Detection of respiratory pathogens in oral fluid; sampling recommendations in commercial conditions.

Juan Hernández-García*, Nardy Robben, Damien Magnée, Ian Dennis, Sara M. Kayes, Jill R. Thomson, and A.W. (Dan) Tucker**

Department of veterinary medicine, University of Cambridge

In collaboration with: Thermo Fisher scientific

SAC consulting (Penicuik, Scotland, UK)

Royal Veterinary College (London, UK)

Presenting,

Juan Hernández-García*, DVM, Resident ECPHM

* jh937@cam.ac.uk
**awt1000@cam.ac.uk

IMPACT OF PORCINE RESPIRATORY COMPLEX

- Respiratory problems produced lots of **losses** to the pig industry.
    - Drives increased use of **antimicrobials**, need of vaccines.
    - Drop of **performances**.
- It is complicate to completely solve some problems.
- **Complex diagnostic approach**.
    - Multiple agents are involved. Environmental factors.
    - Limitations of each scenario.
- **FORTHCOMING ISSUES**.
    - **Need to reduced antimicrobial** use will need to refine the management of PRDC
        - Quick (an accurate) diagnosis.
        - Improve interventions (antibiotherapy, treatments).

New Options in Oral Fluids (OF) for respiratory disease diagnostics.

- **MULTIPLE TYPES OF TEST CAN BE USED IN ORAL FLUIDS.**
    - **ELISA**: PRRS, PCV2, SIV
    - **PCR:** PCV2, PRRS, SIV… (some others as APP, HPS, TTV, CSF, ASF, FMD… has been described).
- **WHY WE CHOSE ORAL FLUIDS?**
    - Inexpensive sampling costs. Farmers can sample.
    - Represent large numbers. Uncertainties about sensitivity and specificity.
- **DO ORAL FLUIDS ARE REPRESENTATIVE FOR RESPIRATORY FLUIDS?**
    - Oral fluid = Saliva + retropharyngeal fluid + expected material + crevicular fluid (serum) + nasal material + faecal material.
    - Good correlations between OF and serum viremia have been described for PCV2 and PRRS ($R^2 \approx 0.6$) *.

* Pricket et al. 2008 J Vet Diagn Invest; Kim 2010 J Vet Clin

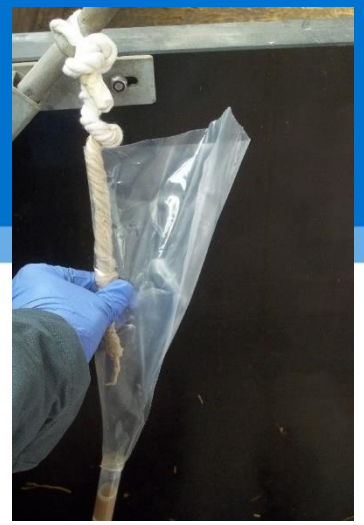
Investigating PRDC problems.

AIM OF THE STUDY

*Explore the **potential** of ORAL FLUIDS to **detect key pathogens** involved in the porcine respiratory disease complex and draw **sampling recommendations**.*

	Primary Pathogens	Opportunistic pathogens.
Virus	**PRRS** **PCV2** **Influenza type A virus** Aujeszky's disease virus Rubulavirus	Porcine coronavirus Porcine cytomegalovirus
Bacteria	***Mycoplasma hyopneumoniae*** *Bordetella bronchiseptica* *Actinobacillus pleuropneumoniae* *Pasteurella multocida* *Mycobacterium spp.* *Salmonella Cholerae-suis*	*Mycoplasma hyorhinis and other Mycoplasma spp.* *Haemophilus parasuis* *Streptococcus suis* *Actinobacillus suis* *Pasteurella multocida* *Trueperella pyogenes* *Escherichia coli* *Klebsiella pneumoniae*
Parasites	*Metastrongylus spp.* *Ascaris suum* (larvae)	

MATERIALS AND METHODS



- **Six wean-to-finish** farms were selected to represent a range of expected severity of **respiratory disease** based on history of previous batches.
- **Six pens** per farm were repeatedly tested
    - **9 time points**
      (every 2 weeks from 4 to 20 weeks of age).
- One or several ropes were hung simultaneously in each pen (1 rope/25 pigs).
- Samples were package and delivered by mail. Transit (overnight) took 18 hours.

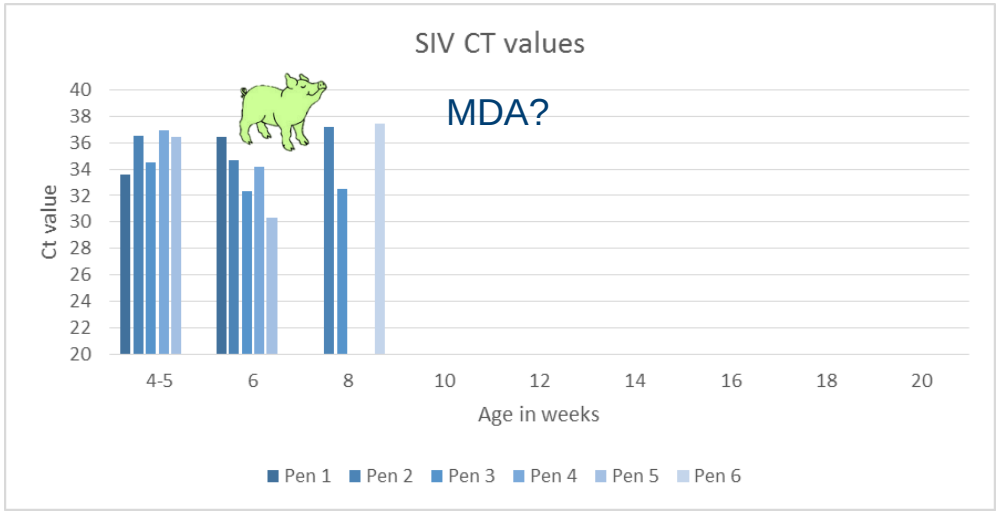
MATERIAL AND METHODS II

- **Nucleic acids** in oral fluids were extracted (MagMax™ Pathogen RNA/DNA extraction kit Thermo Fisher Scientific®
- analysed by real time PCR for PRRS, SIV and *Mycoplasma hyopneumoniae* qPCR for PCV2 (VetMAX™ PCR kit, Thermo Fisher Scientific®).
- **Clinical information** and additional sampling material from sick and dead pigs were collected to corroborate findings in oral fluids.
- **Post-mortem examinations** (casualties/slaughterhouse)

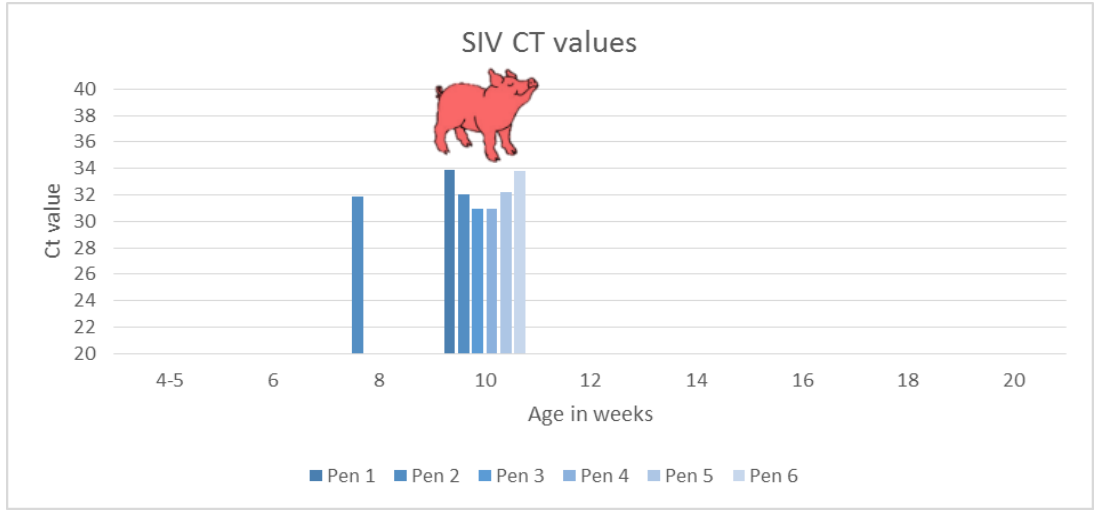
Results SIV.

- SIV in 3 farms. Detected in **2-3 consecutive time points**. 5/6 pens positive on the peak of infection.
- Results agreed with previous reports in literature *.

Case 1. Subclinical SIV.



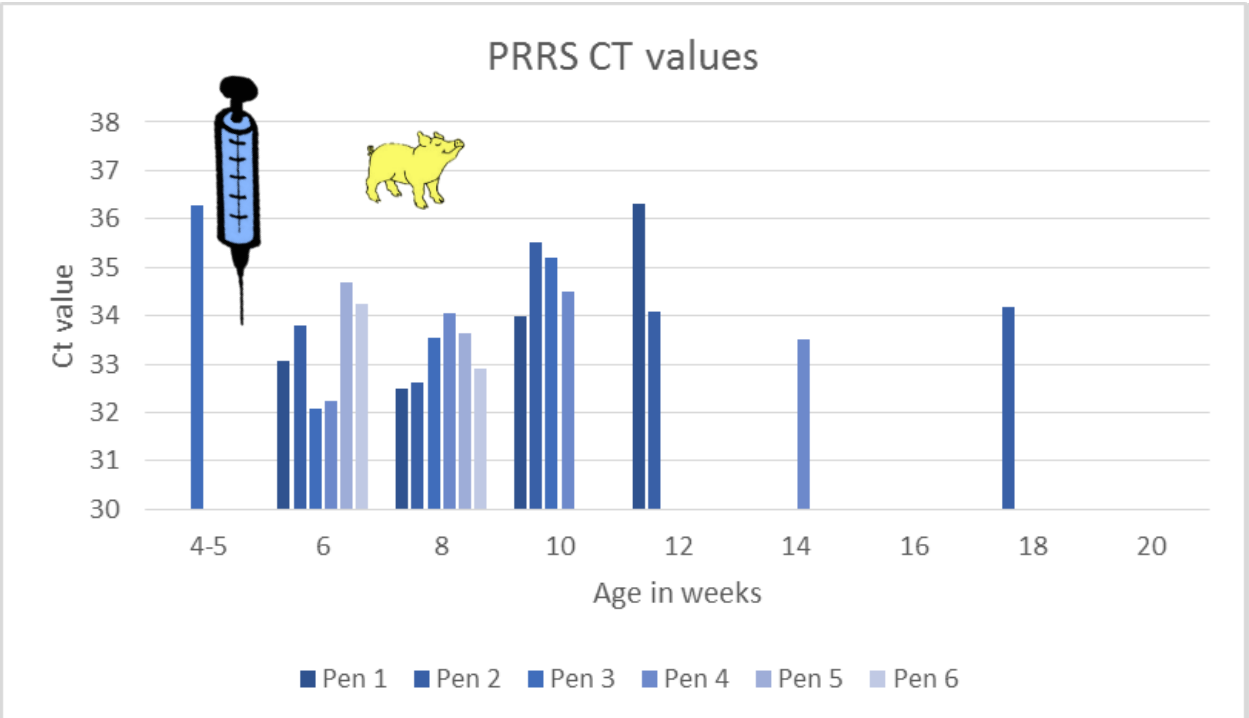
Case 2. Clinical SIV

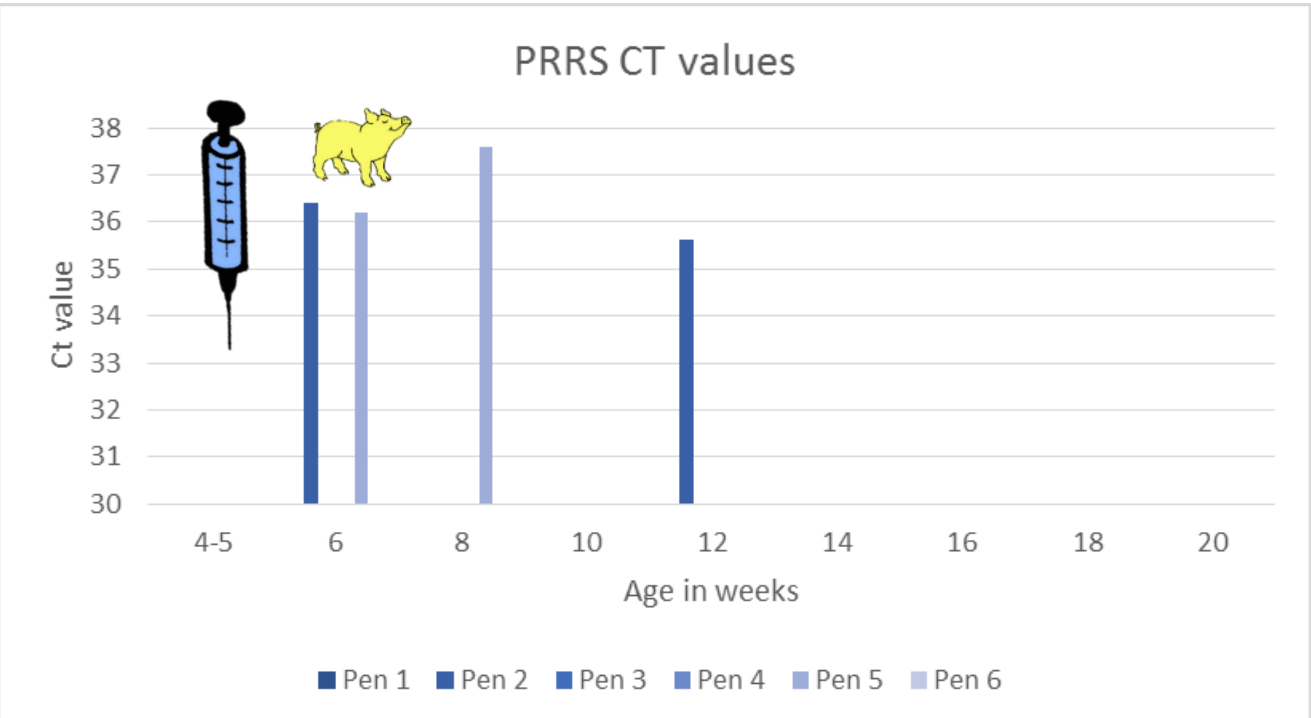


* Romagossa et al. 2011 Vet Res; Panyasing et al 2013. Vaccine

Results PRRS (EU)

- 2 batches were PRRSv +ve at weaning and they were vaccinated with MLV.
- One batch had positive detection in OF at weaning (prior to vaccination).
- Detection patterns last for several weeks. PCR products were not of sufficient quality for sequencing.
- Sensitivity of the method (degradation between the farm and lab) could be a problem.

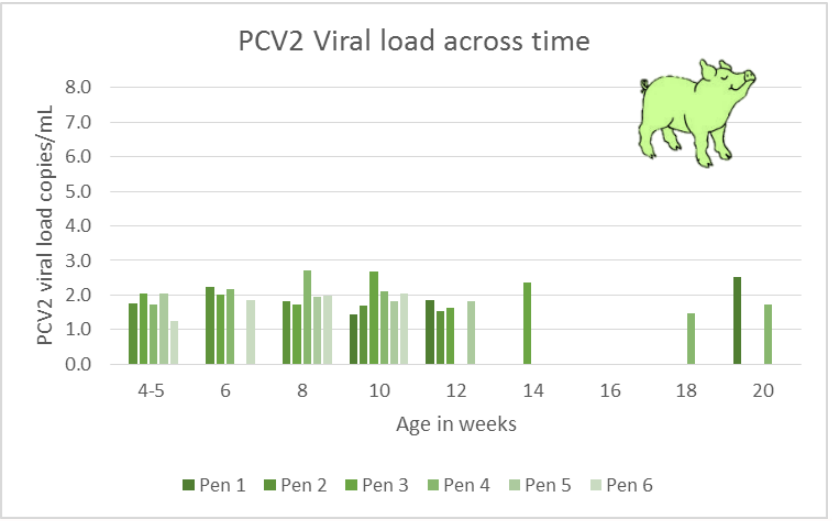




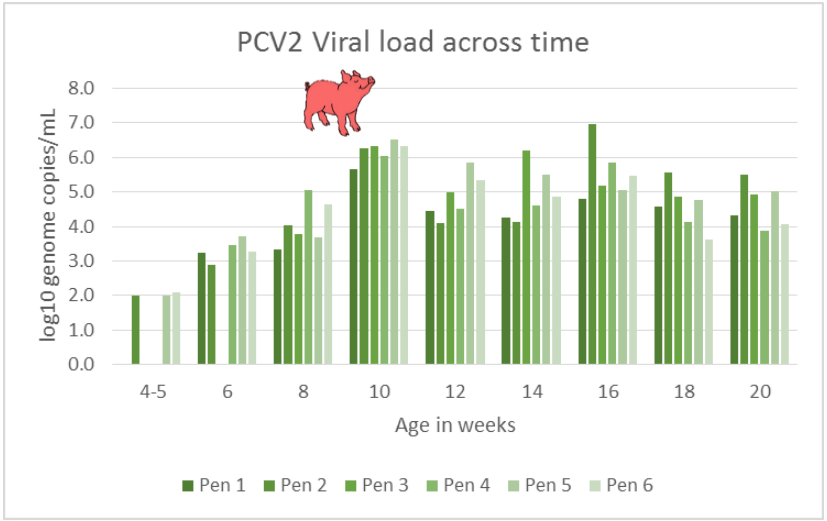
Results PCV2

- Results for PCV2 viral load agreed with previous reports in literature*
- PCV2 was detected in al farms at 5/9 to 9/9 time points. All farms were PCV2 vaccinated.
- One farm presented clinical PCVD, another farm with subclinical PCVD (considering diagnostic criteria in Segalés 2012 Virus research)
- In PCVD cases, viral load over was 10^3 - 10^4 copies per mL in all the pens.

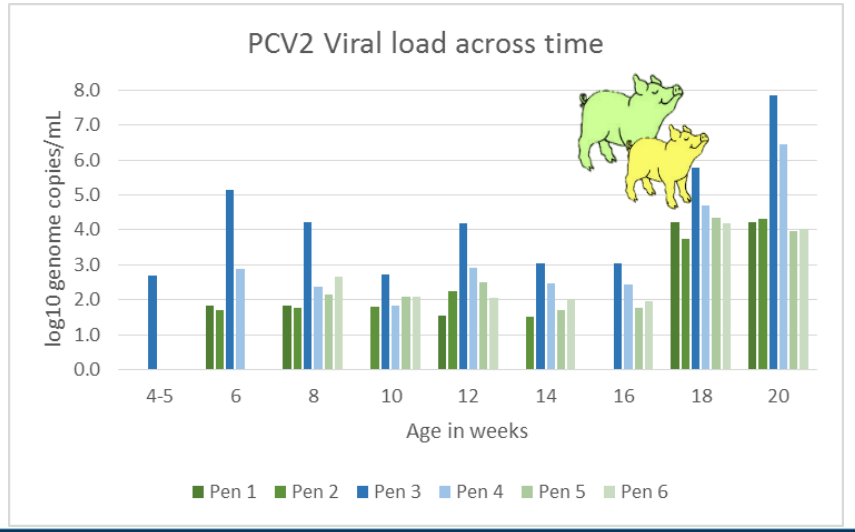
Non affected batch



Clinical PCV2



Subclinical PCVD?

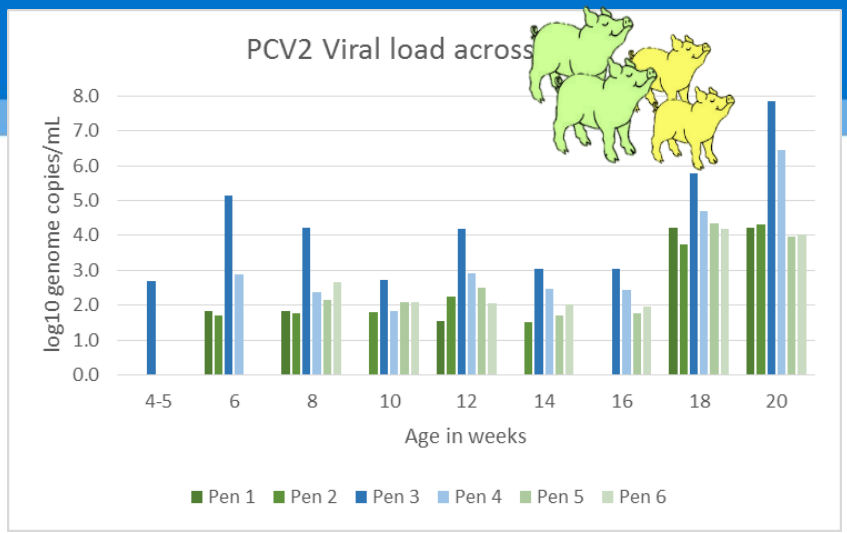


* Kim 2010 J Vet Clin; Ramirez et al. 2012 Preventive Veterinary Medicine

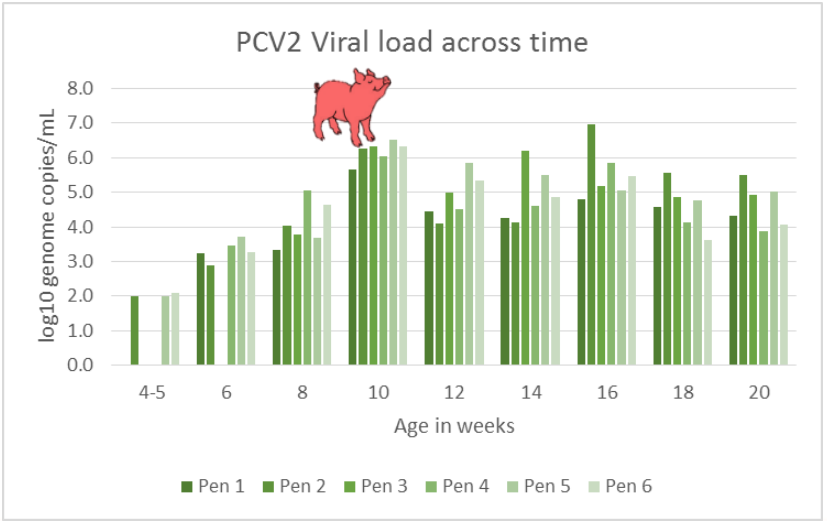
PCV2 in serum samples.

- Serum samples were collected at week 15.
  - <u>subclinically</u> affected farm: 12 samples were collected in pen 3 and 4 (blue) and all <u>were negative</u> for PCV2 PCR.
  - <u>clinically</u> affected farm: PCV2 was detected by PCR in 4 out 12 of the serum samples.
    - Viral load in **serum ranged from $10^{2.5}$ to $10^{3.5}$** while oral fluids were much higher over **10^{5}** copies per mL

Subclinical infection?



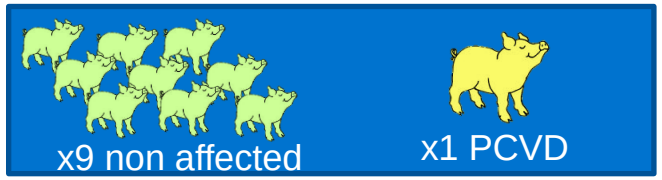
Clinical PCVD



Viral load in collective samples

- Oral fluids can detect seedders even when prevalence is low.
  - Large number of animals interact with the ropes.
  - There will be a dilution of the virus concentration in the collective sample.
  - Nonetheless, shedding pigs material can exceed 10^8 genome copies/mL.

Example: 10 pigs per pen, there is only 1 pig affected

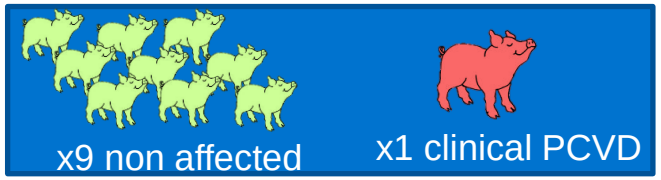


Viral load $<10^3$

e.g. Viral load 10^5-10^6

Assuming similar contribution

Dilution of the viral load.
i.e. 1:10 so final result could be between 10^4 to 10^5



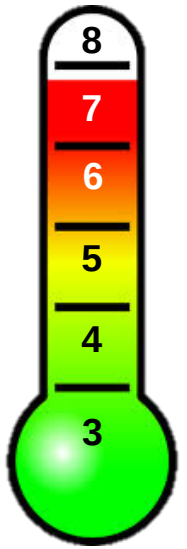
Viral load $<10^3$

e.g. Viral load 10^7-10^8

Even with a very poor contribution

Final result is likely to be $>>10^5$
Actions may be considered.

Log $_{10}$ Viral load in serum (genome copies/mL)



Frequently associated to PCVD

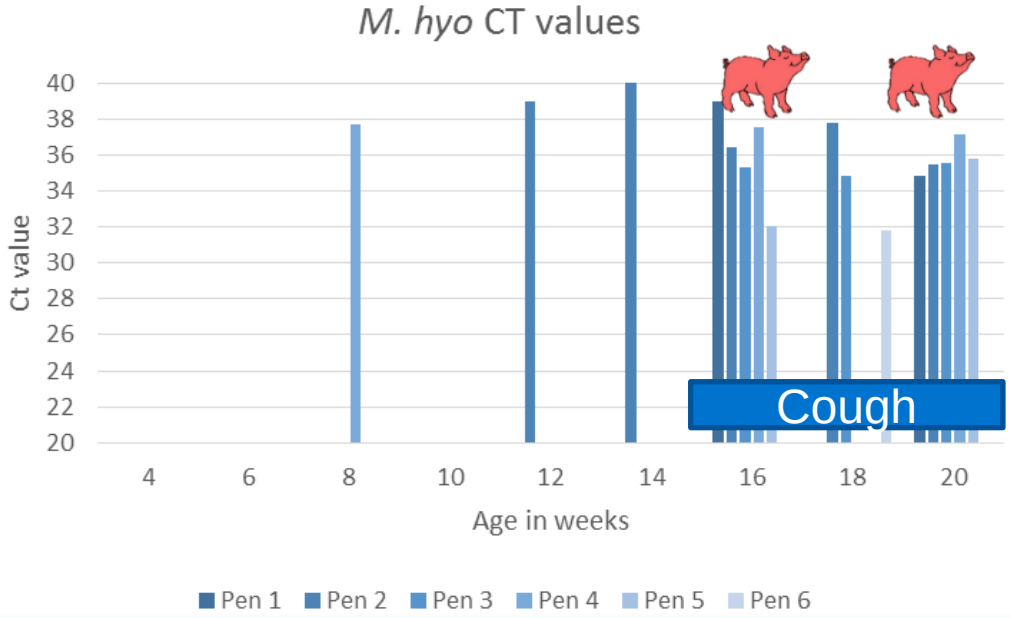
Potential impact in pig health

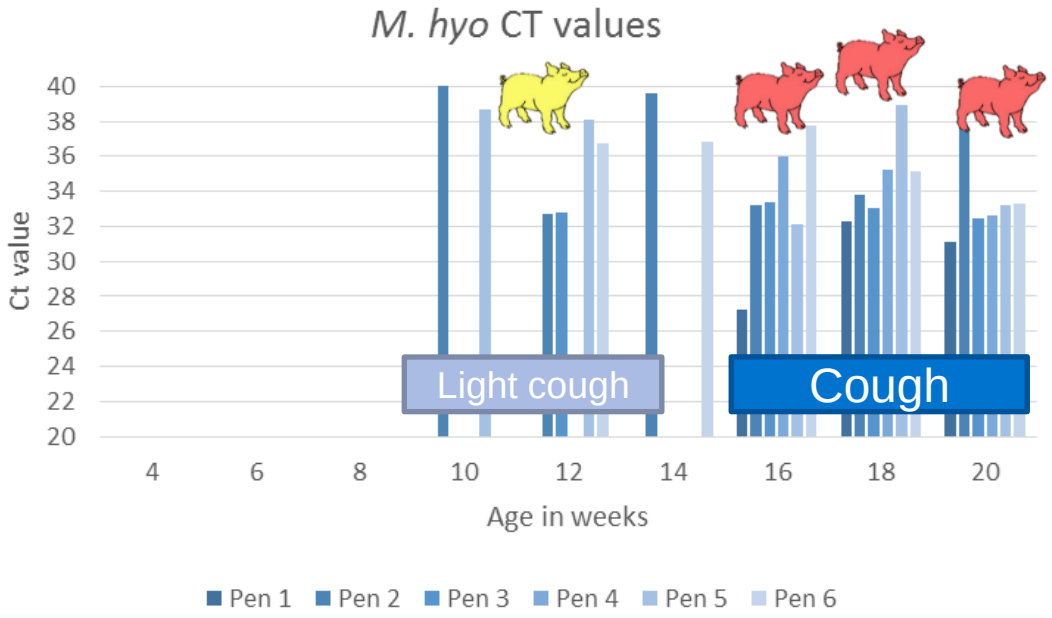
Very limited or none impact in pig health and performance

- In a pen with shedding pigs > 10^6 even when viral load is diluted 1000 times, results are higher than 10^3 so it is –at least- suspicious.

Results *M. hyo*

- *M.hyo* was detected in 4 out of 6 farms. Number of positive pens was related to CT values and clinical signs as respiratory problems.
- Detection was directly related to clinical respiratory problems (prevalence, CT values).
- Low CT values in late stage were related to abattoir lesions.
- Fewer than 6 samples were needed to detect it when coughing and EP-like lesions were present.





RESULTS: Correlations between ropes and pens.

- Significant (P<0.01) correlations of the Ct values between pairs of ropes collected in the same pen were observed for
  - PRRSV (R^2=0.92), PCV2 (R^2=0.98), SIV (R^2=0.87), *M. hyo* (R^2=0.92).
- Correlations between ropes in a pen were higher than correlations between pens in a barn.
  - Multiple ropes need to be hang in a pen with more than 25 pigs.
  - Better to test more pen rather than ropes from the same pen, but they should be hung anyway
- No spatial distribution patterns were detected.

DICUSSION

- Oral fluids contributed to better understand the involvement of different pathogens causing respiratory problems.
- Sampling **six pens** for these respiratory pathogens appeared to be a reasonable number,
    - maybe it is not enough for PRRSv in MLV vaccinated pigs.
- **SIV** Oral fluids testing was sensitive and useful for clinical and subclinical infection.
- **PRRSv** It was useful for determining the infection status (+/-) but sensitivity may be an issue.
- **PCV2** Potentially very useful for understanding infection dynamics and possibility of clinical and subclinical disease.
    - much more work is needed to understand relationships between viral loads in oral fluid and clinical/subclinical disease.
- ***M. hyo*** Useful for confirmation of the involvement of *M. hyo* in a respiratory disease cases. Given sensitivity issues may be less useful for confirming absence of *M. hyo* in herds believed negative.

TAKE HOME MESSAGE

- Oral fluids are **valuable platform to monitor** and assist diagnosis in **PRDC**.
    - They can also be useful to study infection dynamics
- **PRDC investigation** frequently requires to consider several pathogens.
    - Oral fluids allow to test some pathogens **just one type of samples**.
    - To carry out just **one DNA/RNA extraction** significantly reduce PCR testing cost.
    - Then they can be sequentially analysed for several pathogens depending on results.
    - Number of required ropes is different depending the target pathogen.
- Testing in more pens is more valuable than testing some ropes from the same pen.
- Current collective oral fluid tests present some **limitations to be taken into account** when testing.
    - Collective samples, unknown dilution, sample quality problems, nucleic acid degradation…

Acknowledgements

UNIVERSITY OF CAMBRIDGE
Department of Veterinary Medicine

Dan Tucker
Tom Wileman

ThermoFisher SCIENTIFIC

Nardy Robben
Damien Magnée

SAC CONSULTING

Jill Thomson
Sara Loeffen

RVC Royal Veterinary College University of London

Tom Eley
Henny Martineau
Dirk Werling

zoetis

Cambridge / Zoetis ECPHM Residency Program

Farmers and furthermore people involved in this study.