Opportunities and limitations of *Mycoplasma hyopneumoniae* PCR testing in oral fluids to confirm involvement in respiratory disease.

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Introduction

There are some doubts about the sensitivity of PCR testing in oral fluids for different bacterial respiratory pathogens including *Mycoplasma hyopneumoniae* (*M. hyo*) and this study re-evaluated the potential use of *M. hyo* PCR tools in oral fluids.

Material and Methods

Oral fluids were collected in six pens per farm at 9 time points, each two weeks apart, from 5 to 21 weeks of age in six commercial all-in all-out wean-finish pig units (farm 1 to 6) where pigs were *M. hyo* vaccinated just after weaning (28-32 days old pigs, week 5).

DNA was extracted from oral fluids and analysed for *M. hyo* by real time PCR (MagMax™ Pathogen DNA/RNA kit and VetMAX™ M. hyo AB Design Reagents, Thermo Fisher Scientific®). Ct values >40 were considered negative.

Severity of respiratory disease was monitored clinically at sampling. Prevalence of lung lesions compatible with enzootic pneumonia (EP-like score) and pleurisy were evaluated on farm casualties and in abattoir.

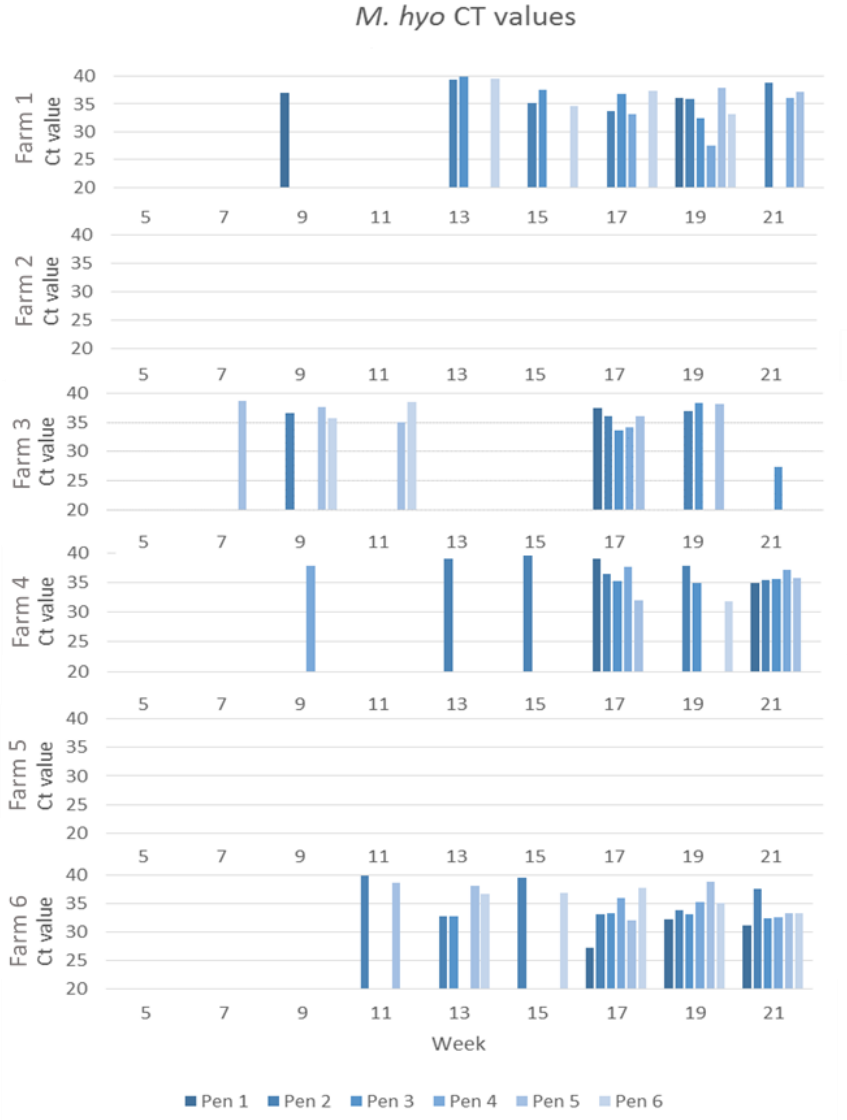

Figure 1. Mycoplasma hyopneumoniae pen level PCR results and Ct values. Note values over Ct 40 were considered as negative so they are not plotted in this figure.

Results

M. hyo was detected in oral fluids in 4/6 tested farms, with positive results at least in 6 of the 9 time points, but **detection patterns were discontinuous** at farm and pen level (see table and figure 1).

M. hyo detection in oral fluids coincided in most of the cases **with clinical respiratory signs** and high EP-like lesion scores.

Where *M. hyo* was not detected few or no respiratory problems or severe slaughter lesions occurred.

Table 1. Clinical signs and lesions associated to respiratory disease

	Respiratory clinical signs	Abattoir evaluations EP-score	Abattoir evaluations Pleurisy (%)
Farm 1	Respiratory problems on weeks 7 to 9 were caused by porcine respiratory complex with active involvement of PRRS and *Streptococcus suis*. Respiratory signs on weeks 15 and 19 as well.	**4.7**	**1%**
Farm 2	Respiratory problems on week 11 to 17 were caused by other pathogens that also caused meningitis and polyserositis as well.	**1.4**	**6%**
Farm 3	Hacking cough was present from week 7 to 21. It was more evident on weeks 7, 9, 17 and 19. Pleurisy lesions were frequent in casualties and also in abattoir (12%)	**1.2**	**12%**
Farm 4	Pigs were healthy until week 16 when cough became really prevalent until slaughter. After week 16, the prevalence of positive pens increased and presented lower Ct values.	**1.9**	**2%**
Farm 5	It only had some slight respiratory problems were caused by influenza virus on week 5-6.	**1.4**	**1%**
Farm 6	Respiratory problems were remarkable on weeks 17th to 21st, when *M. hyo* presented higher prevalence and lower Ct values. It was also present in weeks 5, 9, 13 and 15 but there was evidences of other pathogens that could be involved.	**>4.5**	**1%**

CONCLUSIONS

Based on the conditions employed in the present study, oral fluids for surveillance of *M. hyo* by PCR may be useful for confirmation of involvement in respiratory disease.

Relationships between *M. hyo* detection patterns (pen prevalence and Ct value), clinical respiratory problems and prevalence of lesions in the respiratory tract were observed in this study.

The marked discontinuity of positive sampling time points on known positive farms highlighted the unsuitability of the present oral fluids testing methodology to rule out *M. hyo* infection at a herd level.

However, results in this study highlight the correlation of *M. hyo* detection by real time PCR with clinical and pathological findings, and its potential use in respiratory disease diagnostics in vaccinated herds.

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