Galleria mellonella research hub: a new *in vivo* infection model at GHTM/IHMT-NOVA

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Background: *Galleria mellonella* represents a sustainable invertebrate infection model that is gaining interest for pathogenicity and infection, host-pathogen interaction, and pharmaco-toxicological assays. Here we describe (i) the implementation of a *G. mellonella* colony at GHTM/IHMT-NOVA to provide standardized larvae for the research community and (ii) the optimization of virulence assays for relevant human pathogens.

Methods: *G. mellonella* was acquired from a commercial house at the last larval stage and the species was confirmed by *COI* sequencing. The four life cycle stages are reared and maintained in the dark, at 28ºC, with a high-nutrition diet. Infection assays were optimized for reference strains of four major pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae* and *Escherichia coli*.

Results: A *G. mellonella* colony was established with optimized parameters such as diet and routine maintenance. In-house reared *G. mellonella* had a life cycle of 31-34 days (egg to adult). Each female laid on average > 1,500 eggs, with an egg hatching rate of ~34%. Currently, the colony has high productivity (~20,000 larvae/generation). Compared to the commercially acquired larvae, in-house reared larvae are healthier and more resistant to *S. aureus* infection. Our optimized infection protocols (pre-incubation conditions, bacterial inoculum, infection procedures) enabled the ranking of the virulence potential of the four pathogens, as follows: *P. aeruginosa* >> *S. aureus* ~ *E. coli* >> *N. gonorrhoeae*.

Conclusions: This work represents an expansion of the animal infection models available at GHTM/IHMT-NOVA for collaborators and the research community. Ongoing work focuses on expanding this model to fungal and parasite pathogens, as well as additional functional assays, such as pharmaco-toxicological studies.

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