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PP-057 [Bee Health]

Exploring the role of opportunistic pathogens in honeybee health: the case of *Serratia marcescens* synergy with *Nosema ceranae*

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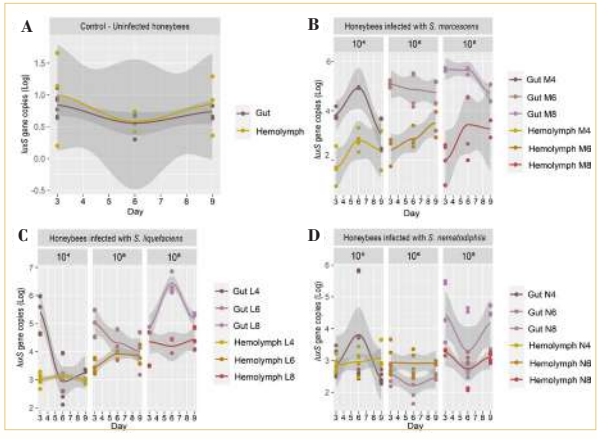
Background & Aim

Honeybee health is threatened by multiple pathogens, including the gut parasite *Nosema ceranae* and opportunistic bacteria such as *Serratia*. **This study wants to investigate a potential synergy between these pathogens, with *N. ceranae* promoting *Serratia* proliferation.** This interaction may exacerbate gut epithelial damage and lead to systemic infections. Understanding this relationship is key to improving disease control strategies in beekeeping.

What we did

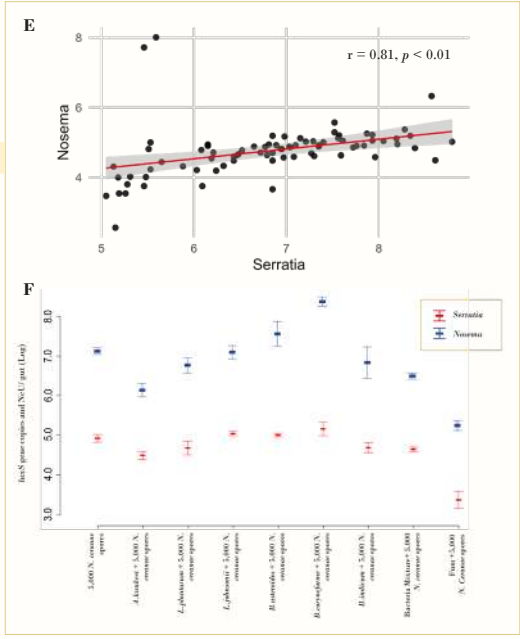
Three *Serratia* strains (*S. marcescens* [M], *S. liquefaciens* [L], *S. nematodiphila* [N]) were isolated from honeybee guts and orally administered to newly emerged bees at increasing doses. Parallel infections with *Nosema* spores were performed to evaluate potential synergistic effects. *Serratia* abundance in gut and hemolymph was quantified by qPCR targeting the *luxS* gene. Additional groups received fumagillin or beneficial bacterial strains to assess their effect on pathogens proliferation.

Panel for honeybee artificial-infection with *Nosema* spores and *Serratia* strains.



Serratia Systemic Spread Dynamics

Quantification of *luxS* gene copies revealed negligible *Serratia* levels in control bees (panel A). In bees infected with *S. marcescens* (panel B), a dose-dependent increase was observed, especially in the hemolymph, indicating systemic spread. *S. liquefaciens* (panel C) and *S. nematodiphila* (panel D) showed gut colonisation mainly at the highest dose (2.5×10^8 CFU/mL), but limited hemolymph proliferation. Among tested strains, *S. marcescens* showed the highest invasiveness and virulence. Moreover, *Serratia* exhibited a clear gut-to-hemolymph translocation, confirming its ability to breach the gut barrier and establish systemic infection.



The synergy between *Nosema* and *Serratia*

Serratia exhibits a strong positive association between bacterial proliferation (*luxS* gene copies) and *Nosema* load (NcU), indicating a synergistic interaction. This is especially evident under high *Nosema* doses, where a compromised gut barrier likely enhances bacterial translocation to the hemolymph. The Pearson correlation (+0.81, $p < 0.01$, panel E) supports this synergism. Moreover, microbial treatments influenced this dynamic (panel F): bees receiving beneficial strains such as *Apilactobacillus kunkeei* and a bacteria mixture showed significantly reduced levels of both *Nosema* and *Serratia*, highlighting a protective effect on gut integrity. In contrast, untreated or fumagillin-only groups did not prevent systemic spread (even if fumagillin decreased *Nosema* load). These findings underscore how both *Nosema* infection intensity and microbiota modulation critically shape the outcome of bacterial co-infections.

Our main findings

This study demonstrates that *Nosema* enhances the proliferation of opportunistic *Serratia* strains, particularly *S. marcescens*, by compromising gut epithelial integrity and promoting haemocel colonization. The strong correlation between *Nosema* load and *luxS* gene abundance supports a synergistic pathogen–pathogen interaction. Some beneficial bacteria treatments significantly reduced both pathogens levels, highlighting their potential as alternative strategies to mitigate infection spread and improve honeybee resilience. These findings underscore the importance of maintaining gut microbiota balance in the context of multi-pathogen stress scenarios in apiculture.

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Honeybee's population losses show periodic fluctuations in intensity at global level, with their decline often driven by specific pathogens, such as the gut microsporidia Nosema ceranae and a multitude of viruses. However, many of these pathogens alone rarely bring honeybee colonies to death. Indeed, negative synergies with other pathogens or environmental factors often occur and get the colonies to collapse. The study of honeybees' pathogen synergies at the colony level is a little explored aspect and has focused especially on the pathogens-pesticides negative effects. However, other biological synergies, especially with pathogen and non-core opportunistic gut bacteria such as Serratia marcescens, is rarely explored and may explain important drawbacks in colony losses and may help beekeepers with a more accurate diseases management. To achieve this goal, our research wants to explore the development dynamics of S. marcescens within the co-infection of N. ceranae, determining the incidence of S. marcescens on the survival rate of honeybees and proposing possible solution (e.g. beneficial bacteria uptake and medicaments) on controlling these pathogens. Briefly, different Serratia strains were isolated from weak honeybees and inoculated into new-born, healthy honeybees' in co-infection with N. ceranae. Infected honeybees were reared in cages, and the impact of the multiple infections on their survival was observed in controlled lab conditions. Quantitative PCR was used to quantify Serratia and N. ceranaein the honeybee gut and hemocoel. Moreover, by feeding specific additives based on beneficial bacteria such as Lactobacillaceae or antimicrobial agents such as fumagillin, a possible co-infection control strategy was tested. Our results, showed that all tested Serratia strains were found capable to cross the gut epithelium and proliferate in the hemocoel and the co-infection with N. ceranae facilitated the surge of Serratia population within the gut, leading honeybees to a rapid death. Beneficial microbes from flowers, particularly Apilactobacillus kunkeei, effectively reduced Serratia load, suggesting it as a potential organic microbial resource management, easily applicable in commercial apiaries. These findings underscore the complexity of microbial interactions in the honeybee, recalling the need for wider research in determining multifactorial interactions between pathogens and environmental bacteria within the honeybee's gut.