IPVSBb

In-depth & functional characterization of the lower airways' microbial community in sick finisher pigs using a novel shotgun metagenomics approach

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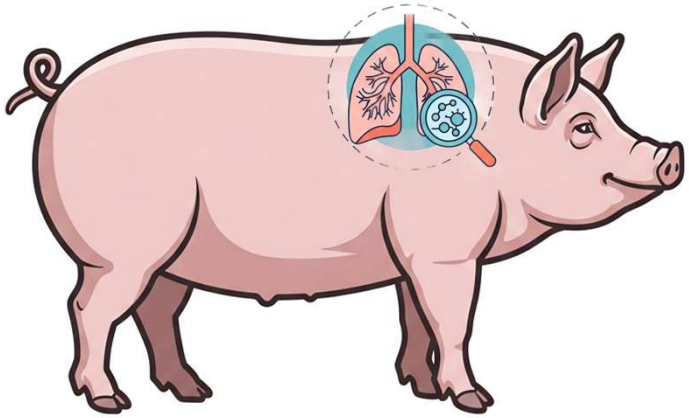
Introduction

- MSc in Molecular and Cellular Biology, University of Pisa (2021)
- Currently: **PhD student** at **PathoSense** & **Antwerp University** (promoters Prof. Sarah Lebeer and Dr. Sebastiaan Theuns) with a strong focus on the **respiratory microbiome of pigs** and **interactions between gut and lung microbiome**
- **Research Scientist** at PathoSense

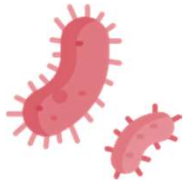
Introduction

What do we know about disease-causing microorganisms in pigs?



Viruses

Bacteria → pathogenic?
→ part of microbiome?



Viral-bacterial interactions?

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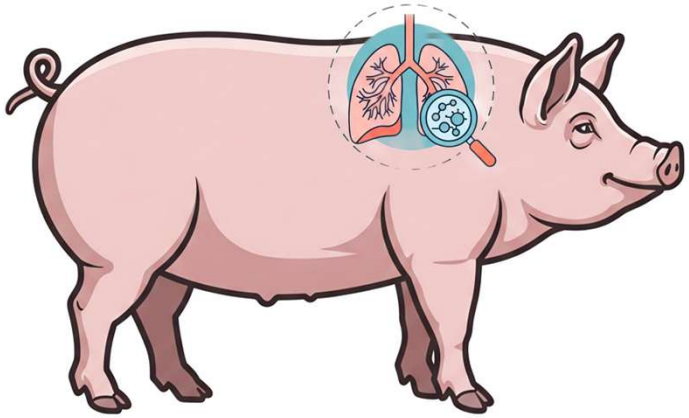

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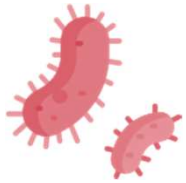
→ part of microbiome? 

Viral-bacterial interactions?

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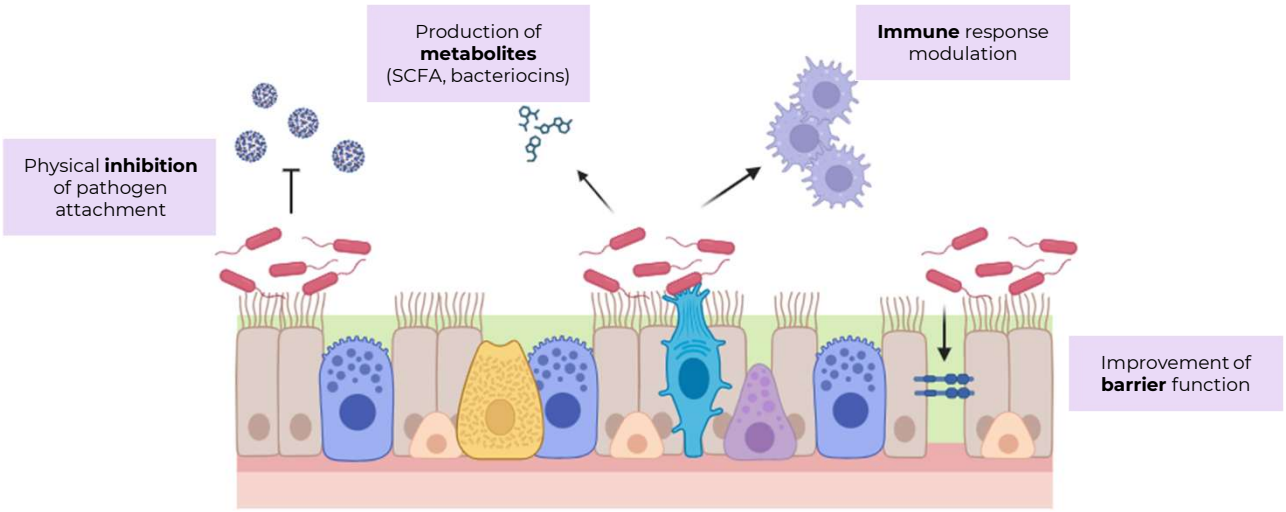
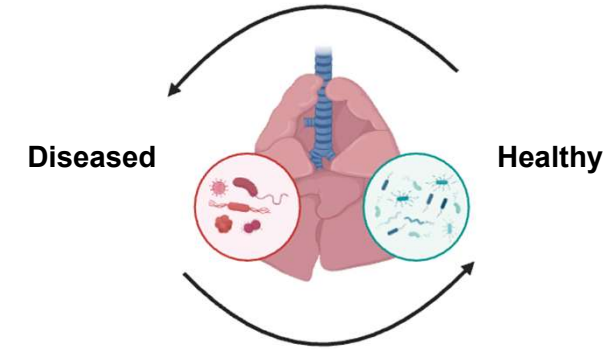
What do we know about disease-causing microorganisms in pigs?

Respiratory microbiome → Bacteria commonly present in the respiratory system





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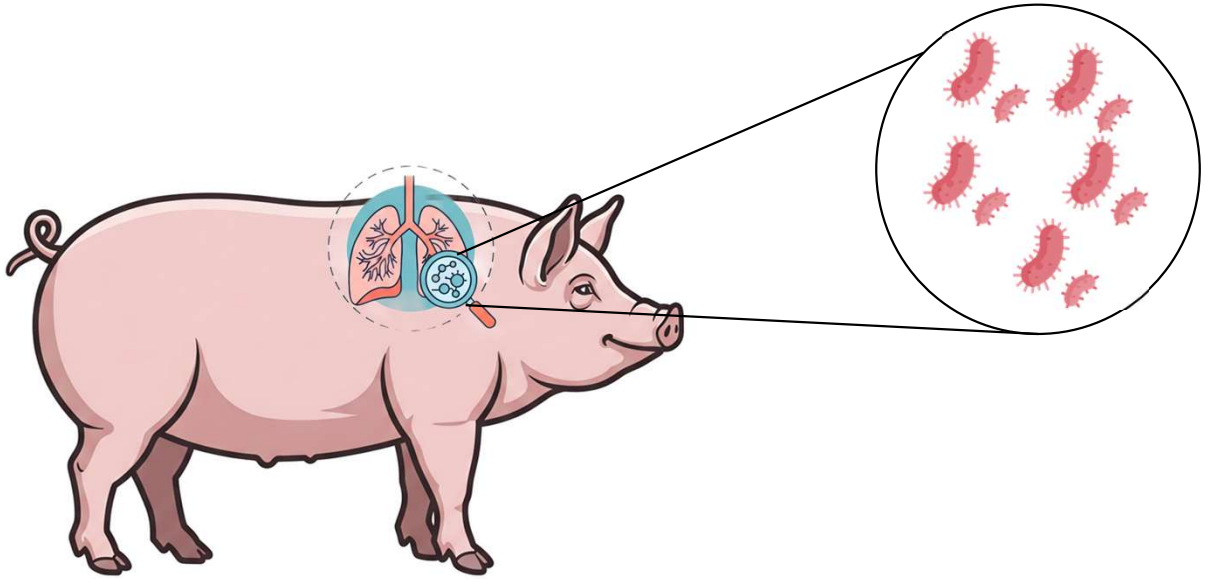

What is the actual role of the lower respiratory tract microbiome in respiratory diseases in finisher pigs?

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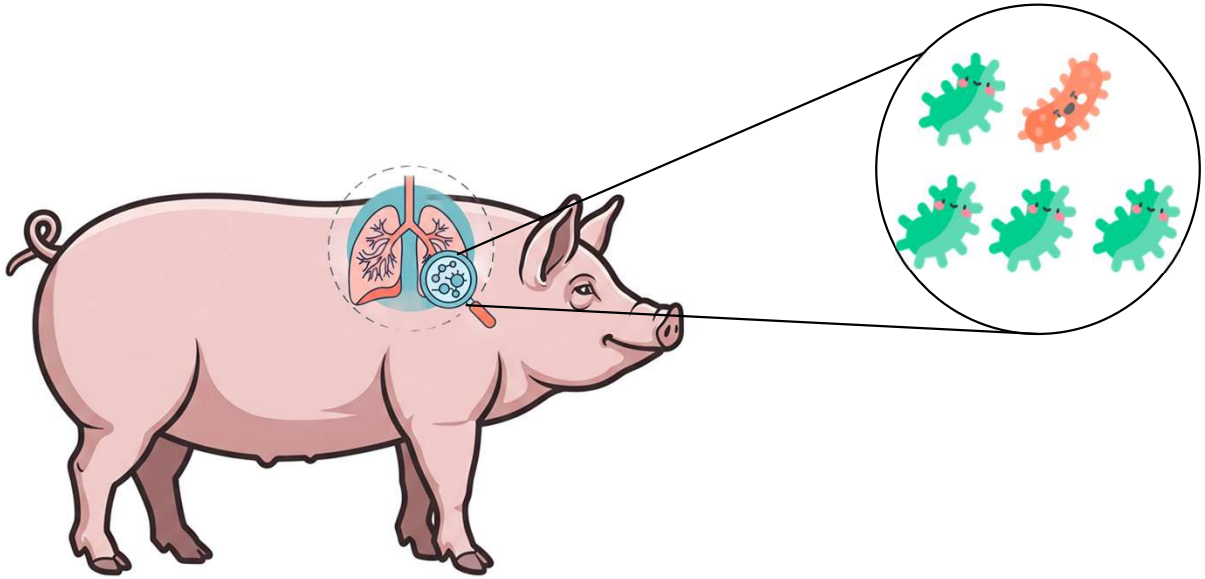
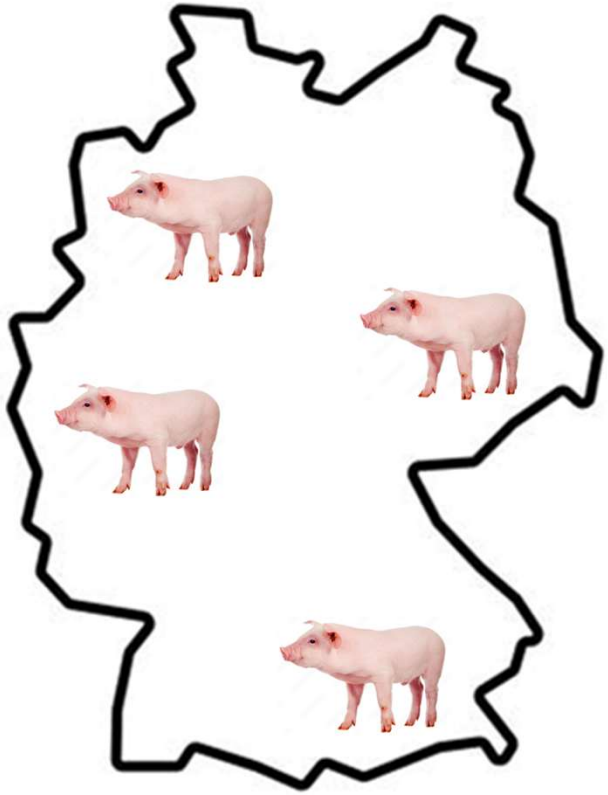


What is the actual role of the lower respiratory tract microbiome in respiratory diseases in finisher pigs?

↓

Importance of distinguishing ‘good’ bacteria from ‘bad’ bacteria

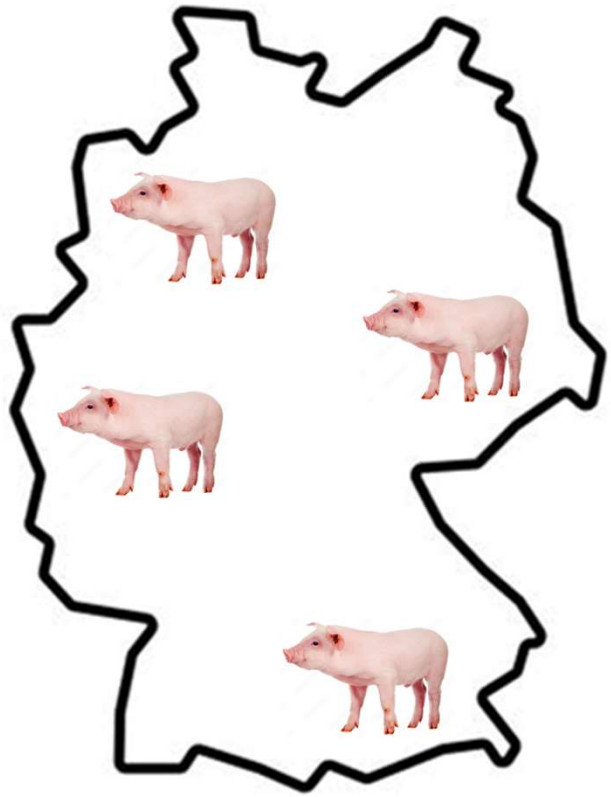
Experimental setup & Methods



- 15 German farms
- Winter period (November 23 - March 24)
- 15 pigs sampled in each farm
- Tracheo-bronchial swabs (TBS)
- Pigs aged 3-5 months old with respiratory symptoms

Paper soon to be released > Panattoni et al., 2025

Experimental setup & Methods



- PathoSense **diagnostic assay**
  - → Detection of viral infections
- Full-length **16S rRNA** gene sequencing
  - → Bacterial profile characterization
- **Shotgun** metagenomics
  - → Functional characterization (*e.g.*, virulence factors detection)

Experimental setup & Methods

Full-length 16S rRNA gene sequencing

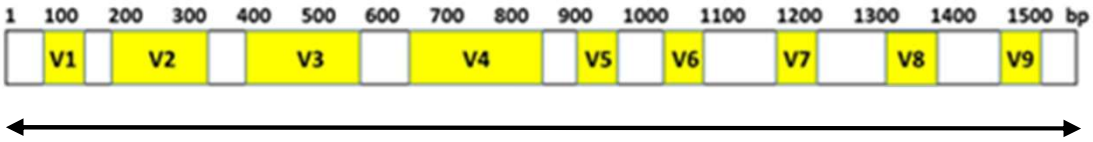
Starting sample

Bacterial DNA extraction

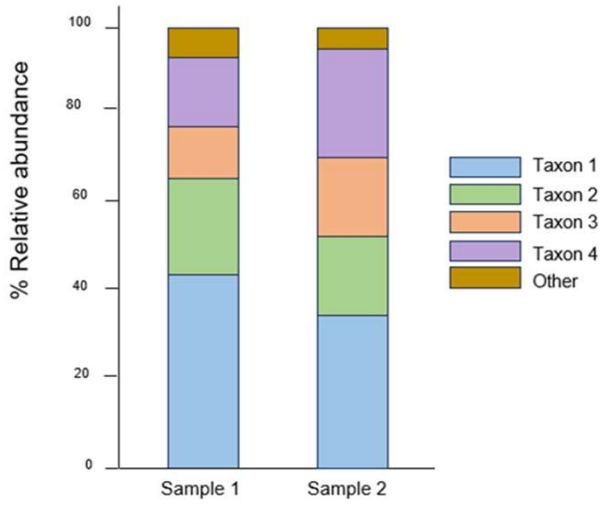
Targeted amplification

Sequencing

Taxonomic identification



Long reads → full 16S rRNA gene (appr. 1,500 bp)



Experimental setup & Methods

Shotgun metagenomics

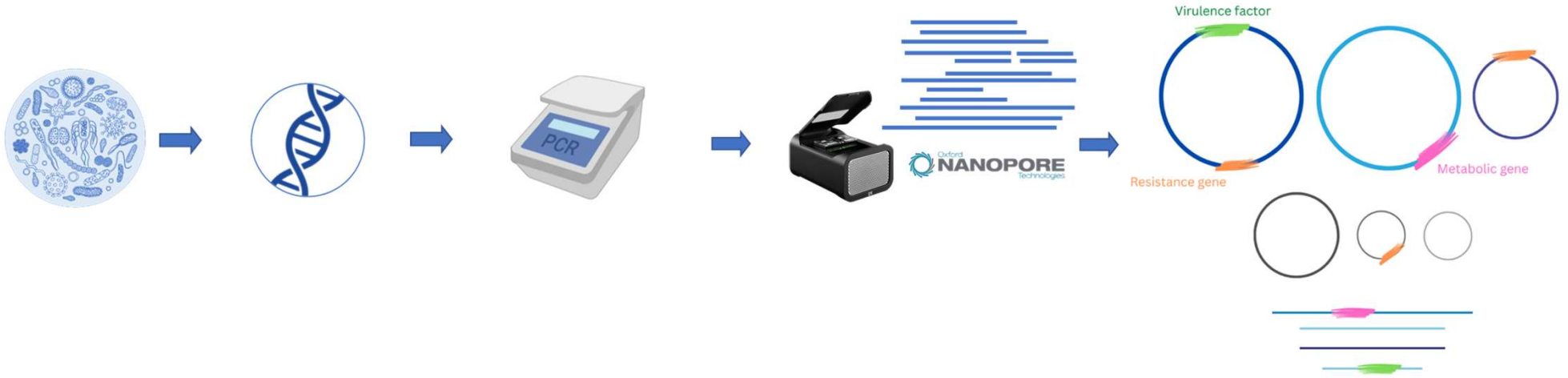
Starting sample

Bacterial DNA extraction

Random amplification

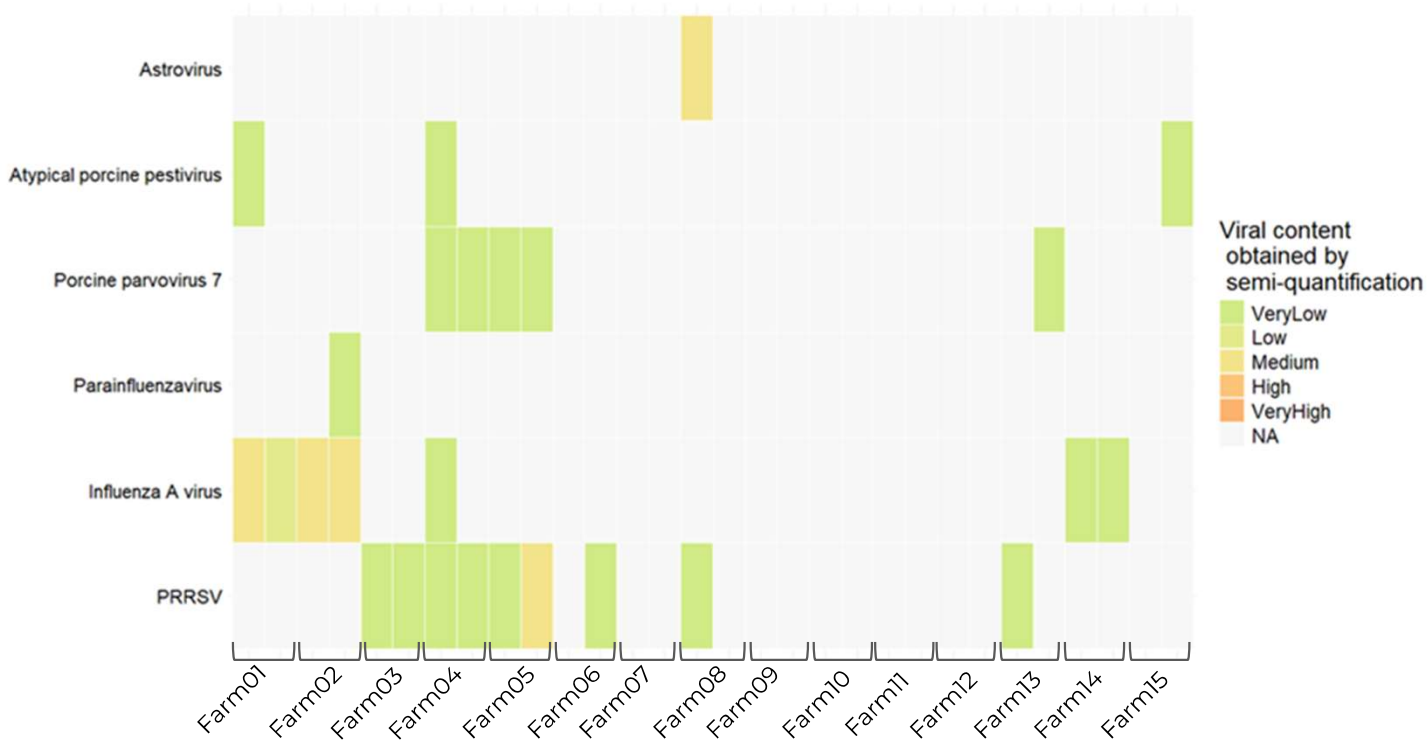
Bacterial shotgun sequencing

Metagenomic assembly / VF detection



No pre-selection!

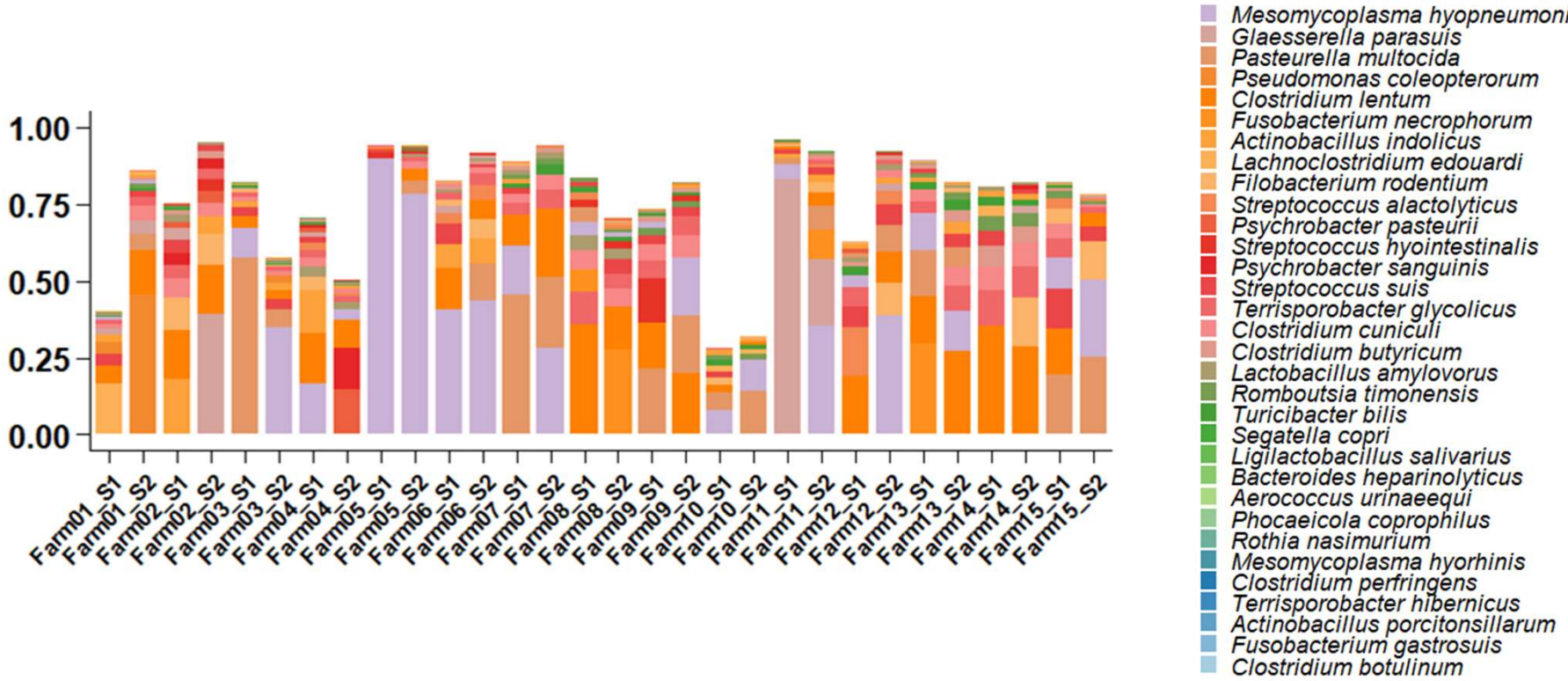
PathoSense diagnostic assay: highest prevalence of PRRSV and Influenza A



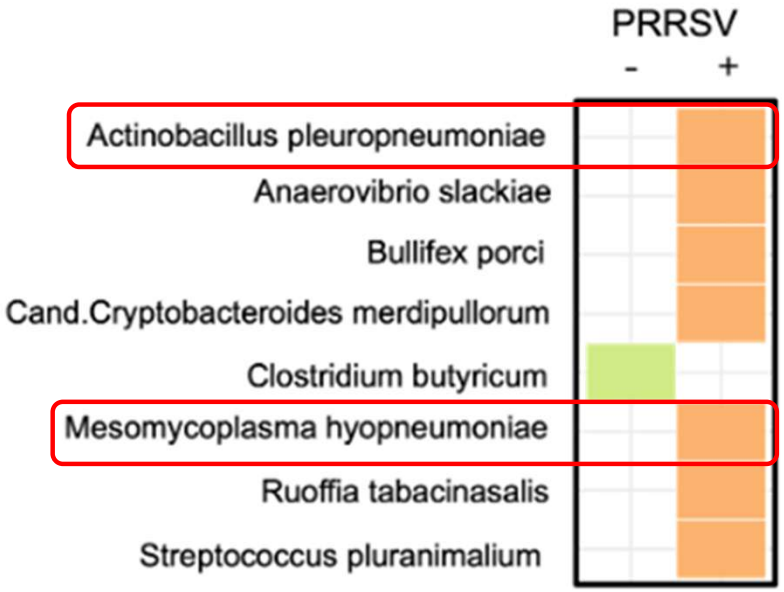
19 out of 30 samples (~ **63%**) presented **both a viral and bacterial infection** going on

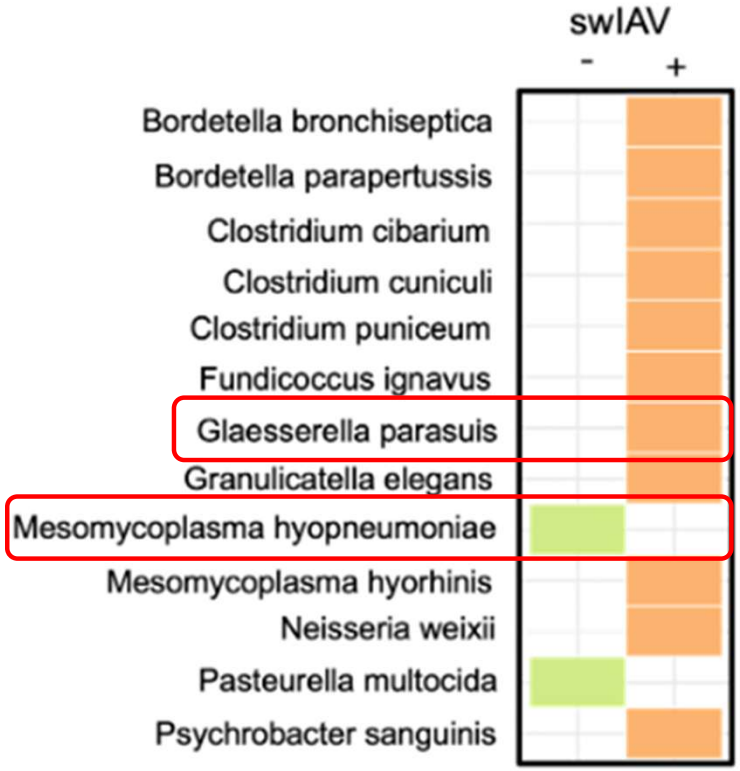
Abundances are indicated from very high to very low based on a semi-quantification method

Bacterial opportunistic pathogens dominate the lower airways' microbiome of sick finisher pigs

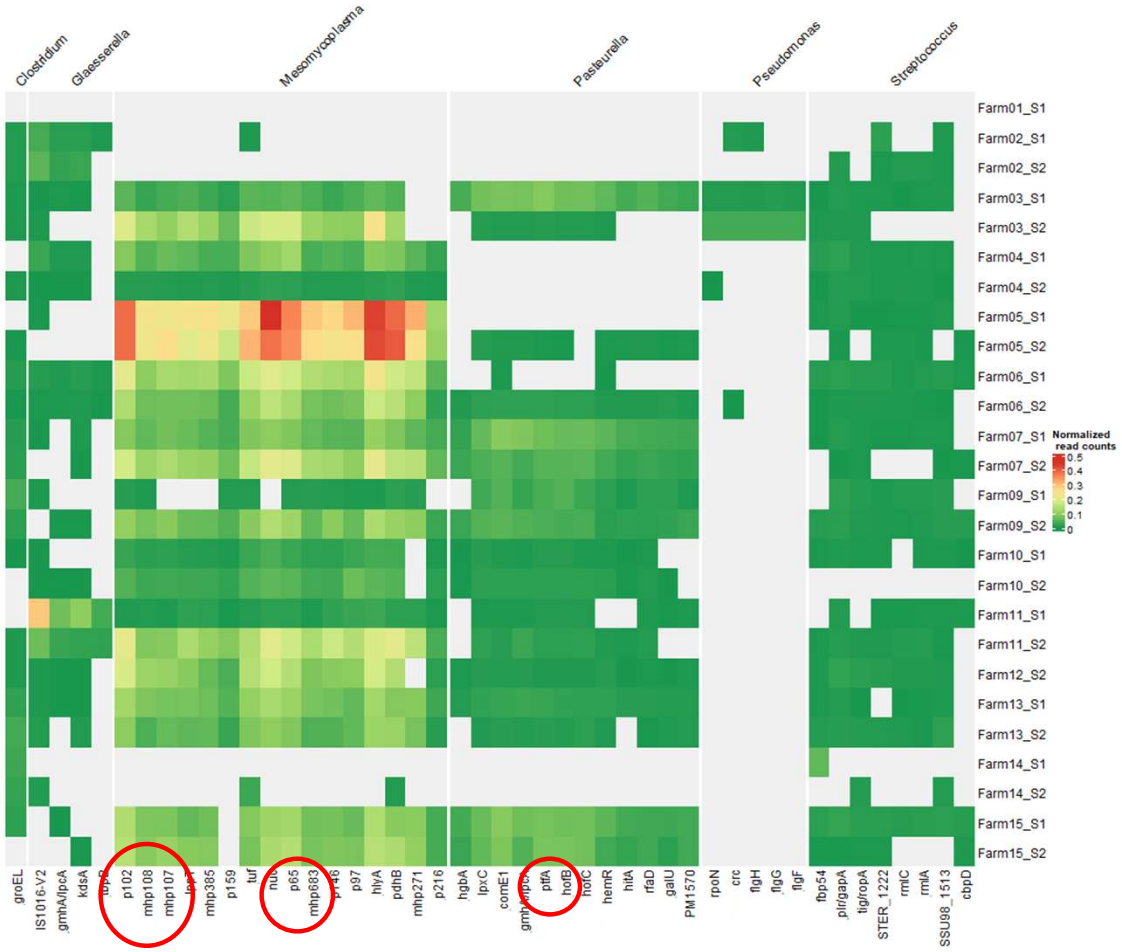


Viral-bacterial co-infections are widespread in the lower respiratory tract of diseased pigs





Virulence factors are detected in respiratory samples through untargeted and culture free detection



Technical advancement

→ low bacterial load

→ high host material

→ difficult to lyse mucus

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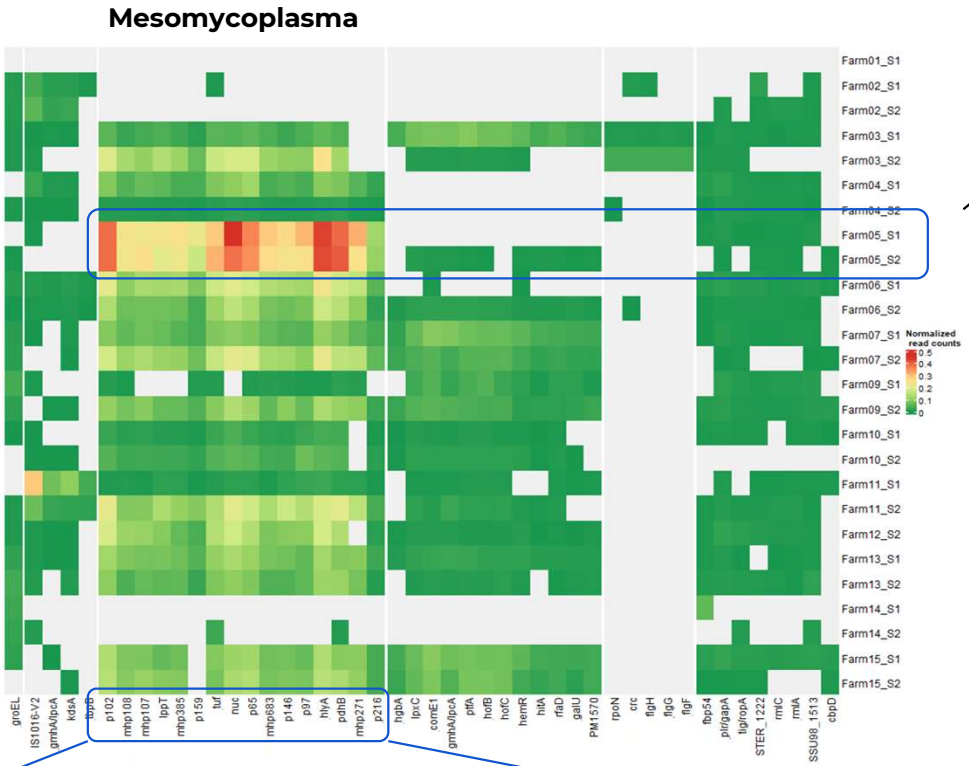
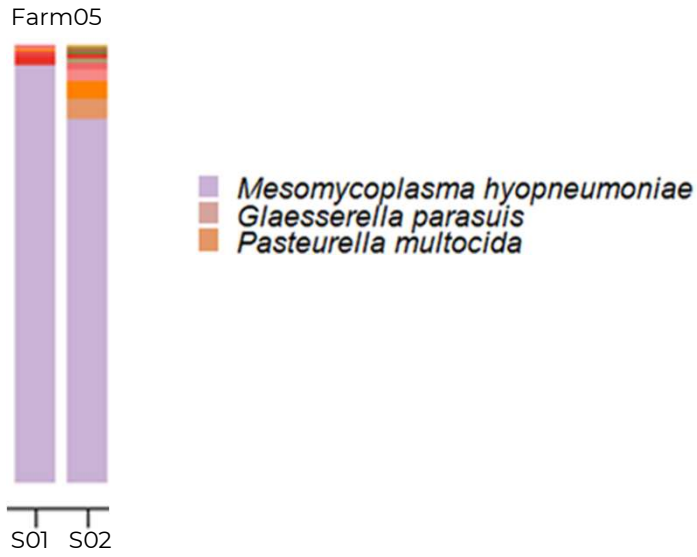


p102, *p97*, *p146*, *mhp108, mhp107*:
adhesion factors belonging to *M. hyopneumoniae*

Highest load detected in **Farm05**



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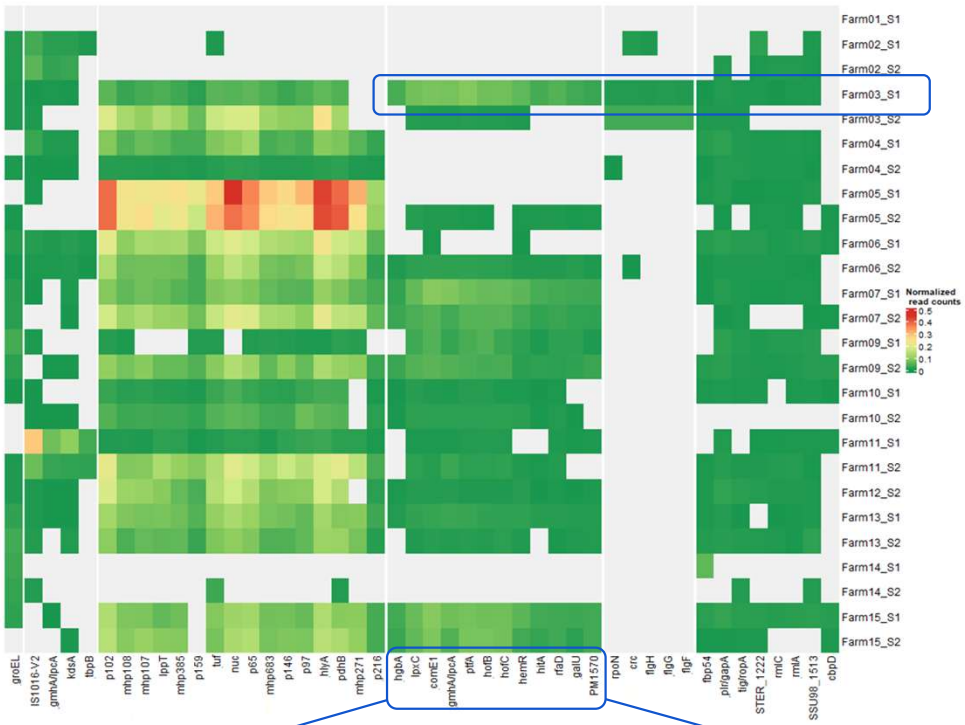
Pasteurella



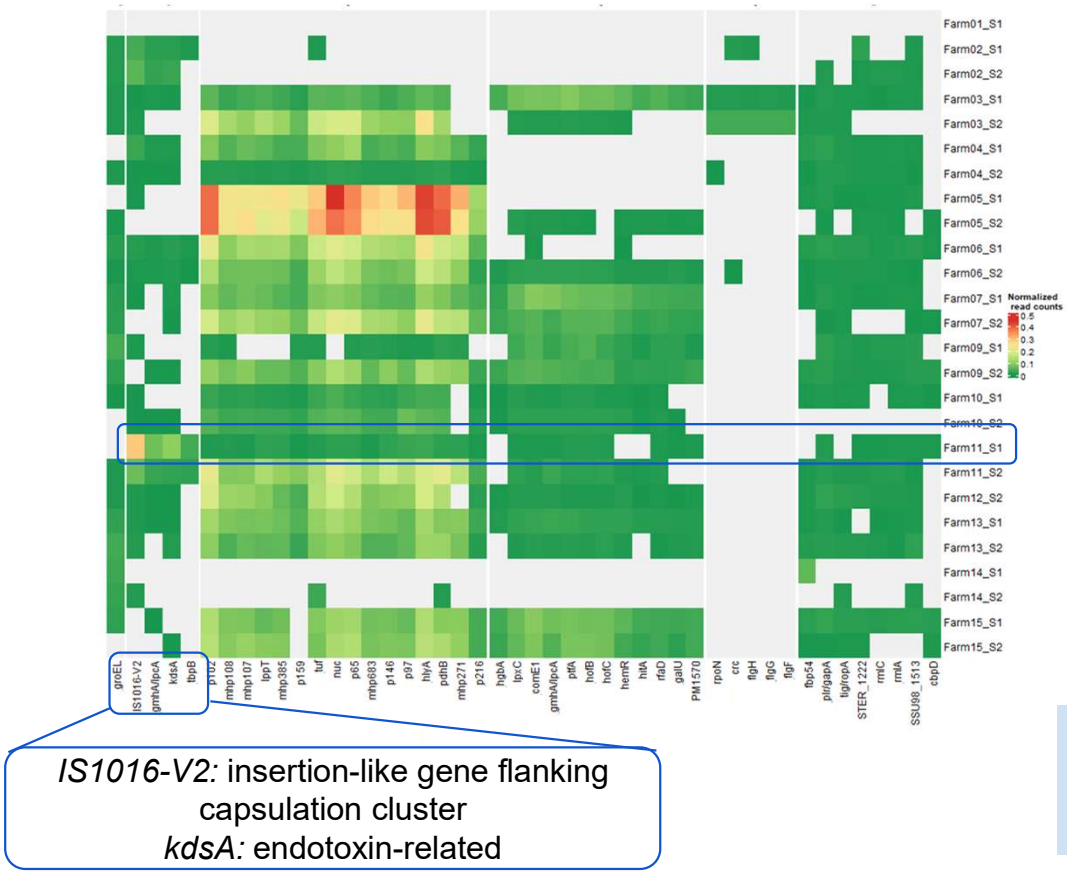
ptfA: adhesion factor
lpxC: endotoxin-related

Highest load detected in **Farm03 - S01**

Mesomycoplasma hyopneumoniae
Glaesserella parasuis
Pasteurella multocida

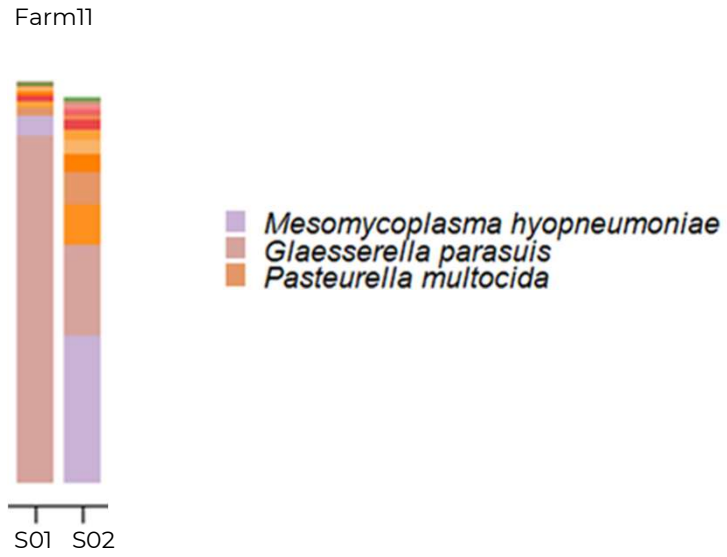
Virulence factors are detected in respiratory samples through untargeted and culture free detection

Glaesserella



IS1016-V2: insertion-like gene flanking capsulation cluster
kdsA: endotoxin-related

Highest load detected in **Farm11 - S01**

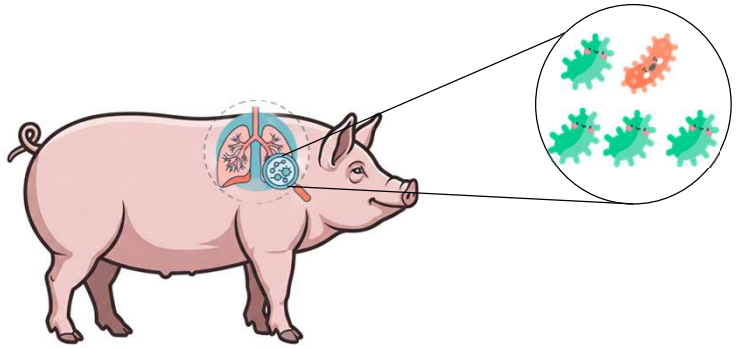


Are these virulence factors contributing to the high relative bacterial abundance?

Final considerations

- More research needs to be done to understand which virulence factors can be discriminating in a disease state

- Appropriate **detection of virulence factors** can improve the currently available diagnostics tools



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Sarah Lebeer
Sebastiaan Theuns

PathoSense

Universiteit Antwerpen

AGENTSCHAP INNOVEREN & ONDERNEMEN

Vlaanderen is ondernemen

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Ceva

Thank you for your attention!