# Metagenomic analysis of the pig gut microbiota and association with Salmonella status

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# Metagenomic Analysis of The Pig Gut Microbiota and association with *Salmonella* status

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**Background**

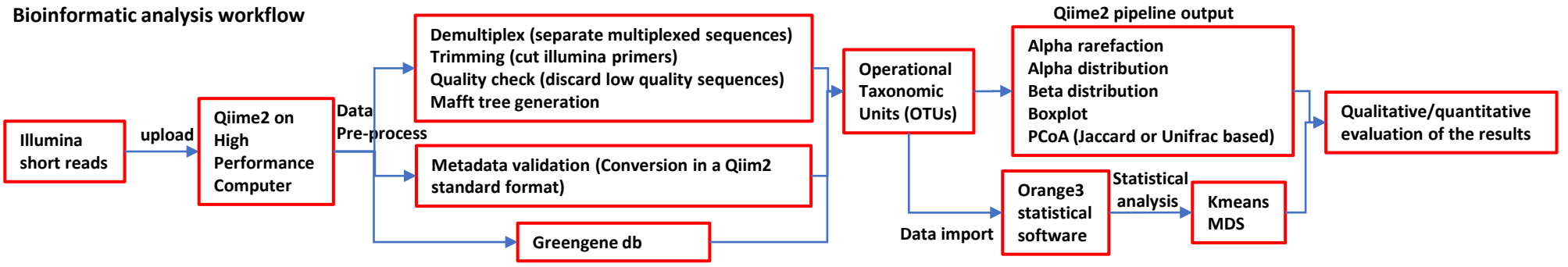
Intestinal microbiota species richness and relative abundance can be linked with the health state of the animals. Recent studies have uncovered the importance of host heterogeneity in infection with zoonotic pathogens, and it has been shown that a minority of the infected individuals are responsible for the majority of the infections (known as 'super-shedders'). A better understanding of the composition of the microbiota of super-shedders may facilitate targeted interventions with, for example, pre and pro-biotics, to reduce colonisation and shedding.

**Aim of study:** to investigate whether there was any association between *Salmonella* shedding status and microbiota heterogeneity.

**Methods:** 16s metagenomic analysis was conducted on samples (faeces and GI contents collected *post-mortem*) from two different studies (666 samples in total). Microbiota species richness and relative abundance were compared with clinical and husbandry data using software for metagenomic and statistical analysis (Qiime2 and Orange3).

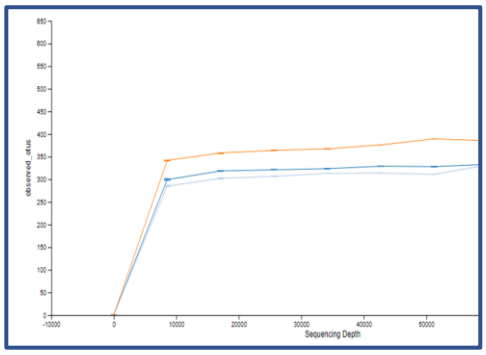
**Results:** The study detected small, but statistically significant differences between sample types, and between the different groups of pigs with regard to the bacterial species richness with implications for our understanding and potential mitigation of foodborne zoonoses.

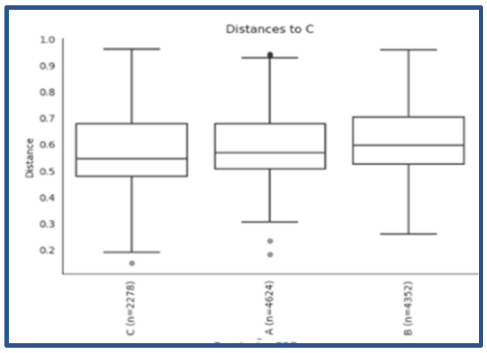
## Bioinformatic analysis workflow




## Study 1:Assess the microbiome composition in experimental infected piglets (ISS/IZSLER)

- Three groups: 30 piglets in total. Two separate Groups (A and B) were challenged with *S.* Typhimurium (10 piglets each group), and 10 piglets used as a control group.
- Faecal samples were taken at T0 to T3. *Post-mortem* samples were also taken from ileum, caecum and colon.
- Temperature, serological data, MRSV (+-) and CFU data were collected at T0 to T3.
- Samples taken from faeces (different dates), ileum caecum and colon (*post-mortem*).
- **Usable Metadata:** Challenged pigs, type of sample. Temperature, serological data, MRSV (+-) and CFU data were collected from T0 to T3.



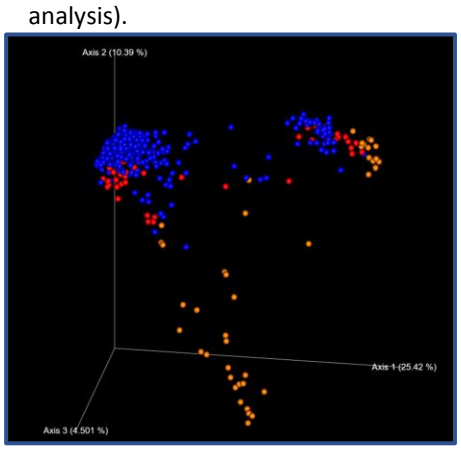



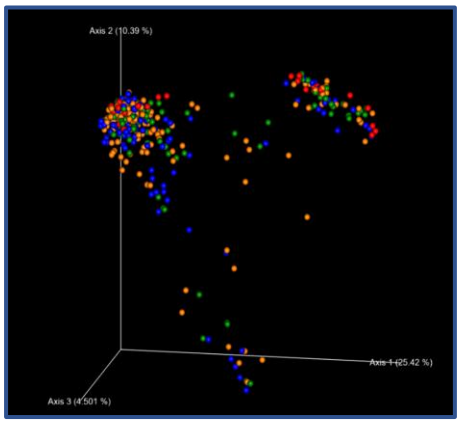

***Salmonella* challenged pigs VS control group - Left image - the α – rarefaction curve, right image - the β-distribution of the microbiota from challenged pigs (Orange= control; light blue= group A; dark blue= group B.**

- Both analyses show a small, but statistically significant difference between control and challenged group (A->B= H 1.98, p 0.007; A->C= H 4.47, p 0.001; B->C= H 4.84, p 0.001 in β-distribution (Permanova pseudo-F values). In conclusion both A and B differs from the control group C, and the difference between A and B, however statistically significant, is minimal.

## Study 2: Explore the microbiota composition in different shedding classes (Low, Intermediate, High) (ANSES)

- 45 piglets challenged by a monophasic variant of S. Typhimurium.
- Samples taken from faeces (different dates before and after inoculation, during a 3-week follow-up.), ileum caecum and colon (post-mortem).
- Temperature, serological data and CFU data were collected for the 45 piglets before and after inoculation during the 3 weeks.
- **Usable Metadata:** Type of sample, location of pigs during the experiment, sex, CFU, class (LS,IS,HS), place of birth. Temperature, serological data, CFU (currently under analysis).

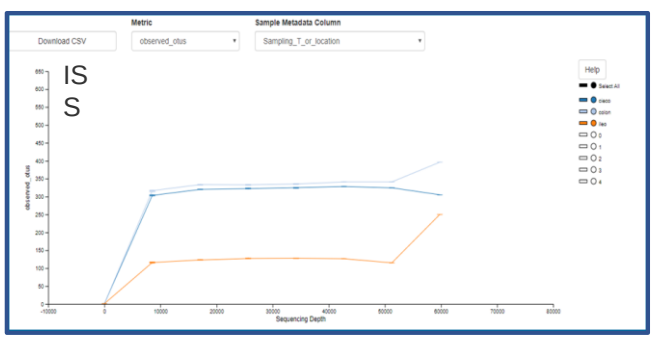


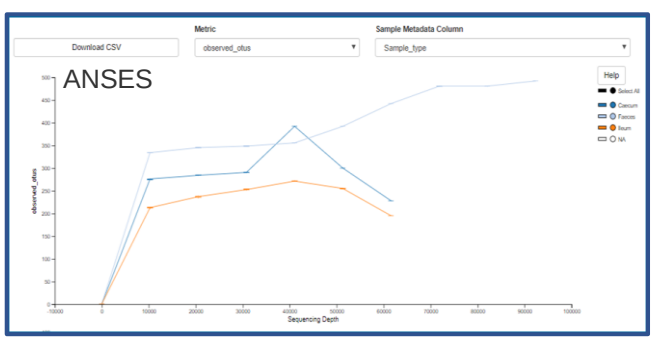




**Unweighted Unifrac PCoA: Comparison between sample type and shedding classes Comparison of two PCoA considering sample type (left) and shedding class (right).**

- It is possible to see a clear grouping of the samples isolated from ileum (orange) respect to the ones isolated from caecum (red) or faeces (blue) (left image).
- On the right image, (shedding class) it is not possible to see any clear pattern even if the β-distribution suggested small, but significant differences between control group and shedding classes.
- It was not possible to find differences in OTUs composition between the proposed High, Low, and Intermediate shedding classes.

## Study 1 (ISS/IZSLER) and Study 2 (ANSES) comparable metadata: Sample Type.




α – rarefaction curves for sample type showed that it is possible to notice a difference in the OTUs number in ileum environment which is much less than what found in caecum and faeces. All the other analysis conducted (β-distribution, Unifrac PCoA, Kmean and MDS) confirmed this distribution.

## Discussion and future plans

- Statistically significant variations were detected in the relative abundance of bacteria in different intestinal locations (ileum, caecum and faeces), between the control and challenged groups.
- This study concurs with previous studies demonstrating the composition of microbiota varies in different parts of the intestinal tract.
- The biological importance of these small variations requires further investigation.
- To date, for all the other metadata it was not possible to find statistically significance in the OTUs composition/abundance.
- Shotgun metagenomic data may provide more detailed information on species level differences.
- A combined gene-targeted bacterial and virologic metagenomic analysis could shed a light on the interactions (synergic or divergent).

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