*Epidemiology and Infection*

Consequences of organ choice in describing bacterial pathogen assemblages in a rodent population.

P. Villette, E. Afonso, G. Couval, A. Levret, M. Galan, C. Tatard, J.F. Cosson, P. Giraudoux

**Supplementary Material 3 - Case study**

We used a case study to explore the consequences of organ choice in detecting host-population-level differences in bacterial assemblages. We used pooled bacterial assemblages for each animal to test for population-level differences, and then conducted the same test with each organ to determine if all organs generate the same conclusion. We predicted that host-population-level differences in bacterial assemblages would be more easily detected in the liver and spleen because these two organs are likely to host the greatest bacterial richness, and that lower bacterial richness in the other organs would make detecting population-level differences, if they existed, more challenging.

We used nonmetric multidimensional scaling ordination (NMDS) to visualize Jaccard dissimilarities between individual organ bacterial assemblages and the bacterial assemblages of each animal when organs were pooled together. We conducted PERMANOVAs with each organ individually with a Bonferonni correction of α= 0.01 to determine if each organ’s OTU community differed between sites. We then pooled organ data within animals to determine if using all organs changed the PERMANOVA results (ie. whether OTU assemblages differed between Arc-sous-Montenot and Censeau).

NMDS ordination of Jaccard dissimilarity indices shows that when organ assemblages are pooled, Arc-sous-Montenot and Censeau animals form distinct clusters (Supplementary Figure 2). Clustering within organ types is less clear, but the PERMANOVA analyses detected location effects when all organs are pooled together, and when the kidney was considered alone, but not when the spleen, liver, lung, or heart assemblages are viewed alone (Supplementary Table 4).

****

Supplementary Figure S2. Jaccard dissimilary-based NMDS ordination of potentially pathogenic bacterial assemblages in the heart, liver, lungs, kidneys, spleen, and pooled organs of 13 male Arvicola terrestris collected in November 2014 in the communes of Arc-sous-Montenot (A) and Censeau (C) in Franche-Comté, France. Individual animals are indicated by number; in instances where points overlap, numbers are shown next to their respective points (e.g. animals 1,4,5,8 and 9 had identical lung assemblages and therefore map to the same point in the lung panel).

|  |
| --- |
| Supplementary Table S4. PERMANOVA results for location effects for each organ individually, and for organs pooled within animals. A bonferonni correction of α=0.01 was applied to the individual organ PERMANOVAs. |
| Organ | F | df | R2 | p | significant |
| Heart | 2.94 | 1,10 | 0.23 | 0.045 | no |
| Liver | 3.53 | 1,6 | 0.37 | 0.02 | no |
| Lung | 10.29 | 1,7 | 0.60 | 0.011 | no |
| Spleen | 1.77 | 1,10 | 0.15 | 0.06 | no |
| Kidney | 3.20 | 1,9 | 0.26 | 0.009 | yes |
| Pool | 2.92 | 1,11 | 0.21 | 0.008 | yes |