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A Metabolic Pattern of Influenza A Virus Infected *Sus scrofa:* Perturbations on Eicosanoids and Gut Metabolism

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Introduction:

- Acute infections of the upper respiratory tract are associated with 4 million deaths per year one of the most frequently causes of death world wide[1].

- **Influenza A virus** infections in combination with secondary bacterial (*S. aureus*, *S. pneumoniae*) infections can lead to even higher mortality rates.

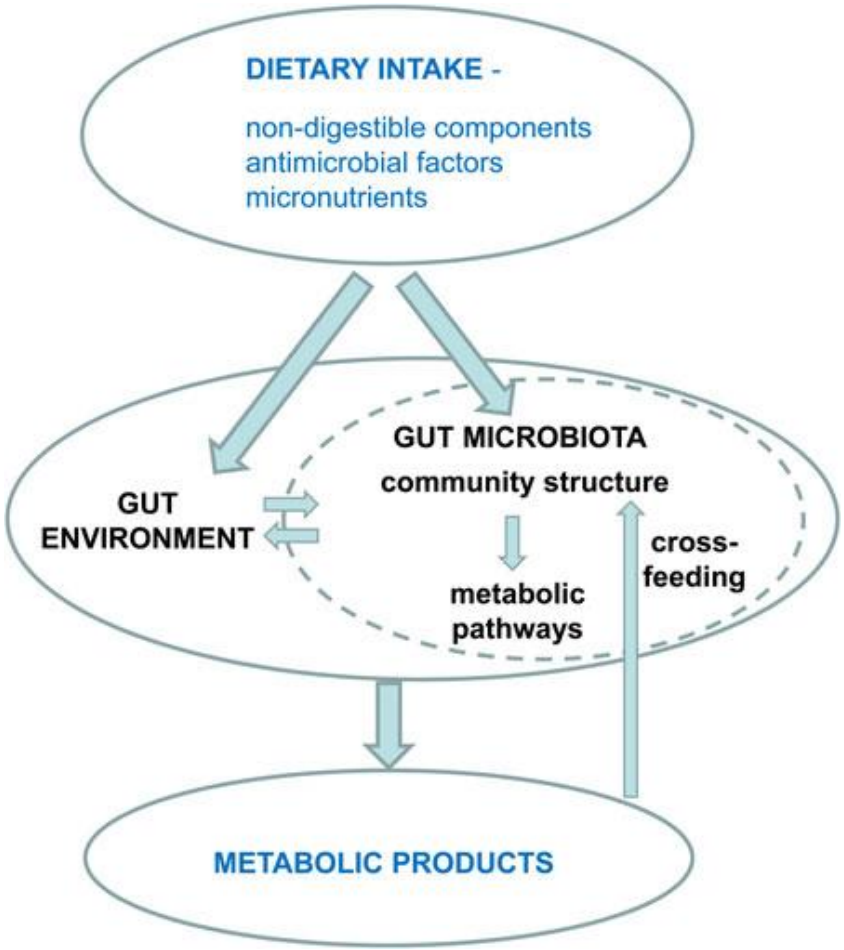
- The **pig** as a new animal model is **more close to humans** (microbiome, genetics, immune system, organ structure and function[2]) compared to mouse or cell culture experiments.

→Hypothesis I: Are there infection-related perturbations in the pig fecal metabolome?

→Hypothesis II: Is the eicosanoid profile altered in infected pigs?

[1] Walker CL, Rudan I, Liu L *et al.* Global burden of childhood pneumonia and diarrhoea. *Lancet* 381(9875), 1405-1416 (2013).
[2] Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. The pig: a model for human infectious diseases. *Trends Microbiol* 20(1), 50-57 (2012).
[3] Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol* 13(5), 321-335 (2013).

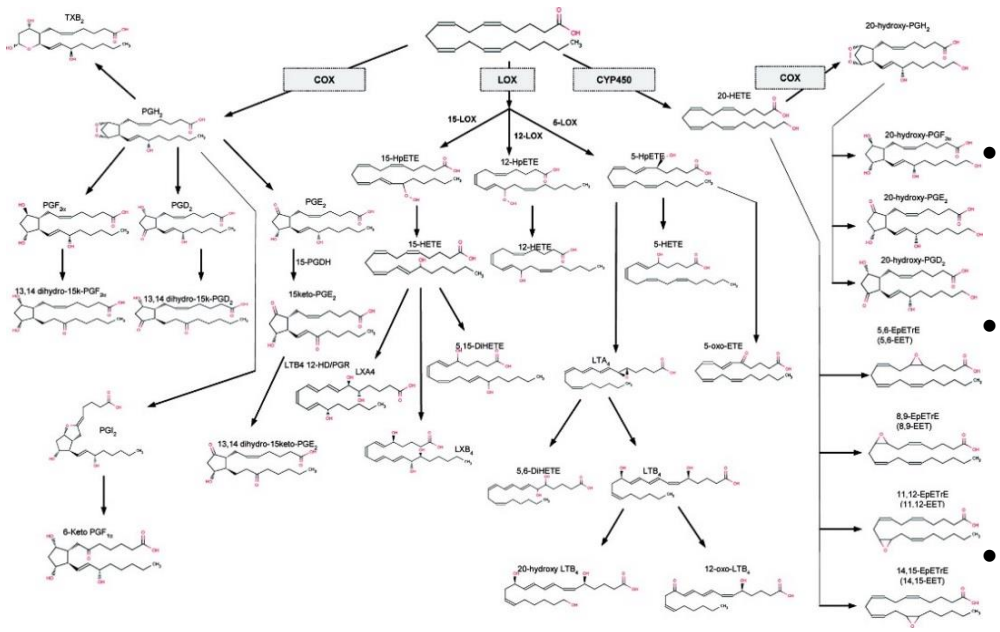
Hypothesis I: Interplay between host and microbiota



- protocol optimization for homogenization and extraction of metabolites from fecal material
- GC-MS and ^{1}H-NMR measurement
- difficult distinction between metabolites from **gut microbiota** (e.g. short chain fatty acids) and **host metabolites**
- **aim: specific metabolic pattern related to infection diseases (mono-infection and co-infection)**

Flint et al: Links between diet, gut microbiota composition and gut metabolism, The Nutrition Society, 2015

Hypothesis II: Role of oxidated lipids (eicosanoids) in infection



- eicosanoids are part of the immune response (activation **and** resolving)
- play a role in: inflammation, fever, allergy, pain, cell growth or blood pressure
- extraction and purification steps[1] needed for LC-MS/MS measurement using dynamic multiple reaction monitoring
- **aim: eicosanoid profile as marker for immune response (mono-infection and co-infection)**

Figure: Masoodi et al: Comprehensive Lipidomics Analysis of Bioactive Lipids in Complex Regulatory Networks, Anal. Chem. (2010)

[1]Gomolka *et al*. Analysis of omega-3 and omega-6 fatty acid-derived lipid metabolite formation in human and mouse blood samples. *Prostaglandins Other Lipid Mediat*. 94, 81–87 (2011)

Infection experiment conditions:

animals	• Group of pigs (german landrace) from a commercial • high health status (negative tested for influenza infection) • control and infection group • free access to water and standard diet
infection	• Influenza A virus • nasal administration
sample material	• fecal material, lung, spleen, blood plasma and bronchoalveolar lavage [BAL]
timepoints	• 0, 2, 4, 7, 14 dpi for feces • 4, 7, 14 and 31 dpi for tissues and body fluids
replicates	• 4 for fecal material • at least 5 for infected tissues and body fluids

Results: Analysis of fecal material

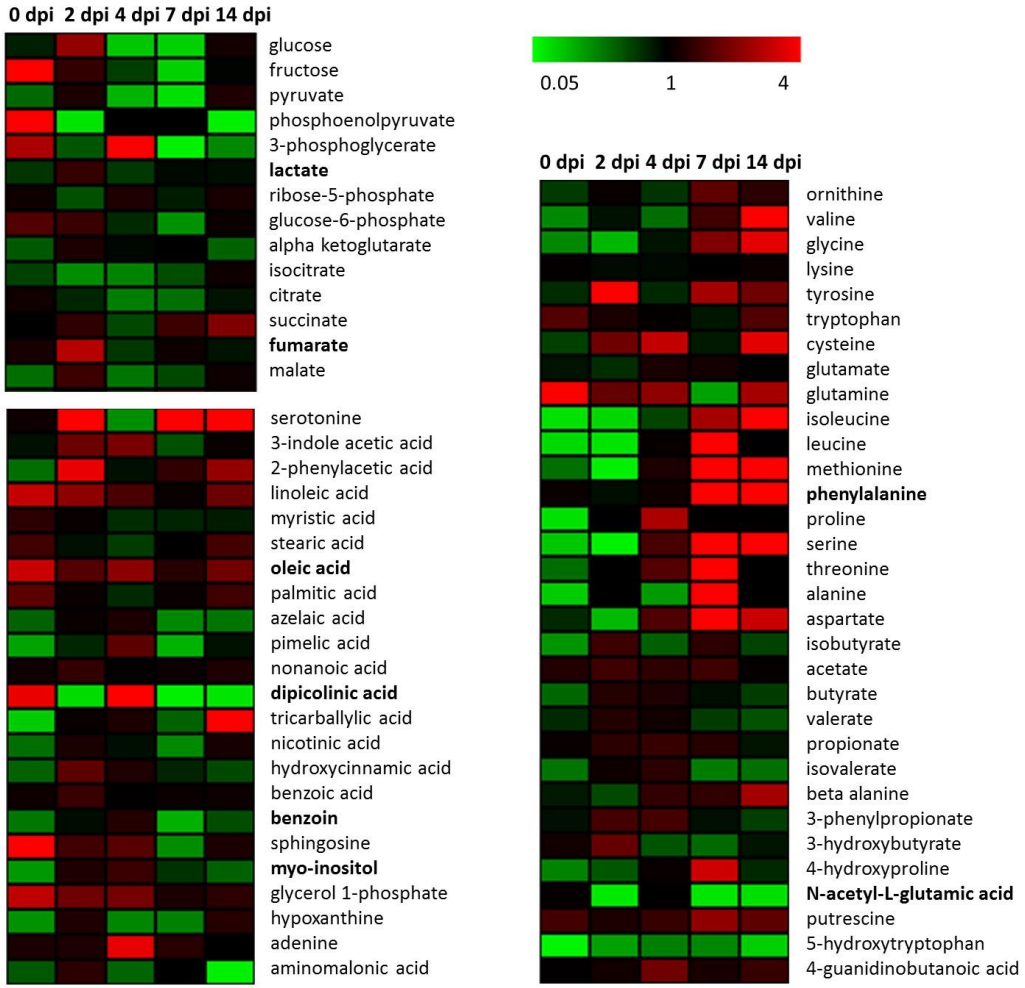


Figure 1. Heatmap displaying fold changes (infection/control) of all detected metabolites from ^{1}H-NMR and GC-MS analysis of feces. Bold names of metabolites indicate significant changes ($p<0.01$, unpaired t test) for at least one time point.

Results: Eicosanoid profile

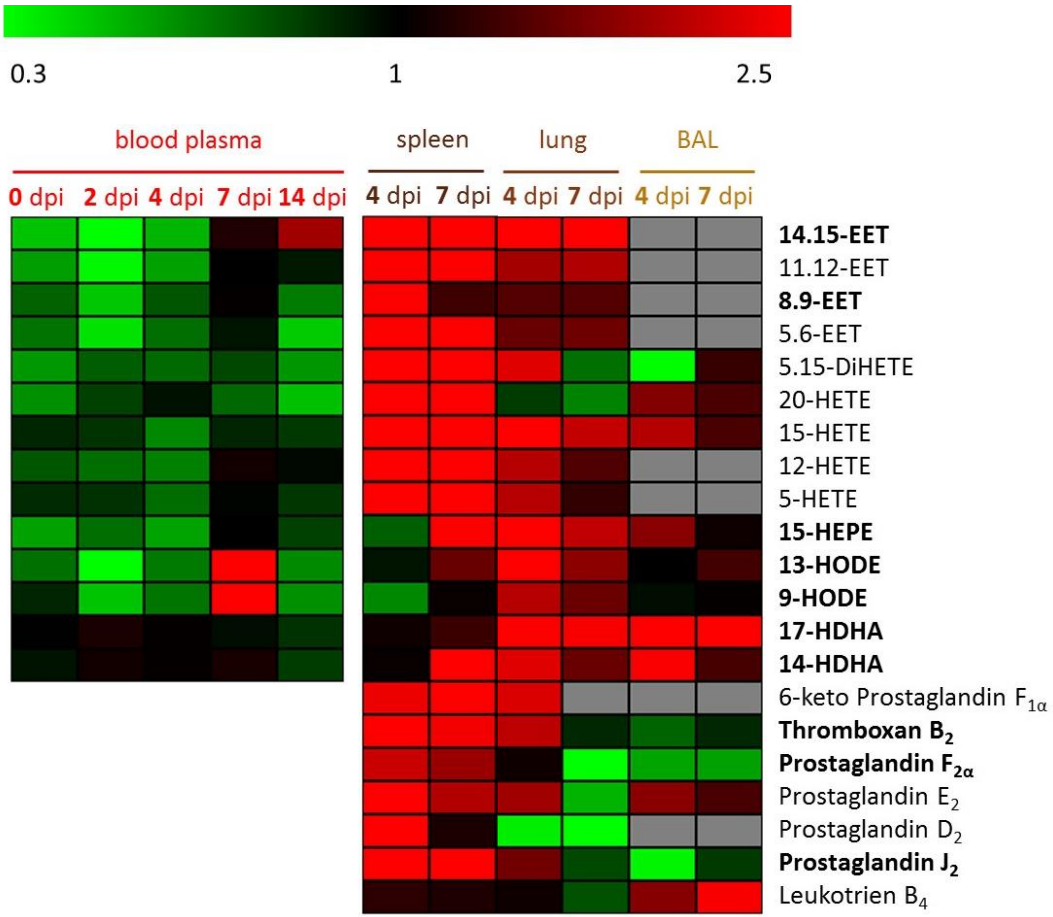


Figure 2. Heatmap displaying fold changes (infection/control) of detected eicosanoids from LC-MS/MS measurement of organ and biofluid. Bold names of eicosanoids indicate significant changes ($p<0.05$, unpaired t test) during infection at least in one sample type and time point. Grey fields: below quantification limit.

Discussion:

- Pigs infected with a low pfu of Influenza virus **didn´t show any clinical scoring** (like increased temperature, body weight loss), but a positive virus titer.
- Analysis of fecal metabolome reveals a **high dynamic range** for detected metabolites concerning **time and single animal**.
- Eicosanoid profiling delivers a hint for **acitivated immune response in the spleen** at 4dpi (increased level of pro-inflammatory prostaglandin $F_{2\alpha}$ and thromboxane B_2).
- **Increased level of anti-inflammatory 17-HDHA** in the lung could be an evidence for resolution of the immune response at 4 dpi. ThIS lipid is also known to mediate specific antibodies against Influenza A.

Eicosanoid analysis in cell culture and mice experiments infected with *S. pneumoniae* strains

	cell culture	mouse
host strain	• 16-HBE	• B6 mice
infection	*S. pneumoniae*	*S. pneumoniae* • colonization (low dose) • acute infection (high dose)
replicates	• 4 (control and infection)	• 10 for control after 7 days • 10 for colonization at 7 dpi • 12 for infection at 2 dpi

16-HBE cells infected with *S. pneumoniae*

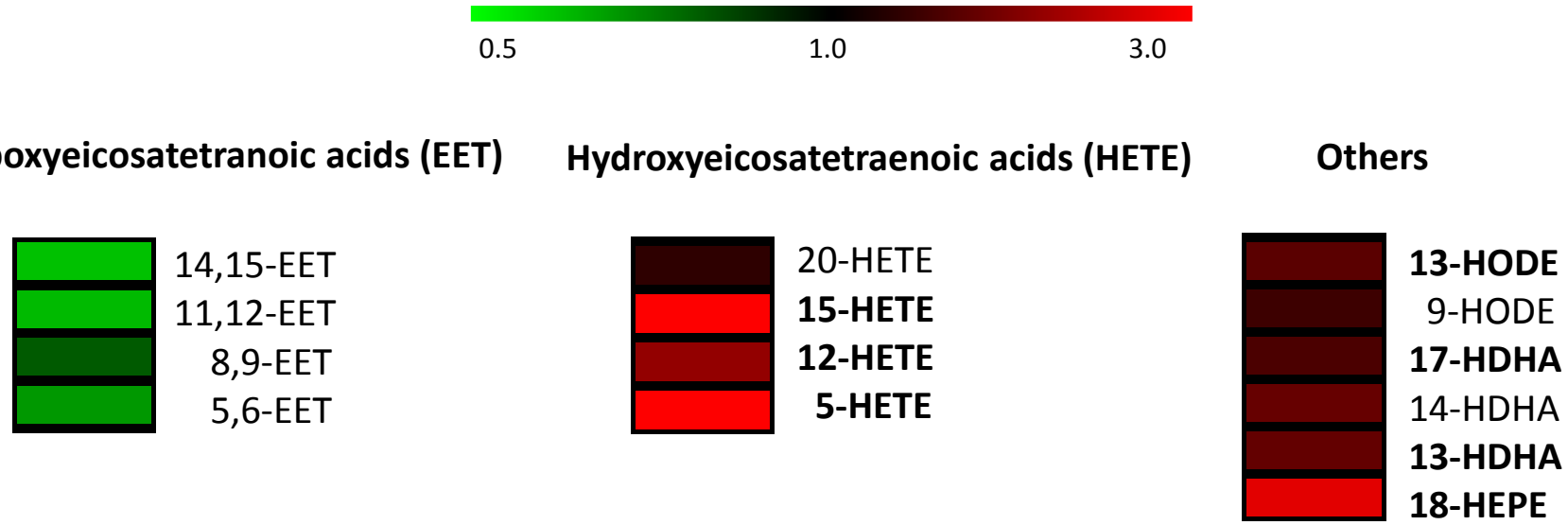


Figure 3. Heatmaps displaying fold changes (infection/control) of detected eicosanoids from LC-MS/MS measurement normalized for $1x10^7$ cells. Bold names of eicosanoids indicate significant changes ($p<0.1$, multiple t test, Holm-Sidak correction) during infection.

Mice infected with *S. pneumoniae*

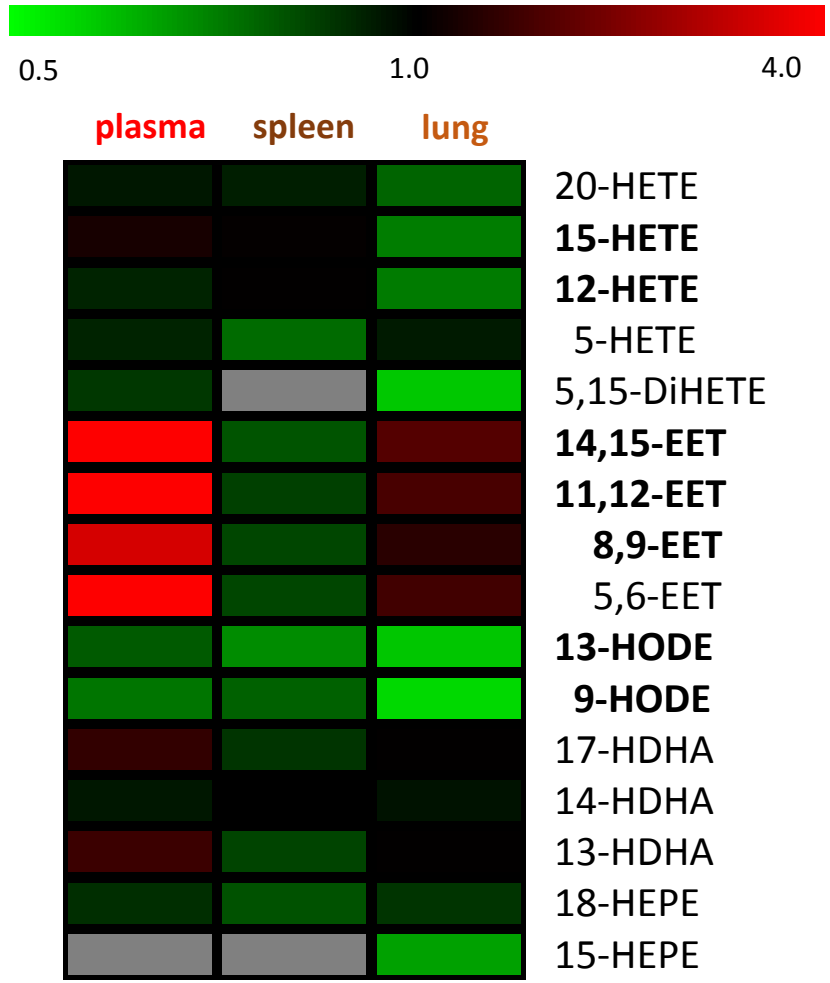


Figure 4. Heatmaps displaying fold changes (infection/control) of detected eicosanoids from LC-MS/MS measurement. Bold names of eicosanoids indicate significant changes ($p<0.05$, multiple t test) during infection at least in one sample type.

Discussion and summary:

Cell culture:

- Infection with *S. pneumoniae* leads to **numerous changes in the eicosanoid profile** of 16-HBE cells.

→ increase of different anti-inflammatory lipid mediators like 13-HODE and 17-HDHA

→ strong **activation of 5-LOX pathway**

Mice:

- Colonization of mice with *S. pneumoniae* has no influence on the eicosanoid profile
- Acute infection of mice influences the amount of eicosanoids.

→ **high** levels of anti-inflammatory **EETs in plasma samples**

→ perturbations in the HETEs level for spleen and lung tissue

Outlook:

- pig mono-infection experiment with high pathenogenic bacteria
- **co-infection** with virus and bacteria in pigs
- virus mono-infection experiments in cell culture and mice
- **co-infections** with virus and bacteria in cell culture and mice
- MSI to localize special lipid mediators in mice lung and spleen

→ Metabolomics to elucidate host pathogen interaction (multi-omics approach)
→ Is there an impact on the microbiota?
→ How is the immune system stimulated by the infections?
→ Are there differences between the host metabolome of mono-and co-infections?

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