Efficacy assessment of PEV2 phage on Galleria mellonella larvae survival after inoculation with a Pseudomonas aeruginosa dog otitis isolate

Laforêt F.1,2, Antoine C.1,2, Glonti T.3, Kutter E.4, Pirmay J.P.3, Mainil J.1, Delcenserie V.2*, Thiry D.1*

Introduction

Dog external otitis is a recurring and worrying problem in veterinary medicine due to the emergence of antibiotic resistance. Phage therapy is increasingly put forward as a promising alternative or addition to the current anti-microbials. In this context, the efficacy of PEV2 phage against a Pseudomonas aeruginosa strain (PAV237), isolated from a dog otitis, was assessed in an in vivo Galleria mellonella larvae (Figure 1) survival model. The immune response against pathogens in Galleria mellonella being similar to the innate immunity in mammals.

Materials and methods

To study the impact of PEV2 on the survival of the infected larvae, the optimal P. aeruginosa inoculation dose, resulting in the death of 50% of the larvae within 4 days, was assessed in a preliminary experiment. A total of 210 larvae were inoculated with different bacterial dilutions and the optimal inoculation dose was found to be 4 CFU/µL. In the main experiment, 270 infected larvae were injected either with different concentrations of PEV2 (Multiplicity Of Infection 50000, 5000, 500 and 50) or with marbofloxacin (Figure 2). Larvae survival was checked at 24, 48, 72 and 96h post inoculation (HPI). Survival curves were generated with R Commander software and the log rank test was used to evaluate the statistical significance of the results.

Results

No significant survival improvement was observed with PEV2 therapy in the infected larvae groups. Indeed, the generated Kaplan-Meier curves (Figure 3) showed that the rate of living larvae was significantly higher in the control groups (non-infected larvae) compared to the infected-treated group irrespective of phage MOI. The only exception was the group with infected larvae treated with marbofloxacin, in which the survival rate was higher than for the other infected larvae groups.

Conclusion

These results show an ineffectiveness of PEV2 phage on the survival of Galleria mellonella larvae infected with P. aeruginosa. One hypothesis could be that the phage is able to kill the bacteria but the bacterial lysis release some toxic materials such as endotoxins. Further studies are needed to assess the phage titer evolution within this model.