

Pen oral fluids as an alternative to faecal sampling to detect low bacterial load of *Lawsonia intracellularis* in subclinical infections

Rubén del Pozo Sacristán¹, Hanny Swam², Amy Elizabeth Taylor³,

¹MSD Animal Health, Madrid, Spain, ²Center for Diagnostic Solutions, MSD AH Boxmeer, The Netherlands, ³University of Leeds, UK

Background and Objectives

Oral fluids (OF) have been demonstrated as a reliable sampling method to detect and semi-quantify bacterial load of *Lawsonia intracellularis* (LI) at group level using qPCR (1, 2). However, it is still unclear whether this sample type would be suitable to detect subclinical infections with low bacterial load. Therefore, the aim of this study was to evaluate whether pen OF could detect LI subclinical infections in a herd with a low bacterial faecal shedding.

Material and Methods

For this aim, a herd with a history of subclinical LI infection was selected. A subclinical infection was defined by the absence of ileitis-like symptoms and by a low bacterial load ($<10^5$ copies/ μ L) of this bacterium in faeces quantified by qPCR (BactoReal *Lawsonia* kit, Ingenetix). A cross-sectional sampling was performed, including pigs sampled at 4, 7, 10, 13, 16, 19 and 22 weeks of age (woa). Fresh faecal samples were collected individually from the anus at defecation (minimum 2 pigs/pen of 10 pigs). OF samples were taken at the same time points on a pen basis. Both faecal and saliva samples were tested for LI by qPCR [results given in 10Log copies/ μ L (faeces) and Ct-values (saliva)]. The association between the number of positive specimens detected by both type of samples was calculated by Chisquare test and Cohen's kappa. A Pearson correlation was performed to compare bacterial load in faeces and saliva.

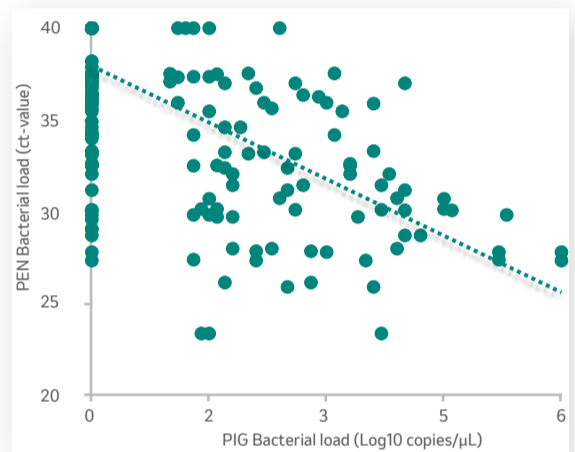
Results

In total, 367 individual faecal and 120 pen OF samples were collected. A significant ($P<0.001$) moderate agreement ($\kappa=0.636$) was found between the number of positive specimens detected by both type of samples. OF sampling identified correctly (sensitivity) 93.5% of all the pens with at least one sample pig with a positive faecal test. Only three pens tested negative while at least one positive pig (1.2-2.4Log₁₀ copies/ μ L) was housed in there. A significant ($P<0.001$) moderate correlation ($r=-0.626$) CI[-0.684; -0.559] was detected for bacterial load between both type of samples.

Table 1. Bacterial load of *Lawsonia intracellularis* agreement between Pen Oral fluids and Individual Faecal samples (Interrater Reliability Cohens Kappa test). $\kappa=0.636$; $P<0.001$.

		FAECES		
		NEG	POS	
ORAL FLUID	NEG	55	3	58
	POS	19	43	62
		74	46	120

Figure 1. Correlation for Bacterial load at Pen vs Pig level ($r=-0.626$; CI[-0.684; -0.559]; $P<0.001$).



Discussion and conclusion

Under the conditions of this study, it was concluded that pen OF is an excellent alternative to faecal sampling to detect LI subclinical infections at group level, even in the face of low bacterial load.

¹ Kamlage, et al. Investigation on the use of single or pooled faecal samples and comparison with group saliva samples for *Lawsonia intracellularis* diagnostic in Fattening Pigs. ESPHM 2021.

² Wertenbroek et al. Saliva sampling as an alternative method besides pooled faeces samples for measuring qPCR *Lawsonia* levels. ESPHM 2022.