Improvement of surveillance of atrophic rhinitis in pigs by using qPCR on oral fluid samples from individual and grouped pigs.

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INTRODUCTION

Atrophic rhinitis (AR) in pigs is caused by toxigenic strains of *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* type D (PMT). Ante mortem AR surveillance is usually carried out by direct detection through bacterial culture, PCR or antigen detection in nasal swabs (NS).[3] Oral fluid (OF) testing offers an opportunity to easily collect herd-level disease data on a periodic basis, and has been evaluated for monitoring various bacterial and viral diseases of swine.[4] To our knowledge, there is no previous report on the use of OF for the detection of Bb and PMT on commercial farms. Therefore, this study was conducted to determine if OF samples collected from pigs housed individually or in groups contain Bb and PMT, capable of being detected by qPCR.

MATERIALS AND METHODS

Two assays originally developed as conventional PCRs[1,2] for the detection of Bb (*fla*A gene) and PMT (*tox*A gene), were adapted to real time format with SYBR Green I detection and melting curve analysis (qPCR). Analytical sensitivities and specificities were assessed using reference strains of Bb and PMT, along with a panel of viral and bacterial respiratory pathogens of pigs, respectively.

Pen samples came from 46 pens (20 to 25 pigs, 4-16 weeks old) in 10 growing-finishing farms, and consisted of one OF and one pool of 4-7 NS per pen (4-6 pens per herd). Individual samples consisted of 32 OF and NS collected from the same number of adult sows in two breeding-gestation units.

Herds with at least one sample testing positive in the qPCR were considered positive to Bb or PMT, as appropriate.

RESULTS

The PCR assays were successfully adapted to the qPCR format, allowing the detection of Bb and PMT in a sensitive and specific way, with no cross-reactivity to other pig pathogens, and detection limits of 1-10 ufc/ul.

Of the 10 growing-finishing units, seven were positive to Bb, and one to PMT. The two breeding-gestation units were negative to PMT, and one positive to Bb. Positive qPCR results for each target and sample type are summarized in Table 1. In all cases both NS and OF samples tested positive in different proportions (data not shown). Only one sow tested positive to Bb in both OF and NS; none tested positive to PMT.

Table 1. *Bordetella bronchyseptica* and *Pasteurella multocida* qPCR positive oral fluid and nasal swabs, collected from pigs in 46 pens and 32 sows in individual stalls.

	Pen Samples[1] (n=46)		Individual Samples (n=32)	
	Bb	PMT	Bb	PMT
OF	23/46	3/46	1/32	0/32
NS	24/46	2/46	1/32	0/32

OF= oral fluid; NS= nasal swab; Bb= B. *bronchyseptica*; PMT= *P. multocida*; [1]Samples were taken simultaneously from pens of 20-25 pigs: One OF and 1 pool of 4-7 nasal swabs were obtained from each pen;

CONCLUSIONS AND DISCUSSION

This study demonstrates that pig OF may contain genetic material of Bb and PMT, which is detectable by qPCR. The detection rate was similar in OF and NS from the same animal or group of animals. These results suggest that OF could be used for monitoring of AR in pigs, reducing labor and animal stress during sample collection, and providing valuable information about the health status of breeding and growing pigs in regards to AR.

The findings also suggest that there may be a significant proportion of pigs free from Bb infection, although it is considered a normal inhabitant of the respiratory tract in pigs. However it is noteworthy that the small number of animals tested may have had an influence on the results presented. Nevertheless, these findings encourage to carry out further studies using the methodology described, along with herd production data, to look for possible links between the prevalence of Bb and PMT in OF and the incidence of the disease.

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REFERENCES

1. Hozbor D *et al.* 1999. Res. Microbiol. 150: 333–41.
2. Kamp EM *et al.* 1996. J Vet Diagn Invest 8:304-09.
3. OIE Terrestrial Manual. 2012. Chapter 2.8.2.
4. Prickett JR *et al.* 2010. Anim Health Res Rev 11:207-16.

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