Acknowledgements Supported in part by the Agricultural Produce Commission – Pork Producers' Committee.

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doi: 10.1016/j.anscip.2023.09.007

7. Use of piggery effluent for point-of-management diagnostics: A proof of concept

M.N. Naseem [a], L. Omaleki [a], J.M. Templeton [b], M. Muralidhar [a], J.R. Botella [c], P.J. Blackall [a], C. Turni [a,*]

[a] Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD 4072, Australia
[b] Department of Agriculture and Fisheries, Ecosciences Precinct, Dutton Park, QLD 4102, Australia
[c] School of Agriculture and Food Sciences, The University of Queensland, St Lucia, QLD 4072, Australia

* Corresponding author: Conny Turni. E-mail: c.turni1@uq.edu.au

Introduction During the COVID-19 pandemic, detection of the virus in sewage water was used to determine disease spread in the community. Piggery effluent or waste is composed of faeces, urine, oronasal secretions, feed residues and other miscellaneous components which are diluted in water and released into the environment (Rate, 1997). This blended composition of effluent, along with evidence that several pathogenic bacteria have already been isolated from effluent (Chinivasagam et al., 2004) suggest that it could be useful to monitor pathogen prevalence in a herd at a given point in time. This monitoring could help in early detection and improved control of disease outbreaks that could potentially cause significant economic losses and decreased animal welfare. This point-of-management (POM) approach and better control of infectious diseases would also reduce the need for antimicrobials, improving the sustainability of intensive pig production and reducing the risk of antimicrobial resistance genes entering the food chain or the environment. The nucleic acid amplification-based assay known as Loop-mediated isothermal amplification (LAMP) has been widely adopted for POM diagnostics due to its high sensitivity, accuracy, affordability and quick turnaround time. Therefore, this study was designed to produce a proof-of-concept effluent testing methodology through LAMP-based POM assay, to determine if this tool is useful to monitor pathogens at the shed or herd level. Due to the composition of effluent and the ease of implementation and low cost of LAMP assays, it was hypothesised that LAMP assays could be used to detect a common pathogen in pig effluent for the purpose of POM diagnostics.

Material and methods *Campylobacter coli* detection was performed on effluent samples using an already-established and validated LAMP assay by Mason et al. (2020). Briefly, effluent samples were collected from two commercial piggeries. The presence of *C. coli* via culture was initially confirmed. DNA was extracted using 15 mL of effluent ($n = 20$) by using multiple approaches including commercial DNA extraction kits (QiaAmp Power Faecal Kit, QIAGEN, Clayton, VIC, Australia and NucleoSpin Soil Kit, MACHEREY-NAGEL, Düren, Germany) and a laboratory-based extraction method developed in this study (using chemical and thermal lysis). The extracted DNA was tested for the presence of *C. coli* with both a conventional PCR and a LAMP assay as originally reported by Wang et al. (2002) and Mason et al. (2020) respectively, as well as with slight modifications in these methods (including the use of Platinum Taq II DNA polymerase for the conventional PCR and *Bst* 3.0 DNA polymerase for the LAMP assay).

Results Even though the samples were positive for *C. coli* by culture, the conventional PCR and LAMP assays failed to produce a positive result, irrespective of the extraction method. However, when Platinum Taq II DNA polymerase was used in the conventional PCR assay, four of the samples from which DNA was extracted with the laboratory-based method together with one extracted with QiaAmp Power Faecal Kit were positive for *C. coli.* After optimising the LAMP assay using *Bst* 3.0 DNA polymerase, all samples irrespective of the extraction method returned a positive result for the presence of *C. coli.*

Conclusion and implications This study has shown that effluent testing with LAMP assay can be used for POM monitoring of pathogens in pig herds. The improved results are presumably due to the fact that Platinum Taq II DNA polymerase and *Bst* 3.0 DNA polymerase are better able to tolerate the polymerase inhibitors present in effluent as compared to the polymerase enzymes used in the original reported methodologies of these assays. In current work, we are seeking to demonstrate that effluent testing can also be used to detect a key respiratory pathogen, *Actinobacillus pleuropneumoniae.* This study aims to develop LAMP-based POM assays for testing the presence of bacterial pathogens in both the respiratory and enteric systems using effluent. The laboratory-based DNA extraction method developed in this study requires minimal instrumentation and can be performed on farms, enabling on-farm testing of infectious diseases using POM assays. Following the success of this assay, POM assays can also be developed for routine monitoring and early detection of exotic diseases, such as Foot and mouth disease and African swine fever.

Acknowledgements Supported in part by Australian Pork Limited.

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doi: 10.1016/j.anscip.2023.09.008

8. Exudative epidermitis-causing *Staphylococcus hyicus*: Insights and education for industry optimisation

A. Truswell [a,*], T. Laird [a], J. Blinco [a], S. Pang [a], D. Jordan [a], D. Hampson [a], S. Adsett [b], R. Abraham [a], S. Abraham [a]

[a] Antimicrobial Resistance and Infectious Diseases Laboratory, Murdoch University, Murdoch, WA 6150, Australia
[b] Rivalea (Australia) Pty Ltd, JBS Australia Pork Division, Corowa, NSW 2646, Australia

* Corresponding author: Alec Truswell. E-mail: alec.truswell@murdoch.edu.au

Introduction Exudative epidermitis (EE) is a disease characterised by a distinct greasy appearance, resultant from the production serum sebum and sweat by exudative lesions. The disease is of economic significance to the pig industry, boasting high morbidity and mortality rates in piglets under three weeks and reaching up to 90% mortality in severely affected litters, where death is often seen within 24 to 48 hours (Frana and Hau, 2019). The swift and dynamic nature of EE demands that its pro- and meta-phylactic management is both equally rapid and sufficiently informed. *Staphylococci* are notorious for their ability to acquire and exchange genetic material (Schwarz et al., 2018), emphasising the need to incorporate antimicrobial resistance (AMR) testing as a routine component of their treatment, particularly in a time-critical disease such as acute EE. Efficacious autogenous vaccination relies on genomic data, of which little currently exists, and as such there is little documentation of vaccination attempts (Arsenakis et al., 2018). To adequately cope with the pace of EE, the authors applied the high-throughput Robotic Antimicrobial Susceptibility Platform (RASP) to a recent EE outbreak, demonstrating its value in outbreak scenarios. The authors hypothesised that the outbreak would be clonal, with low levels of AMR.

Material and methods The RASP was applied to rapidly characterise *Staphylococcus hyicus* isolates from a single outbreak of EE in weaner pigs. In this study, individual lesion swab samples were collected from 20 weaner pigs and subjected to multi-isolate (eight per swab) phenotypic and genetic characterisation using RASP.

Results Exudative epidermitis lesions were found to be comprised of several organisms, including three different *Staphylococcus* species, however, *S. hyicus* was the most highly represented throughout (19/20 swabs, 88/160 isolates). The *S. hyicus* isolates were found to be predominantly clonal, with a high degree of genetic similarity between 24 of the 27 isolates (Fig. 1). Despite their genetic homogeneity, three different antimicrobial resistance genotypes were identified between them (Fig. 1).

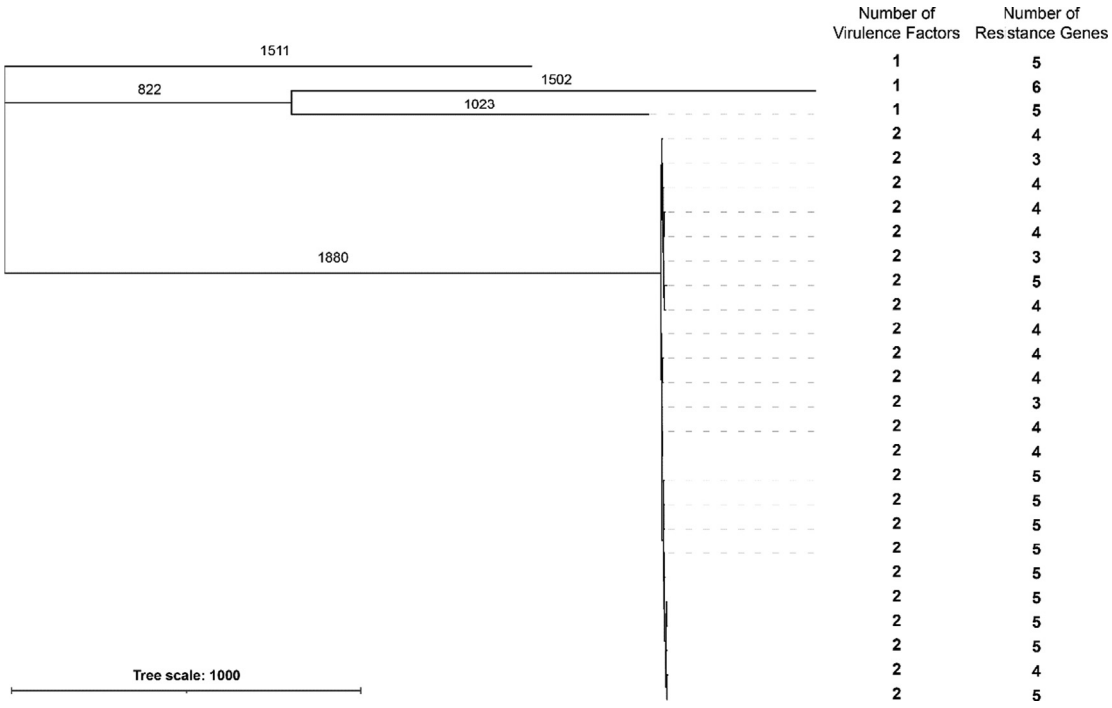

Fig. 1. Phylogenetic comparison of *Staphylococcus hyicus* ($n = 27$) from an exudative epidermitis outbreak in Australian pigs.

Conclusion and implications This study demonstrates the possibility for EE outbreak isolates to exhibit differing antimicrobial resistances. This finding should prompt higher-resolution sampling and investigation of *S. hyicus* outbreaks, to provide accurate data on which