Assessment of the efficiency of the phage PEV2 against *Pseudomonas aeruginosa* by titration in the *Galleria mellonella* model

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**Introduction**

*Pseudomonas aeruginosa* is a ubiquitous Gram-negative bacteria forming biofilms and living in soil and water. *P. aeruginosa* is the first bacterial species isolated from dog’s ear canal that cause secondary infections during chronic otitis. The management and treatment of *P. aeruginosa* in dog otitis is particularly complicated given the high antimicrobial resistance. The aim of this work is to evaluate the efficacy of one bacteriophage (PEV2) against a clinical isolate of *P. aeruginosa* using an in vivo model of *Galleria mellonella* larvae.

**Materials and methods**

A preliminary experiment was performed to assess the optimal inoculation dose of *P. aeruginosa* (PAV237) which was 4CFU/4µL. In a second experiment, the optimal multiplicity of infection (MOI) was assessed by injecting larvae with the optimal inoculation dose and 4 different MOI (50000, 5000, 500, 50). The MOI 5000 and 50000 were selected for the main experiment, which consisted in monitoring the evolution of the concentration of phages and bacteria contained into the larvae. For this experiment, 6 groups of 10 larvae were injected (Fig. 1). All groups were separately mixed and the titration of *P. aeruginosa* and PEV2 was performed every 24h during 72h (Fig. 2). Marbofloxacin, frequently used in treatment of chronic otitis, was used as control (1µg/4µL).

![Figure 1: Groups of larvae injected. PAV237 inoculation dose: 4CFU/4µL. Marbofloxacin inoculation dose: 1µg/4µL](image)

**Results**

An increase in phage titer was observed after 24h and 48h with both MOI (5000 and 50000). No phage was detected in the phage control group (group 3) (Fig. 3a). At the same time, there was a smaller increase in the titer of *P. aeruginosa* after 24h for MOI 50000 compared to 5000. The *P. aeruginosa* titers were lower with MOI 50000 and 5000 compared to the positive control after 24h and 48h. (group 2) (Fig. 3b).

![Figure 2: Experimental design of the titration. HPI: hours post inoculation](image)

**Conclusion**

These results show that PEV2 is active against *P. aeruginosa* in an in vivo model, *Galleria mellonella*, even if it did not result in a bacterial elimination at the MOI tested.