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 (R² ≈ 0.6) *.

### 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.

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

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 all 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 was $10^3$ - $10^4$ copies per mL in all the pens.

* Kim 2010 J Vet Clin; Ramirez et al. 2012 Preventive Veterinary Medicine
PCV2 in serum samples.

- Serum samples were collected at week 15.
  - **Subclinically** affected farm: 12 samples were collected in pen 3 and 4 (blue) and all were negative for PCV2 PCR.
  - **Clinically** affected farm: PCV2 was detected by PCR in 4 out of 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.
Viral load in collective samples

- Oral fluids can detect seeders 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.

- 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…
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