Implementation of a *Galleria mellonella* infection model at GHTM/IHMT-NOVA

Telma Rodrigues, Mariana Andrade, Joana Marques, Liliana Rodrigues, Miguel Viveiros, Henrique Silveira, Isabel Couto and Sofia Santos Costa

Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal

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Introduction

***Galleria mellonella* larvae** has gained scientific relevance as an *in vivo* model to study virulence properties of pathogens (bacteria, fungi, viruses and other parasites), host-pathogen interactions, for evaluation of new antimicrobial compounds, among other applications.[1] The advantages of this model include its low-cost, a *G. mellonella* immune response similar to the mammals innate immune system and allowing infection protocols at 37 ˚C.[2] It is also a sustainable model, replacing and/or reducing significantly the use of vertebrate models in preliminary studies, in agreement with the 3R principles (Replacement, Reduction, Refinement). [2] Yet, its use is compromised by the lack of a research-oriented supplier of standardized reared larvae with stable traits that strengthen the quality of the research data to be obtained.

Aims

To implement a *G. mellonella* research hub to support ongoing and future studies on virulence and drug discovery at GHTM, IHMT-NOVA. The specific aims were: (i) to create a dedicated area and (ii) implement a standardized protocol for *G. mellonella* larvae rearing; (iii) to establish optimal conditions for assessing virulence properties of relevant bacterial pathogens.

Methods & Results

1 Establishment of a *G. mellonella* colony

- Acquisition of a batch (n = 80) of *G. mellonella* from a pet-food store

- ID confirmation by COI (Cytochrome c Oxidase subunit I) sequencing [3]

Creation of a dedicated room at IHMT's Insectary [4,5]

- temperature ≈ 28 ˚C
- darkness
- relative humidity 60 – 80%
- high nutrient diet

Figure 1. **Partial view of the insectary room dedicated to the *G. mellonella* colony.**

GHTM/IHMT's *G. mellonella* traits (in average)

- oviposition: ~1500 eggs/female
- egg hatching: ~34%
- less susceptible to *S. aureus* infection than commercial larvae
- high productivity of last instar larvae: ~ 500/week

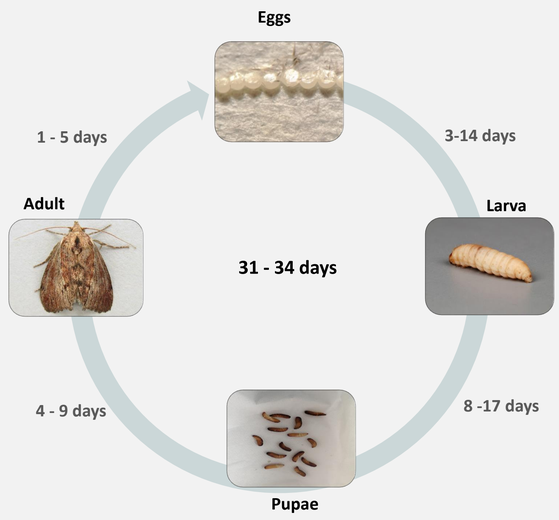

Figure 2. **Life cycle and duration of each stage of *G. mellonella* reared at IHMT-NOVA.**

2 Optimization of infection protocols

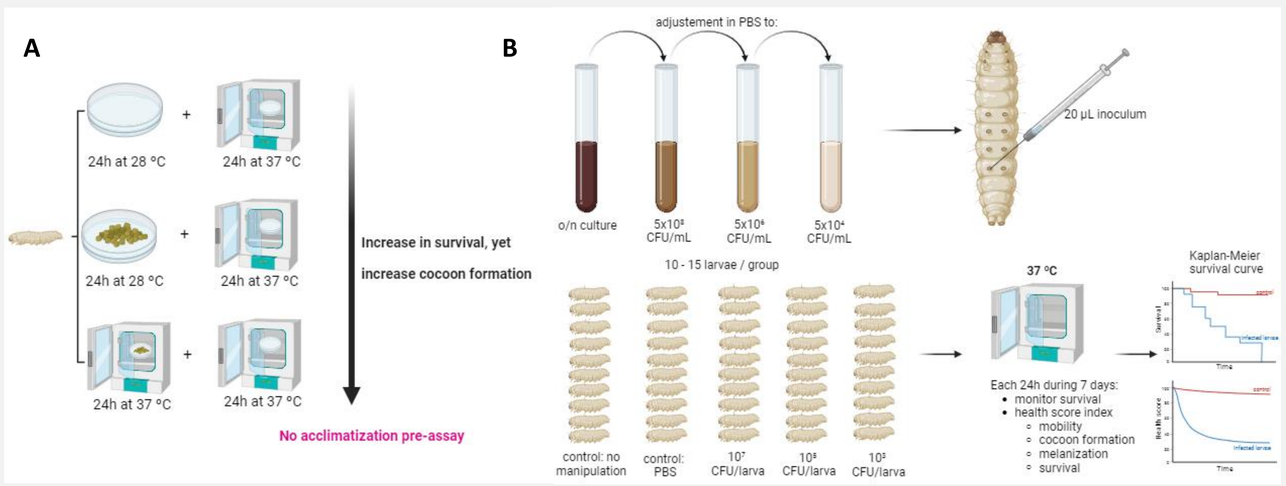

Figure 3. **Optimization of the *G. mellonella* infection protocol, namely pre-assay conditions (A) and testing different bacterial inocula (B).**

3 Virulence assays for relevant pathogens

Increased virulence potential

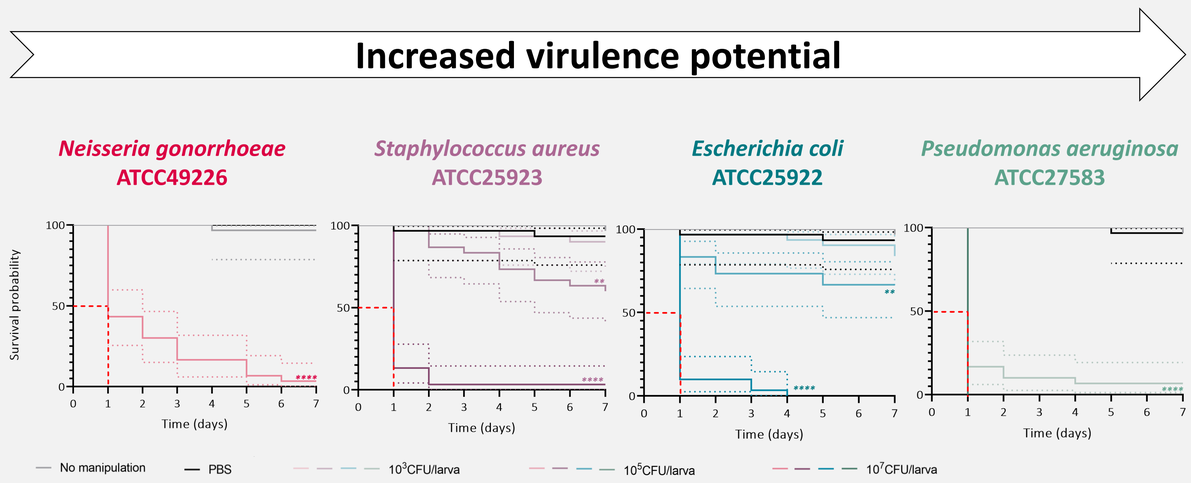

Figure 4. **Kaplan-Meier survival analysis of *G. mellonella* infected with four bacterial pathogens.** Dotted lines: 95% confidence intervals; red lines: median survival time. Statistically significant differences between each inoculum and the control (PBS) are identified as follows: ** $p < 0.01$ and **** $p < 0.0001$.

Conclusions

- ✓ A ***G. mellonella* colony has been implemented at GHTM/IHMT-NOVA** (currently at the 10th generation) with a **standardized rearing protocol** to supply last instar larvae in optimal conditions.
- ✓ ***G. mellonella* infection assays have been optimized** for main human and animal bacterial pathogens.
- ✓ Current work: testing of protocols for evaluation of drug toxicity and efficacy; additional protocols focusing on pathogen-host-antimicrobials interactions.
- ✓ **GHTM/IHMT-NOVA *G. mellonella* research hub**: a platform for drug discovery and microbial pathogenesis studies, envisaging establishment of collaborations and services to the scientific community.

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Contact information

Sofia Santos Costa
scosta@ihmt.unl.pt