**Supplementary Text1**

Specific versions and compositions of the DBs used for each software in this study, as well as the computational resources and time used to build each software is available in Table 1. For DBs comparisons, the maxikraken2 DBs, includes both complete and draft genomes of the microbe, requires over 150 GB of memory available on the workstation for the downstream analyses, while the standard DB requires only 53 GB and minikraken DB requires 8GB. Customized DB (60 GB) was built with the same composition of the standard DB, with the addition of the genomes of two Rattus species the dataset was collected from. With 12 threads of CPU used on UGA’s high memory computing node, the building of the customized database took ~15 hrs (Table I) to complete the building process. Time of the analyses also changes with the selection of the databases, but the differences is only in the range of minutes. For rest of the software, pre-built DBs were chosen to perform the profiling, if these were provided by the software. CLARK, CLARK-s, Diamond, and Kaiju were the only four software in our study without a pre-built software provided. With 12 threads of CPU used on UGA’s high memory computing node, building of CLARK’s database took over 42 hours to complete, with over 400 GB memory utilized (Table I). CLARK-s database, required to build on top of the CLARK’s database, took around 40 additional hours to complete, with around 300 GB memory utilized (Table I). Building of Diamond’s database, with the same computational setting, completed in ~2.4 hours utilizing ~ 8 GB, while Kaiju’s database took ~ 5 hours to complete utilizing ~115 GB of memory (Table I). As for analysis time, using 12 CPU on UGA’s high memory computing node, Diamond used ~5 hour on average to classify one sample and BLASTN used ~2 hours to classify one sample. Rest of the software could finish classifying one sample within 5 minutes at most (Table I).

**Supplementary Text2**

*Lung and spleen sample comparison*

The DA taxa identified between lung and spleen samples were similar with those identified between lung and kidney samples (Table SII.8, Figure S2).

Kaiju identified the highest number of DA species (484 taxa), while Diamond identified the lowest (44 taxa) (Figure S2a). All the DA taxa were more abundant in the lung than in the spleen samples (Figure S2b). Six species (*Mycoplasm pulmonis*, *Mycoplasma bovoculi*, *Mycoplasma neurolyticum*, *Bordetella pseudohinzii*, *Bordetella bronchiseptica*, and *Bacteroides uniformis*) were identified as the DA taxa by all software (Table SII.8). Kaiju has the highest number of distinct DA species taxa (335), followed by centrifuge (268), and BLASTN (46) (Figure S2a).

At the Phylum level, “p\_\_Bacterodietes”, “p\_\_Tenericutes”, “p\_\_Cyanobacteria” ,“p\_\_Protebacteria”, and “p\_\_Firmicutes” were as DA identified by all the software (Figure S2c). Taxa "p\_\_Aquificae”, "p\_\_Actinobacteria”,and “p\_\_Fusobacteria” were identified by all software except for Diamond. Archaea phylum, "p\_\_Euryarchaeota”, was still the Archaea taxon identified by BLASTN, Centrifuge, and Kaiju, however, the rest of the Archaea taxa were either only identified by Kaiju and Centrifuge, or Kaiju alone. Virus taxon, “p\_\_Negarnaviricota”, was only identified by Centrifuge as differentially abundant, while Kaiju only identified the virus taxa “p\_\_Nucleocytoviricota” and “p\_\_Uroviricota”. Morever, CLARK also reported the virus taxon, “p\_\_Uroviricota”, as significantly abundant.

*Kidney and Spleen sample comparison*

Finally, we identified the DA taxa between Kidney and Spleen samples (Table SII.9, Figure S3). The number of species identified ranges from 6 by Diamond and 57 by BLASTN (Figure S3a). More taxa were identified significantly abundant in the Kidney samples than in the Spleen samples, especially at the genus level (Figure S3b). Kaiju, the software that identified the second highest number of distinct DA taxa at the species level, has five out of ten distinct taxa reported as viruses (Figure S3a). In general, only 1 species (*Leptospira interrogans*) and 4 phylum taxa (“p\_\_Spirochaetes”, “p\_\_Bacteroidetes", "p\_\_Cyanobacteria”, and “p\_\_Proteobacteria”) were reported by all software (Table SII.9, Figure S3c). The Phylum taxon “p\_\_Firmicutes” was identified as the DA taxon by all software except for Diamond. Kaiju solely identified the virus taxon, “p\_\_Negarnaviricota”, as DA taxon in this anlysis.