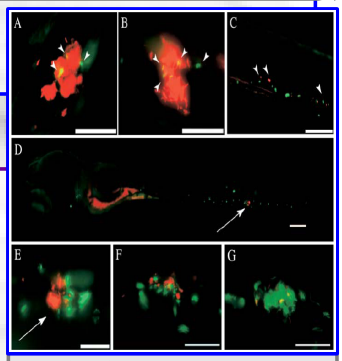


**Figure 3. Superinfection with wt bacteria ΔRD1 aggregation defect.** Embryos with red-labelled aggregates are shown 4h after superinfection with green-fluorescent strains of ΔRD1 (A) or wt (B) and followed for 24h post-secondary infection. (C) Embryo infected with green-fluorescent ΔRD1 and then (D) infected with red-fluorescent wt. (E, F and G) Higher magnification of superinfections. Scale bar, 200μm [5].

## Latency, dormancy and reactivation studies in zebrafish

Induction of a persistant infection in adult zebrafish — Parikka, M. *et al.* (2012)

- Status followed by
  - qPCR-based method
  - Histological analyses
  - Vast majority of the fish showed
    - 4wpi bacterial burdens and granulomas stopped growing
    - Asymptomatic disease
- Dormant state test
  - *Ex vivo* plating experiment
  - Latent disease charaterized by a high proportion of dormant mycobacteria
- Adaptive immunity role
  - *rag1* deficient zebrafish line (T and B cells not functional)
  - Comparison between *Nos2b* and *IFNγ*1-2 expression levels
  - Initial macrophage activation before latency is madiated by adaptive response inducing *Nos2b* not *IFNγ*
- Reactivating the tuberculosis
  - Two doses of radiation (25Gy each one)
  - Quantification of mycobacteria during 24h and survival rate
    - Survival rate dropped fast
    - Amount of mycobacteria doubled
  - Mortality increased and huge bacterial areas were found outside the granuloma

**References**

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4. Erica L., *et al.* 2012. Infection of Zebrafish embryos with intrecellular bacterial pathogens 61: 1-8
5. Volkamn H.E., *et al.* 2004 Tuberculous granuloma formation is enhanced by *Mycobacterium* virulence determinant 2: 1046-1956.
6. Berg R.D., *et al.* 2012. Insights into tuberculosis form the zebrafish model 18: 689-690
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## Advantages

- Optical transparency during embryonal and larval stages [2]. ⇨ Great possibilities for *in vivo* imaging
- The immune system maturates at different stages [1,2].
- Relatively resistant to acute adverse effects caused by irradiation [3].
- A single pair of fish can have hundreds of progeny every week [2].
- Increasing availability of transgenic lines with immunity cell fluorescently labelled [6].

## Disadvantages

- Some pathogens cannot be studied at zebrafish maintained temperature, 28ºC [7].
- Lack of monoclonal antibodies to surface antigens of zebrafish immune cells[7].
- Comparing and validating human infection models can be difficult due to the unknown characteristics of zebrafish immune system[7].
- The maintenance of zebrafish requires a significant commitment of funds and personnel[8].

## Conclusions and Future prospects

- Zebrafish has made possible to reproduce tha hallmarks of host-pathogen interactions.
- The model advantages can be critical points during the proces of choosing the animal model.
- Immune system needs to be fully studied.
- The characterization of similarities and differences between zebrafish and mammalian immune system need to be complete.
- Zebrafish future in drug and inflammatory disease research is bright [2].