Respected Editor of PLOS Biology Journal,

Reviewer #1

This study presents an interesting and thoughtful analysis on the effects of antibiotic treatment on the size and composition of the honeybee gut microbiome. The results show a significant alteration of the honeybee gut microbial community structure post antibiotics. The primary hypothesis is that the increased susceptibility to infection by opportunistic pathogens leads to increased mortality in the hive. Although the results are compelling, few issues must be resolved prior to publication:

**Major revisions**

Results:

Page 6 (last paragraph) and page 7 (first paragraph)

A bacterial challenge experiment was performed using 4 strains namely, i) *Serratia* kz11, ii)

*Escherichia coli* K-12, iii) *S*. *alvi* wkB2, iv) *Lactobacillus* sp. wkB8. As noted by the study, the latter two species are part of the core bee gut microbiome and *E*. *coli* K-12 is a nonpathogenic lab strain. Quite a bit of discussion and interpretation hinges on the results from one opportunistic pathogen (*Serratia* kz11). Testing the susceptibility of the antibiotic treated bees to another pathogenic strain would make the results stronger.

Methods:

Hive recovery Experiment (page 12)

Both Paragraphs under this section: An outline of the experimental procedure with a simple diagram or flowchart would be highly helpful to the reader. This study is very strong as it compares multiple groups with many replicates. The beginning of this section involves collecting 800 worker bees. It is a little unclear from the explanation if all of them were included in the study. It gets very confusing as the reader has to keep track of how many bees are included in the study, number of bees divided into different groups, how many bees are included in the replicates etc.

The same issue continues for replicate hive survival experiment and the laboratory recovery experiment (Page 12 and 13).

**Minor Revisions**

Discussion:

Page 10, Paragraph 1, line 2-3:

The study states that *G*. *apicola* is an exception to the trend followed by other microbes, where its relative abundance as well as strain-level diversity increases after treatment. Discussions explaining this observation are missing, considering the recent reports that indicates an important role for *G*. *apicola* in tetracycline resistance.

Methods:

Hive recovery Experiment (page 12)

Paragraph 2, line 11-15: It would be interesting to see how long it took the researchers to separate the two groups of bees from the hive (after being reintroduced to the hive). It is not explicitly stated if the two groups of bees were kept separately during their temporary storage in cup cages.

In summary, I recommend the manuscript for publication in PLOS Biology after the concerns have been addressed.