**Practice review of a scientific publication: *Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees*, Rayman, et al.**

**Summary**

The gut microbiome is known to be linked to health and honeybees can serve as a model organism for the study of host-microbiome interactions, due to their simple microbiota comprising mainly of eight core species. Honeybees acquire their microbiota through social contact, are composed of host-specialized bacterial species, and share common features with mammalian microbiomes. Honeybees have been experiencing elevated mortality, which may be partially linked to the use of antibiotics which are known to disrupt the usual gut microbiome, causing dysbiosis. The use of tetracycline was examined in the honeybee model to see how antibiotic treatment effects the composition and community size of the microbiome, honeybee mortality, and susceptibility to the opportunistic pathogen *Serratia* kz11. The honeybees were exposed to either sucrose syrup (controls) or tetracycline antibiotic (treatments) for 5 days, after which they were either returned to the hive or maintained in the laboratory under sterile (only kept with other tetracycline-treated bees) or exposed (with normal workers collected from their hive) conditions. Bees were sampled at several time points post-treatment, the gut microbiome was assessed for size and composition using qPCR and 16S rRNA sequencing. Other laboratory experiments were conducted to include recovery experiments to control for age and a germ-free bee experiment to determine the effects of tetracycline.

Antibiotic exposure was shown to effect survivability, both in the hive and in the laboratory experiments where the bees were exposed to the opportunistic pathogens. Also, treated bees had a decrease in absolute abundance, a decrease in the relative abundance of some core species of the microbiome, and the alpha and beta diversity were altered. The infection with the opportunistic infection with *Serratia* kz11 resulted in increased mortality in bees treated with tetracycline. Also, it was illustrated that dysbiosis alone, rather than treatment with tetracycline, can impact bee health.

This manuscript has a lot of merit, and is recommended to be accepted with some major and minor revisions.

**Major Revisions**

1. In the introduction, in the first paragraph it states that “complete recovery of initial bacterial community composition is rarely achieved” after receiving antibiotics, and the manuscript later states in the last paragraph of the introduction that “the perturbations caused by tetracycline treatment were still evident one week after bees were returned to their hives following exposure. “ The second paragraph on page six states that the “gut microbial composition…did not return to the baseline composition after one week.” The experiment only follows the recovery of the microbiome for seven days post-exposure to the antibiotic treatment. The length of the experiment should be increased to further follow the long-term effects of antibiotic exposure.
2. In the Discussion on page 11, paragraph three, it states that “Our results suggest that treated bees allowed to recover in the hive without further exposure revert towards their baseline microbiome composition after 1 wk (Fig. 3).” However, as stated in the previous revision suggestion, the manuscript states that the microbiome does not recover after seven days and Figure 3 shows that both the alpha and beta diversity are significantly different at day 7. The statement that the bees returned to baseline microbiome composition is inaccurate based on the data and previous statements in the manuscript.
3. The exposure dosage for this experiment should be altered to encompass other doses of tetracycline. The Discussion, on page 11, the third paragraph, it states that the dose administered was “slightly lower than that used in apiculture” and “it is likely that some bees receive doses as high or higher than those used here.” Also, antibiotics “can persist for long periods of time” and has been detected for “up to 3 mo post-treatment.” If the hive is receiving three doses in the spring and three in the fall, there may be a substantial additive effect of the antibiotic. This study should include a range of doses of tetracycline, from a lower dosage to one much higher than was used in this study to mimic the conditions that are found in the hive.

**Minor Revisions**

1. In the results section, paragraph three (Fig. 2A), it states that recovery of the treatment bees was much lower than the recovery of the control bees. Since the mortality of the bees is a major part of this manuscript, some detail is needed as to how the dead bees were calculated in the recovery of the bees. Does recovery mean that only that percentage of living bees was recovered or that only that percentage of the bees was able to be located in the hive? The Materials and Methods, page 12, second paragraph, does not elaborate on this point. The laboratory experiments on the bees clearly state in the Materials and Methods that any dead bees were removed during a daily census.
2. In reference to Figure 5, on page six, paragraph four, it states that age-controlled and non-age-controlled bees treated with tetracycline and exposed kz11 had increased mortality when compared to control bees, bees exposed to tetracycline only, or bees exposed to *Serratia* only. Figure 5 seems to show that the bees exposed to tetracycline only and those exposed to *Serratia* only exhibit similar results, especially in the not age controlled bees. There needs to be discussion resolving this point.
3. In the Discussion, page nine, it is mentioned that the persistence of the core bacterial species may be due to antibiotic resistance to tetracycline. The manuscript would be stronger if the genes for antibiotic resistance were tested for in the bee population.
4. In the Discussion, page 10, in the first paragraph, it states that the gut microbiome populations were more prominent several days after the treatment, probably due to the lack of defecation of the bees during their time in the laboratory and this was also seen in the bees that were kept in the laboratory. There should be an explanation as to when the bees that were kept in the laboratory began defecating again.
5. In the Discussion, page 10, in the second paragraph, the word “morality” was used instead of the word “mortality.”
6. In the Materials and Methods section, under Illumina sequencing, more detail on the library preparation should be added, in addition to the reference.