A multiplex molecular assay for *Glaesserella australis*

S Yee[1], N Nahar[1], E T Lobo[1], S Jenkin[2] and C Turni[1]
[1]*Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia,* [2]*Apiam Animal Health, Australia*
C.Turni1@uq.edu.au

Application The development of a PCR for *Glaesserella australis* will result in its correct identification and therefore improve its prevention and control.

Introduction A new bacterial species, consisting of 17 isolates, has been previously identified (Turni *et al.,* 2018) and has been unofficially named *Glaesserella australis*. The isolates collected came from different disease scenarios. Some isolates were taken from lungs that displayed acute consolidation of dorsal lung lesions, which affected approximately 50% of the lung with no gross abscesses or pleurisy and were from pigs aged 12 to 20 weeks that died on farm. From some of these lungs, the only bacterial species isolated was *G. australis*. The other isolates came from lungs collected at the abattoir that displayed lung lesions, lung abscesses and pleurisy while on farm no symptoms or only mild respiratory symptoms were observed. The lesions observed at the abattoir were very similar to lesions of *Actinobacillus pleuropneumoniae* in appearance and it was hypothesised that due to the similarity in lesions and time of onset of the disease caused by *G. australis* and *A. pleuropneumoniae,* it might have been that these lesions at the abattoir had been attributed to the wrong bacterial species. To be able to do a study on the abundance of this species, a species-specific PCR has been developed, which is a multiplex PCR for the detection of *G. australis*, *Pasteurella multocida* and *A. pleuropneumoniae*. The hypothesis of this study was that the multiplex PCR developed would be specific for *G. australis* without amplifying any related strains.

Material and methods A total of 26 isolates of *G. australis* including the original 17 isolates and a further 9 field isolates collected from lungs at the abattoir were used. Isolates of the other two species in this PCR were also tested with 15 reference serovar strains and one field isolate of *A. pleuropneumoniae* and 16 reference serovar strains plus the type strain of *P. multocida*. To assure specificity, 47 related species including type strains and field isolates were tested in the PCR. DNA was extracted by suspending a loopful of bacteria into H_2O, heating and boiling twice and spinning at 13 000 x g for 2 minutes. The 24 µL PCR reaction mix contained 1.5 mM $MgCl_2$, 10 mM Tris, 50 mM KCl, 0.24 mM dNTPs, 0.3 µM of each primer (Table 1), 0.5 U of Taq and 1µL of template. The reaction mix was run for one cycle at 94°C for 2 min and then for 30 cycles at 94°C for 30 sec, 65°C for 30 sec, 72°C for 2 min. After this followed a final cycle at 72°C for 10 min. Furthermore, a total of 16 swabs were sampled from 15 lungs that were sampled at the abattoir, cultured and resulting isolates tested by PCR.

Table 1. Primers used in the assay

Primers		Amplicon	Reference
LPF	5' - AAG GTT GAT ATG TCC GCA CC -3'	951	Gram and Ahrens 1998
LPR	5' - CAC CGA TTA CGC CTT GCC A – 3'		
PM1231F	5' - AGA AAG CAC ATG ACC AAA GGG – 3'	601	Liu *et al.,* 2004
PM1231R	5' - GCA GCT ACT CGC AGA AGG TT – 3'		
GA F	5' - AAG ATG ATG ATC GCC CAA TCG 3'	449	This study
GAR1	5' - CCA CGA GAA GCA AGA ACA TCT TTG ATC – 3'		

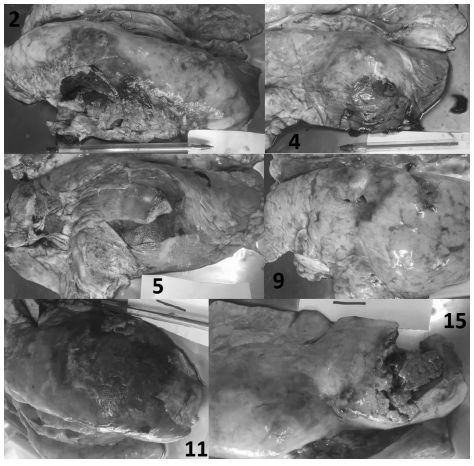

Figure 1. Showing the six lungs with lesions from which *G. australis* was isolated.

Results The multiplex PCR amplified all the *G. australis*, the *A. pleuropneumoniae* and *P. multocida* reference, type and field strains. The PCR was very specific and did not amplify any of the related strains, be that type strains or field strains. The sampling at the abattoir yielded pure culture of *G. australis* from six lungs. The appearance of the lesions of some lungs resembled *A. pleuropneumoniae* lesions (Figure 1).

Conclusion The new multiplex is specific and can identify *G. australis* in pure culture. The assay would appear to be a useful addition to the suite of diagnostic tools for pig respiratory disease diagnostics.

Acknowledgements Supported in part by Australian Pork Limited.

References

Gram T and Ahrens P 1998. Journal of Clinical Microbiology. 36, 443–448.
Liu D, Lawrence ML and Austin FW 2004. Journal of Microbiological Methods. 58, 263-267.
Turni C, Giang N, Wu Y, Omaleki L, Blackall PJ and Christensen H 2018. *In* Australian Society for Microbiology Annual Scientific Meeting. Abstract 385.