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# ERYSIPELOTHRIX RHUSIOPATHIAE EPI-INTERFACE, A NEW APPROACH TO THE MANAGEMENT OF PORCINE ERYSIPELAS

**Report prepared for the Co-operative Research Centre for High Integrity Australian Pork**


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**Executive Summary**

*Erysipelothrix rhusiopathiae* is a major source of economic loss in the pork industry. Given the ubiquitous nature of *E. rhusiopathiae*, elimination of the pathogen is unlikely. As a result, farm management becomes the critical means to control the disease, eliminate ineffective measures and minimize cost. As most cases of erysipelas are dealt with on an animal by animal or farm by farm basis there is limited understanding of this disease on a national scale. Little data is collected cumulatively to assess the true epidemiology of the disease.

In this study one hundred and eighty *E. rhusiopathiae* isolates were whole genome sequenced (WGS) of which 178 were successful. From the whole genome sequences we were able to generate multi locus sequence type (MLST), anti-microbial resistance and virulence genes profiles. By using the WGS approach, we now have 178 assembled genome sequences available for future intensive genomic investigation as new knowledge and/or molecular investigation techniques become available.

The MLST, anti-microbial resistance and virulence genes data were incorporated into the *E. rhusiopathiae* Epi-Interface along with clinical information obtained from historical reports or submitted with samples. As the Epi-Interface is modular by nature, it can be added to and systematically interrogated.

This study has demonstrated that irrespective of the mode of interrogation a clear distinction exists between Australian and international *E. rhusiopathiae* isolates. In addition, we have reinforced international findings with respect to the degree of plasticity found within *E. rhusiopathiae* isolates.

Antibiotic sensitivity screening showed that all strains were susceptible to amoxicillin and single isolates displayed Lincospectin, Penicillin or Erythromycin resistance. Only the observed resistance against Oxytetracycline was confirmed by the presence of the gene. These findings should be taken into consideration during the development of on-farm antibiotic treatment strategies.

All 178 assembled whole genome sequences were screened for 47 putative virulence genes. The number of genes detected ranged from 41 to 47 per isolate, producing 24 distinct profiles (virotypes).

Forty-five farms were associated with suspected vaccine breakdowns. Interestingly 64% of these submissions were attributable to three MLSTs.

A bacteriophage, detected in 14% of the studied isolates, has been previously identified as a potential opportunity for a new anti-*E. rhusiopathiae* agent and may be instrumental in overcoming antimicrobial resistance.

This innovation study has generated a dynamic resource to improve the understanding of this highly variable pathogen in the Australian pig industry. It has also provided insight into the quantity and quality of metadata that is required nationally for improved management of porcine erysipelas. The Epi-Interface developed in this project will have cumulative commercial value as data is added. The genome sequences generated during this pilot study have stand-alone commercial value for data mining in relation to future diagnostic, vaccine and antibiotic development.

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## 1.0 Introduction

Commenting on Aristotle's observation and differentiation of three diseases of swine, Connell *et al*. (1952) stated that it took 22 centuries before additional clarity appeared! Whilst there has been tremendous positive change in the swine industry since 1952, our understanding of erysipelas is still incomplete (Dipietre 2006).

*E. rhusiopathiae* causes economic loss predominantly in the pork and poultry industries. Losses are incurred in the form of sub-optimal growth, mortality and carcass condemnation. Whilst vaccines and/or antibiotics can be used to prevent or overcome infections, the results are often sub-optimal and total protection against or eradication of porcine erysipelas has rarely been achieved. The unit price of delivering these sub-optimal protection/treatment regimens and disease associated losses add to the total cost of production which erodes farm and industry profits. Any additional costs to production are particularly important to the Australian pork industry given current low pig prices and with imports threatening the fresh pork market.

As most cases of erysipelas in the pig industry are dealt with on a farm by farm basis there is little understanding of this disease on a national scale. Whilst *E. rhusiopathiae* has a very wide host range, which includes humans, it is not well understood at the genomic level in comparison to other bacterial pathogens. Attempts to characterise *E. rhusiopathiae* in the past have provided very specific information which has not correlated to or discriminated between differences observed in the disease or the success of its' control. Recent success had been achieved by investigating DNA sequencing of virulence genes and using a technique known as multi locus sequence typing (MLST) (Janssen, *et al*. 2015).

To establish the MLST system for Australian porcine isolates of *E. rhusiopathiae*, we undertook whole genome sequencing (WGS) rather than the more expensive PCR-based sequencing approach. By using WGS we were able to simultaneously profile both anti-microbial resistance and virulence genes. Additionally, the WGS can be repeatedly mined as new insights into the functions of bacterial genomes are elucidated.

The aim of this project was to use this approach, in combination with conventional bacteriological techniques, clinical observations and management field data, to develop a better understanding of erysipelas in Australian pigs and to establish a new epidemiological tool we call an Epi-Interface. Why an interface? An interface is commonly perceived as an interconnection between systems, equipment, concepts, entities and the environment. To that end we want to improve the connection between the laboratory and the farm, drawing on all aspects of the available information to identify associations that go beyond just the causation of disease.

By linking distinct combinations of strain specific laboratory data such as MLST type, virulence and antimicrobial resistance gene profiles with field variables like antibiotic usage and the severity of infection (i.e. acute, subacute, chronic, and/or recurrent) we have developed an "Epi-Interface" which could be used in the control and management of porcine erysipelas. The modular nature of the Epi-Interface allows the incorporation of new modules for interrogation of new and existing data. This makes the Epi-Interface a growing and dynamic epidemiological tool.

## 2.0 Methodology

### *2.1 Collection of Erysipelothrix rhusiopathiae isolates*

In consultation with EMAI's biometrician, Damian Collins, 180 isolates (Table 1) were selected for this analysis. As a pilot project, this number was deemed adequate when considering the cost and time constraints associated with this project. These isolates were divided into two groups: archive (isolates sampled before 2008, n=112) and contemporary (isolates sampled after 2008, n=68 including two isolates that failed). Historical records located at EMAI were mined and freeze-dried *E. rhusiopathiae* isolates were selected according to their host, serotype and disease presentation. These isolates comprised the archive group. The majority of the isolates were from Australia. However, a few international isolates were included. These either were a unique serotype or featured in published studies.

The contemporary group were isolates submitted from a range of pig farms, mostly NSW and Victoria. Due to unforeseen circumstances the number of isolates was well below expectations. Attempts at sourcing samples/isolates from Queensland and Western Australia were unsuccessful. A submission form (Appendix 9.1) for contemporary samples was designed to extract as much clinical data as possible (i.e. region, vaccination history antibiotic use and clinical and sub-clinical histories).

**Table 1: Summary of *Erysipelothrix rhusiopathiae* isolates used in this study.**

| ID No. | Class | Source | Location | Year | ID No. | Class | Source | Location | Year | ID No. | Class | Source | Location | Year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | Pig | NS | 1981 | 61 | A | Pig | NSW | 1997 | 121 | C | Pig | Vic | 2017 |
| 2 | A | Pig | NSW | 1969 | 62 | A | Pig | NSW | 1997 | 122 | C | Pig | Vic | 2017 |
| 3 | A | NS | NS | 1981 | 63 | A | Pig | NSW | 1997 | 123 | C | Pig | Vic | 2017 |
| 4 | A | Pig | NSW | 1982 | 64 | A | Pig | QLD | 1997 | 124 | C | Pig | Vic | 2017 |
| 5 | A | Pig | NSW | 1982 | 65 | A | Pig | NSW | 1997 | 125 | C | Pig | Vic | 2017 |
| 6 | A | Pig | USA | 1982 | 66 | A | Pig | Vic | 1997 | 126 | C | Pig | Vic | 2017 |
| 7 | A | NS | Argentina | 1982 | 67 | A | Pig | WA | 1997 | 127 | C | Pig | Vic | 2017 |
| 8 | A | Pig | NSW | 1983 | 68 | A | Pig | NSW | 1997 | 128 | C | Pig | Vic | 2017 |
| 9 | A | Pig | NSW | 1983 | 69 | A | Pig | NSW | 1997 | 129 | C | Pig | Vic | 2017 |
| 10 | A | Pig | NSW | 1984 | 70 | A | Pig | NSW | 1997 | 130 | C | Pig | Vic | 2017 |
| 11 | A | Pig | NSW | 1984 | 71 | A | Pig | NSW | 1998 | 131 | C | Pig | Vic | 2017 |
| 12 | A | Pig | NSW | 1984 | 72 | A | Pig | NSW | 1989 | 132 | C | Pig | Vic | 2017 |
| 13 | A | Pig | NSW | 1986 | 73 | A | Pig | NSW | 1989 | 133 | C | Pig | Vic | 2017 |
| 14 | A | Pig | NS | 1987 | 74 | A | Pig | NSW | 1998 | 134 | C | Pig | Vic | 2017 |
| 15 | A | Pig | NSW | 1987 | 75 | A | Pig | NSW | 1998 | 135 | C | Pig | Vic | 2017 |
| 16 | A | Pig | NSW | 1987 | 76 | A | Pig | NSW | 1998 | 136 | C | Pig | Vic | 2017 |
| 17 | A | Pig | NSW | 1990 | 77 | A | Pig | NS | 1998 | 137 | C | Pig | NSW | 2016 |
| 18 | A | Pig | NSW | 1994 | 78 | A | Pig | NSW | 1998 | 138 | C | Pig | NSW | 2000 |
| 19 | A | Pig | NSW | 1997 | 79 | A | Pig | QLD | 1998 | 139 | C | Pig | NSW | 1999 |
| 20 | A | Pig | NSW | 1997 | 80 | A | Pig | NSW | 1998 | 140 | C | Pig | Vic | 1999 |
| 21 | A | Pig | NSW | 1998 | 81 | A | Pig | NS | 1998 | 141 | C | Pig | QLD | 2002 |
| 22 | A | Pig | Vic | NS | 82 | A | Pig | NSW | 1998 | 142 | C | Pig | NSW | 2017 |
| 23 | A | NS | Vic | 1998 | 83 | A | Pig | NSW | 1998 | 143 | C | Pig | NSW | 1999 |
| 24 | A | NS | NS | 1999 | 84 | A | Pig | Vic | NS | 144 | C | Pig | NSW | 1998 |
| 25 | A | Pig | WA | 2000 | 85 | A | Pig | NSW | 1998 | 145 | C | Pig | NSW | 2002 |
| 26 | A | NS | NS | 2002 | 86 | A | Pig | NSW | 1998 | 146 | C | Pig | NSW | 1999 |
| 27 | A | Pig | NSW | 2008 | 87 | A | Pig | NSW | 1999 | 147 | C | Pig | NSW | 1998 |
| 28 | A | Pig | NSW | 2010 | 88 | A | NS | NS | 1999 | 148 | C | Pig | NSW | 1998 |
| 29 | A | Pig | QLD | 2010 | 89 | A | Pig | WA | 1999 | 149 | C | Pig | NSW | 1998 |
| 30 | A | Pig | SA | 2010 | 90 | A | Pig | NSW | 1999 | 150 | C | Pig | NSW | 2000 |
| 31 | A | Pig | Vic | 2011 | 91 | A | Pig | SA | 1999 | 151 | C | Pig | QLD | 2006 |
| 32 | A | Pig | Vic | 2011 | 92 | A | Pig | QLD | 2000 | 152 | C | Pig | QLD | 1999 |
| 33 | A | Pig | NSW | 2012 | 93 | A | NS | NS | 2000 | 153 | C | Pig | NSW | 2017 |
| 34 | A | Pig | NSW | 2013 | 94 | A | Pig | NSW | 2000 | 154 | C | Pig | NSW | 2017 |
| 35 | A | Pig | NSW | 2016 | 95 | A | Pig | NSW | 2000 | 155 | C | Pig | NSW | 2017 |
| 36 | A | Pig | NS | 1962 | 96 | A | Pig | NSW | 2001 | 156 | C | Pig | NSW | 2017 |
| 37 | A | NS | NS | 1973 | 97 | A | Pig | Vic | 2001 | 157 | C | Pig | NSW | 2017 |
| 38 | A | Pig | NSW | 1979 | 98 | A | Pig | QLD | 2002 | 158 | C | Pig | NSW | 2017 |
| 39 | A | Pig | NSW | 1981 | 99 | A | Pig | NSW | 2002 | 159 | C | Pig | Vic | 2017 |
| 40 | A | Pig | NSW | 1975 | 100 | A | Pig | NSW | 2005 | 160 | C | Pig | NSW | 2016 |
| 41 | A | Pig | NSW | 1982 | 101 | A | Pig | NSW | 2006 | 161 | C | Pig | Vic | 2017 |
| 42 | A | Pig | Hungary | 1982 | 102 | A | Pig | NSW | 2006 | 162 | C | Pig | Vic | 2017 |
| 43 | A | Pig | NSW | 1982 | 103 | A | Pig | NSW | 2007 | 163 | C | Pig | Vic | 2017 |
| 44 | A | Pig | USA | 1982 | 104 | A | Pig | NSW | 1990 | 164 | C | Pig | Vic | 2017 |
| 45 | A | Pig | NSW | 1983 | 105 | A | Vaccine | NS | 1998 | 165 | C | Pig | Vic | 2017 |
| 46 | A | Pig | NSW | 1983 | 106 | C | Pig | Vic | 2017 | 166 | C | Pig | NSW | 2017 |
| 47 | A | Pig | NSW | NS | 107 | C | Pig | Vic | 2017 | 167 | C | Pig | NSW | 2017 |
| 48 | A | Pig | NSW | NS | 108 | C | Pig | Vic | 2017 | 168 | C | Pig | NSW | 2017 |
| 49 | A | Pig | NSW | 1983 | 109 | C | Pig | Vic | 2017 | 169 | C | Pig | Vic | 2017 |
| 50 | A | Pig | NSW | 1983 | 110 | Fail* | Pig | Vic | 2017 | 170 | C | Pig | Vic | 2017 |
| 51 | A | Pig | NSW | 1984 | 111 | Fail* | Pig | Vic | 2017 | 171 | C | Pig | Vic | 2017 |
| 52 | A | Pig | NSW | 1985 | 112 | C | Pig | Vic | 2017 | 172 | C | Pig | Vic | 2017 |
| 53 | A | Fish | Germany | 1988 | 113 | C | Pig | Vic | 2017 | 173 | C | Pig | Vic | 2017 |
| 54 | A | Pig | NSW | 1988 | 114 | C | Pig | NSW | 2017 | 174 | C | Pig | Vic | 2017 |
| 55 | A | Pig | NSW | 1989 | 115 | C | Pig | NSW | 2017 | 175 | C | Pig | NSW | 2017 |
| 56 | A | Pig | WA | 1992 | 116 | C | Pig | NSW | 2017 | 176 | C | Pig | NSW | 1998 |
| 57 | A | Pig | WA | 1992 | 117 | C | Pig | NSW | 2017 | 177 | C | Pig | Vic | 2017 |
| 58 | A | Pig | WA | NS | 118 | C | Pig | Vic | 2017 | 178 | C | Pig | Vic | 2017 |
| 59 | A | Pig | NS | 1996 | 119 | C | Pig | Vic | 2017 | 179 | C | Pig | Vic | 2017 |
| 60 | A | Pig | NS | 1996 | 120 | C | Pig | Vic | 2017 | 180 | C | Pig | Vic | 2017 |

A: archived isolated; C: contemporary isolated; NS: data not specified;
*Discarded isolates, which failed quality control.

## *2.2 Bacteriology*

### 2.2.1 Lyophilised (archive) cultures

Briefly, lyophilised vials were opened and resuspended in Brain Heart Infusion (BHIB) and horse serum and then transferred to Tryptic Soy Sheep Blood Agar (SBA) (MicroMedia, Australia) as per standard culture procedures for *E. rhusiopathiae*.

### 2.2.2 Field (contemporary) samples

Tissues: Tissues were homogenised in saline using a stomacher and an aliquot of the homogenate was cultured in BHIB with 0.08% w/v kanamycin, 0.01% w/v neomycin and 0.005% w/v vancomycin (KNV) and BHIB only. The culture was then streaked onto SBA and Colistin and Nalidixic Acid (CNA) plates as per standard culture procedures for *E. rhusiopathiae*.

Joints*:* Isolation of *E. rhusiopathiae from* the *joints of* pigs was performed using a hot spatula to sear the outside of the joint to expose the synovial membrane, cartilage and ligaments. Exposed membranes were swabbed and streaked onto SBA, CNA, BHIB and BHIB with KNV as per standard aseptic culture procedures for *E. rhusiopathiae*. Often a 4˚C enrichment step was included if required. Liquid cultures were incubated for 24 h, and then subsequently streaked onto CNA and SBA. Colonies that resembled *E. rhusiopathiae* were sub-cultured onto SBA and genus and species confirmed by PCR.

### 2.2.3 Storage of *E. rhusiopathiae* -80˚C culture collection

Once a colony was confirmed to be *E. rhusiopathiae*, a single colony was grown in liquid culture overnight and suspended in BHIB with 20% glycerol and stored at -80˚C.

## *2.3 Real-time PCR*

All isolates were assessed by real-time PCR to confirm the presence of *E. rhusiopathiae*. PCR was performed using PerfeCTa SYBR® Green FastMix in a final volume of 25 µL. Thermocycling was performed using a magnetic induction cycler (MIC) (Biomolecular Systems, Potts Point, Australia) using the following conditions: 95˚C 3 min, 40 cycles (95˚C for 5 s, 57˚C 10 s and 72˚C 10 s). Results with a Tm value of 74˚C were considered positive. *E. rhusiopathiae* DNA was included as a positive control in all PCR assays.

## *2.4 Serotype*

Serotyping for *E. rhusiopathiae* is not currently available in Australia and it was cost prohibitive to undertake this internationally. Therefore, all serotype data was for archive isolates as this data could be obtained from historical records (bacterial collection records or microfiche).

## *2.5 Antibiotic Sensitivity Profile*

Sensitivity of *Erysipelothrix rhusiopathiae* isolates to antibiotics (Table 2) commonly used in the pork industry was assessed. These antibiotics were chosen following consultation with pork industry experts (Dr. Graeme Eamens and Dr. Pat Mitchell) and the literature (Yamamoto, *et al*. 2001, Opriessnig, *et al*. 2004, Ding, *et al*. 2015)Venditti, *et al*. 1990, Chuma, *et al*. 2010). The antibiotic Tylosin commonly used in the pork industry was not available in Australia as antibiotic disks. A surrogate antibiotic, erythromycin was selected

according to the information supplied in the CDS method (Appendix 9.2) (Bell, *et al*. 2016). Antibiotic sensitivity testing was performed using a modified version of the CDS protocol for disk diffusion (Bell, *et al*. 2016). Briefly, 3-6 colonies were suspended in saline and flooded onto the surface of Sensitest blood agar plates (MicroMedia, Australia). After 15 min any excess inoculum was removed and six antibiotic disks (Oxoid, Scoresby, Australia) (Table 2) were dispensed onto the plate. Plates were incubated at 35˚C with 10% $CO_2$ for 48 hours. *Staphylococcus aureus* (NTC6571) was included as a positive control throughout the testing. The results from the testing were reported as either sensitive (≥ 6mm) or resistant (< 6mm) based on the zone of inhibition as specified in the CDS protocol without exceptions.

**Table 2: Antibiotics utilised in antimicrobial susceptibility testing.**

| Antibiotic | Concentration | Line of defense |
|---|---|---|
| Penicillin | 0.5 units | 1st |
| Amoxicillin | 10 µg | 1st |
| Erythromycin[1] | 5 µg | 1st |
| Oxytetracycline | 30 µg | 1st |
| Lincospectin[2] | 109 µg | 2nd |
| Ceftiofur | 30 µg | 3rd |

[1]Surragote disc used in place of Tylosin
[2]Combination of lincomycin and spectinomycin antibiotics

### *2.6 Whole Genome Sequencing (WGS)*

#### 2.6.1 Extraction and purification of DNA for WGS

DNA was extracted using the QuickGene DNA Tissue kit (Kurabo, Japan) in combination with the QucikGen-810 (Kurabo, Japan). After extraction, the DNA was subjected to the following quality control analyses before submission for WGS; the DNA had to be intact double-stranded DNA, as assessed by agarose gel electrophoresis; the DNA concentration required for NextEra XT sequencing was measured using the Qubit Fluorimeter and a Nano Drop spectrophotometer measured the 260/280 ratio, to check for RNA contamination. Once the quality of the DNA was confirmed, the samples were submitted to the Ramaciotti Centre for Genomics where NextEra XT WGS was undertaken.

#### 2.6.2 Genome sequencing analysis methods

WGS reads were then assembled by Daniel Bogema (Molecular Epidemiologist, Biotechnology EMAI) using the Spades assembler (v3.11.1) software (Bankevich, *et al*. 2012). Additionally, raw sequencing data and assembled WGSs were monitored using stringent checks to ensure genome sequences were of the highest quality. Raw sequencing data was quality checked for accuracy and contamination using fastqc (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and kraken software (Wood and Salzberg 2014). Assembled genome sequences were checked using QUAST (v4.6.3) (Gurevich, *et al*. 2013) and BlobTools (v1.0) (Laetsch and Blaxter 2017) to assess

genome completeness, fragmentation and to further exclude contaminating DNA sequences.

#### 2.6.3 Multi locus sequence type (MLST)

The MLST system of Janssen, *et al.* (2015) was applied to the genome sequences performed using the SRST2 (Inouye, *et al.* 2014) and MLST pipelines (https://github.com/tseemann/mlst). Extraction of novel sequences to identify new sequence types was completed using the novel option of MLST.

#### 2.6.4 Antibiotic resistance gene profiling

Antibiotic resistance gene presence was identified using SRST2 (Inouye, *et al.* 2014) and ABRicate, a bundle of multiple databases, which mass screens contigs for antimicrobial resistance or virulence genes. These databases include Resfinder, CARD, ARG-ANNOT, NCBI BARRGD, NCBI, EcOH, PlasmidFinder and VFDB (https://github.com/tseemann/abricate).

#### 2.6.5 Virulence gene and phylogenetic analysis

The presence of virulence genes were determined using manual blastn searches with *E. rhusiopathiae* virulence genes identified by literature searches (Ogawa, *et al.* 2011, Ding, *et al.* 2015, Janssen, *et al.* 2015). Genome sequences were annotated using Prokka (Seemann 2014). Phylogeny of genome sequences was inferred using protein sequences identified from the Prokka annotation by using the PhyloPhlAn pipeline (Segata, *et al.* 2013). An alignment of marker genes from the PhyloPhlAn pipeline was used to generate a maximum likelihood tree with the genome sequence of *E. larvae* and *E. tonsillarum* as outgroups.

### *2.7 Epi-Interface development*

All bacteriological, genomic and clinical data was then assembled into Microsoft Access (v6) to generate the *E. rhusiopathiae* Epi-Interface. Analysis of data using the Epi-Interface was undertaken in Access and recorded on a work sheet (Figure 1).

The database was assembled from seven sets of data (Blue cells in Figure 1): Clinical Information, MLST, Antibiotic Resistance Genes, Virulence Genes, Quality WGS Report, Quality Control Information and Contamination Report. The data was linked using the WGS ID No. (Orange cells, Figure 1) that is a specific ID code for each *E. rhusiopathiae* isolate in the database. The key information generated within the MLST (MLST ID No.), Antibiotic Resistance Genes (Gene and Gene 2) and Virulence Genes (Virotype) spreadsheets are highlighted in the Yellow cells (Figure 1). These were then used systematically to interrogate the Clinical Information using the information highlighted in the Green cells (Figure 1). The remaining information (highlighted in grey) is the raw data used to generate the different profiles which can be accessed using the WGS ID No. This is for scientific use only, not for disease or epidemiological purposes.

***Erysip Epi-Interface*** ***Query name*** ***Date***

| MLST | Clinical Information | | Virulence Genes | Quality WGS Report | Quality Control information |
|---|---|---|---|---|---|
| **WGS ID No.** | **WGS ID No.** | **Gender** | **WGS ID No.** | **WGS ID No.** | **WGS ID No** |
| LIMS ID No. | LIMS ID No. | **Joints affected** | ERH_1356_K09815_ABC_transporter,_metal-binding_lipoprotein | LIMS ID No. | LIMS ID No |
| DNA Extraction No. | DNA Extraction No. | **Skin lesions** | ERH_0175_K03386_peroxiredoxin\|ahpC;_alkyl-hydroperoxide_reductase | DNA Extraction No. | DNA Extraction No |
| Purpose | Purpose | **Clinical history** | ERH_0407\|cbpA;_choline-binding_protein | Project ID No | Purpose |
| Project ID No | Project ID No | **Differential diagnosis** | ERH_0768\|cbpC;_choline-binding_protein | . contigs (>= 0 bp) | Project ID No |
| **MLST ID No.** | **Serovar or Type** | **Post-mortem** | ERH_0260_K01361_lactocepin\|cell-envelope_associated_proteinase,_subtilase_family | . contigs (>= 1000 bp) | -80C Glycerol stock location Box |
| gpsA | **1st (F7)** | **Histopathology** | ERH_0333_K06131_cardiolipin_synthase_A/B\|cls;_cardiolipin_synthetase | . contigs (>= 5000 bp) | -80C Glycerol stock location row/column |
| recA | **2nd (F8)** | **Bacteriology** | ERH_0075\|collagen-binding_protein | . contigs (>= 10000 bp) | Check this page |
| purA | **Source** | **Management** | ERH_1436\|collagen-binding_protein | . contigs (>= 25000 bp) | DNA extraction Run 1 |
| pta | **Tissue** | **Other disease factors/characteristics** | ERH_0777\|dipeptidase | . contigs (>= 50000 bp) | DNA extraction Run 2 |
| prsA | **Disease 1** | **Vaccination history 1** | ERH_1034\|fibronectin-binding_protein | Total length (>= 0 bp) | DNA extraction Tube .1 |
| galK | **Disease 2** | **Vaccination history 2** | ERH_0221\|glycoside_hydrolase,_family_16 | Total length (>= 1000 bp) | DNA extraction Tube .2 |
| ldhA | **Disease 3** | **Vaccination history 3** | ERH_0561\|glycoside_hydrolase,_family_85 | Total length (>= 5000 bp) | Qubit |
| Naming Origin | **Deidentification No** | **Vaccination history 4** | ERH_0649_K11068_hemolysin_III | Total length (>= 10000 bp) | Agarose gel |
| Depth | **Reference** | **Vaccination history 5** | ERH_0467_K03699_hemolysin-related_protein | Total length (>= 25000 bp) | Confirmatory PCR Run 1 |
| MaxMAF | **Reference M No.** | **Stressors 1** | ERH_0150_K01727_hyaluronate_lyase\|hylA;_hyaluronidase | Total length (>= 50000 bp) | Confirmatory PCR Run 2 |
| **Antibitoic Resistance Genes** | **Reference CM No.** | **Stressors 2** | ERH_0765_K01727_hyaluronate_lyase\|hylB;_hyaluronidase | . contigs Excluding contigs < 500bp | Confirmatory PCR Run 3 |
| **WGS ID No.** | **Reference Other No.** | **Stressors 3** | ERH_1210_K01727_hyaluronate_lyase\|hylC;_hyaluronidase | Largest contig Excluding contigs < 500bp | Confirmatory PCR Result |
| LIMS ID No. | **Brief Clinical Desciption** | **Streptomycin** | ERH_1258\|hypothetical_protein | Total length Excluding contigs < 500bp | Complete |
| DNA Extraction No. | **Interesting cases** | **Chloramphenical** | ERH_1472\|internalin-like_protein | GC (%) Excluding contigs < 500bp | PCR |
| Purpose | **State or Country** | **Erythromycin** | ERH_0728\|leucine-rich_repeat_protein | N50 Excluding contigs < 500bp | PCR result |
| Project ID No | **Region** | **Spiromycin** | ERH_0278\|LPXTG-motif_cell_wall_anchor_domain_protein | N75 Excluding contigs < 500bp | PCR Run Reference No 1 |
| DB | **Town** | **Neomycin** | ERH_1454\|LPXTG-motif_cell_wall_anchor_domain_protein | L50 Excluding contigs < 500bp | PCR Run Reference No 2 |
| **Gene** | **Raw Geographic location** | **Penicillin G** | ERH_1214\|lysophospholipase | L75 Excluding contigs < 500bp | PCR Run Reference No 3 |
| Allele | **Date** | **Bacitracin** | ERH_1433\|lysophospholipase | N's per 100 kbp Excluding contigs < 500bp | Antibiotic |
| Coverage | **Date outbreak first noted** | **Furazolidone** | ERH_0299_K01186_sialidase-1\|nanH.1;_neuraminidase | **Contamination Report** | Pellet |
| Depth | **Date Samples collected** | **Cloxacillin** | ERH_0761\|nanH.2;_neuraminidase | **WGS ID No.** | Glycerol |
| Diffs | **Acute/Chronic 1** | **Methicillin** | ERH_0356\|nrdH;_glutaredoxin-like_protein_NrdH | LIMS ID No. | |
| Uncertainty | **Acute/Chronic 2** | **Tetracycline** | ERH_0072_K07001_NTE_family_protein\|patatin-like_phospholipase | DNA Extraction No. | |
| Divergence | **Amoxycillin (AML)** | **Nitrofurazone** | ERH_0334\|patatin-like_phospholipase | Purpose | |
| Length | **Erythromycin 'E'** | **Linco-spectin** | ERH_0201\|pectin_lyase_fold-containing_protein | Project ID No | |
| maxMAF | **Ceftiofur (EFT)** | **Sulphonamide** | ERH_0161\|peptidase_M14,_carboxypeptidase_A | Contamination report (Kraken) ID No. | |
| Clusterid | **Lincospectin (LS)** | **Sulpha-trimethoprim** | ERH_0347_K06999_phospholipase/carboxylesterase | Bacteria (% homology) | |
| Seqid | **Penicillin (P)** | **Lincomycin** | ERH_0083_K06999_phospholipase/carboxylesterase | Bacteria 2 (% homology) | |
| Annotation | **Oxytetracycline (OT)** | **Novobiocin** | ERH_0388\|phospholipase_D_family_protein | Bacteria 3 (% homology) | |
| DB 2 | **Mortality %** | **Trimethoprim** | ERH_0148\|pldB;_lysophospholipase | Bacteria 4 (% homology) | |
| **Gene 2** | **Affected %** | **Ampicillin** | ERH_1467\|putative_biofilm-associated_surface_protein | Bacteria 5 (% homology) | |
| Allele 2 | **Age** | **Ceftiofur** | ERH_0668\|rspA;_rhusiopathiae_surface_protein_A | Bacteria 6 (% homology) | |
| Coverage 2 | **Breed** | **Sulphafurazole** | ERH_0669\|rspB;_rhusiopathiae_surface_protein_B | Bacteria 7 (% homology) | |
| Depth 2 | | | ERH_1687\|rspC;_rhusiopathiae_surface_protein_C | Bacteria 8 (% homology) | |
| Diffs 2 | | | ERH_1065_K04564_superoxide_dismutase,_Fe-Mn_family\|sodA;_superoxide_dismutase | **MLST ID no.** | |
| Uncertainty 2 | | | ERH_0094\|spaA.1;_surface_protective_antigen_SpaA.1 | | |
| Divergence 2 | | | ERH_0162_K11065_thiol_peroxidase,_atypical_2-Cys_peroxiredoxin\|tpx;_thiol_peroxidase | | |
| Length 2 | | | ERH_0375_K03671_thioredoxin_1\|trxA.1;_thioredoxin | | |
| maxMAF 2 | | | ERH_1500_K03671_thioredoxin_1\|trxA.2;_thioredoxin | | |
| Clusterid 2 | | | ERH_1311_K00384_thioredoxin_reductase_(NADPH)\|trxB.1;_thioredoxin-disulfide_reductase | | |
| Seqid 2 | | | ERH_1541_K00384_thioredoxin_reductase_(NADPH)\|trxB.2;_thioredoxin-disulfide_reductase | | |
| Annotation 2 | | | ERH_1139\|ushA.2;_5'-nucleotidase | | |
| Number | | | **Virotype** | | |

**Figure 1: Worksheet used to record analysis of the *E. rhusiopathiae* Epi-Interface.**

## 3.0 Outcomes

### *3.1 Whole Genome Sequence*

In total 180 *E. rhusiopathiae* isolates were selected for whole genome sequencing (WGS) from 195 isolates identified to be of interest. This was based on their location of origin, serotype, clinical history, vaccine association, culture performance and anti-microbial susceptibility assessed by the CDS method. However, often the associated metadata was limited for an isolate, particularly those sourced from the archive collection. In total 178 workable genomes were assembled and interrogation of MLST, AMR genes and virulence genes were undertaken. These analyses and the metadata were then built into the Epi-Interface database.

#### 3.1.1 Phylogenetic Tree

Phylogenies of *E. rhusiopathiae* isolates were inferred using >400 conserved proteins generated by PhyloPhlAn (Segata *et al*. 2013). This has confirmed that the 178 isolates in this study do belong to the *rhusiopathiae* species, as there is a clear distinction between *E. rhusiopathiae* and the other Erysipelothrix sp. (i.e. *E. tonsillarum*). In addition, we observed agreement between previously sequenced *E. rhusiopathiae* isolates and those sequenced in this current study. Interestingly this phylogenetic analysis also demonstrated that the majority of isolates from this study, which grouped with international reference strains, were the international isolates (6/8) from the archive collection.

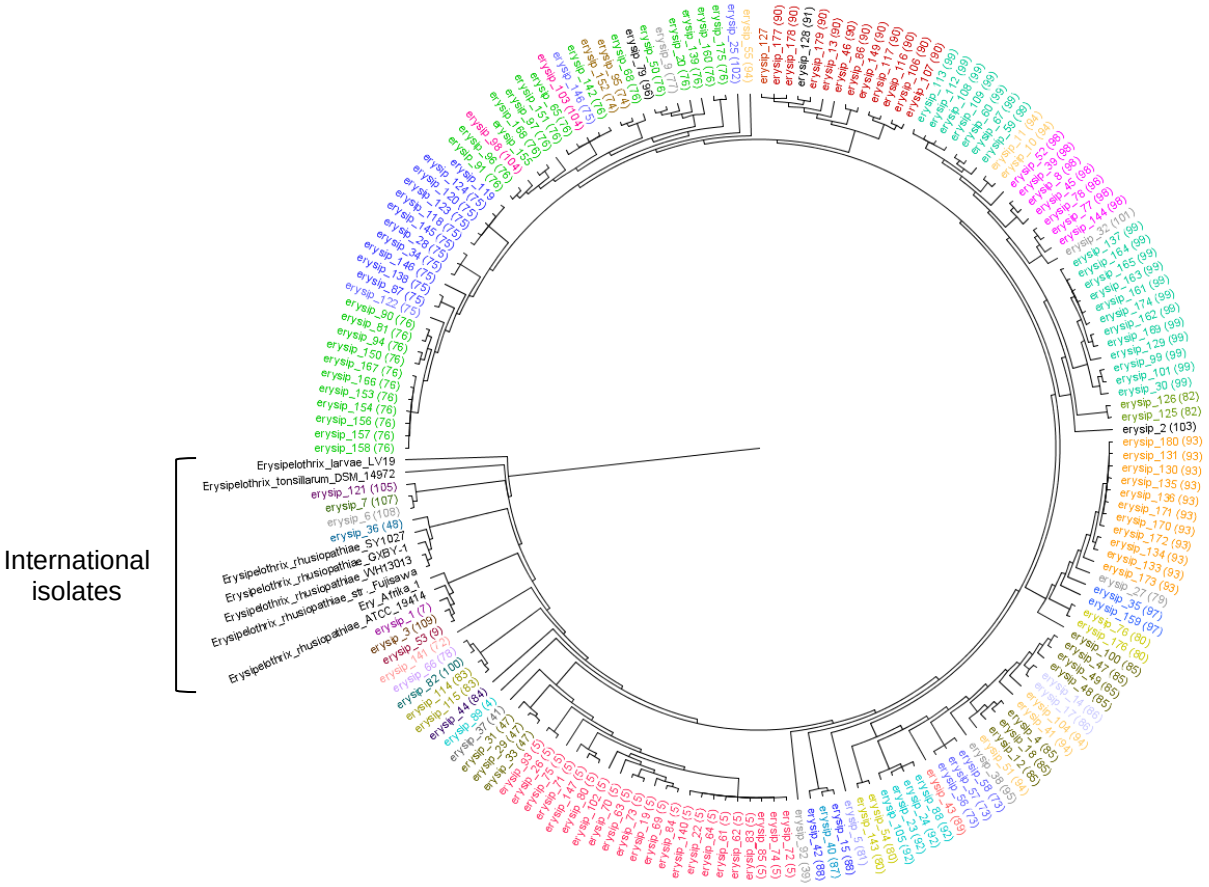



**Figure 2: Phylogenetic relationship between the *Erysipelothrix rhusiopathiae* isolates utilised in this study. This tree was generated using PhylopPhlAn and is based on >400 conserved bacterial protein sequences. *Erysipelothrix tonsillarum* and *Erysipelothrix larvae* were used as the out-group. Isolates are coloured according to their multi locus sequence type (MLST) classification.**

## *3.2 Antibiotic Sensitivity Testing*

### 3.2.1 Technique

Antibiotic sensitivity testing was carried out using a modified version of the CDS method utilizing the guidelines for Erysipelothrix (Bell, *et al.* 2016).

Both the calibration guide and surrogate disk selection tables from the CDS manual (Appendix 9.2 and 9.3) were used to select, undertake and assess the antibiotic disk diffusion testing for Gram positive bacteria. Minimum inhibitory concentrations were not determined as this testing was carried out as a high through-put screening tool. The assessment of clearance in this study has provided a qualitative assessment of resistance only.

### 3.2.2 Selection of Antibiotics and Findings.

- Penicillin was selected as it is the most routinely used antibiotic for treatment of erysipelas in Australian pigs. Amoxicillin was included as an example of an extended spectrum of Penicillin, often used against respiratory disease. Both of these Beta Lactam antibiotics inhibit bacterial cell wall synthesis and are therefore useful against Gram positive bacteria.

There was a single contemporary farm that had an isolate positive for penicillin resistance but all of the isolates from the 133 farms were susceptible to Amoxicillin.

- Tylosin is used against a range of enteric and respiratory diseases, as well as erysipelas in pigs and often results in a positive effect on weight gain and food conversion efficiency. At the time of this testing Tylosin diffusion disks were not available in Australia. Therefore Erythromycin was used as a surrogate, as recommended by the CDS manual (Appendix 9.3); Erythromycin and Tylosin are macrolides which reversibly bind to a ribosome subunit inhibiting protein synthesis. A single contemporary farm produced an isolate with apparent resistance to Erythromycin.
- Oxytetracycline was selected as representative of the tetracycline group. They are active against a wide range of Gram-positive, Gram-negative bacteria and atypical organisms. Tetracycline's also inhibit protein synthesis but are bacteriostatic rather than bactericidal. In this study three contemporary farms and 24 archive farms had isolates that were tetracycline resistant. In the archive isolates Oxytetracycline was the only antibiotic against which resistance was observed.
- Lincomycin is used in the prevention and treatment of dysentery and sometimes in mycoplasma infections. It is not active against Gram negative bacteria and also inhibits protein synthesis. Lincomycin belongs to the group known as the lincosamides.
- Spectinomycin is an aminocyclitol antibiotic structurally related to aminoglycosides which also inhibits protein synthesis. It is effective against Gram positive and negative bacteria. Lincospectin is the common name for a combination of both Lincomycin and Spectinomycin and is classified as a 2nd tier antibiotic in the Australian pig industry. Only one contemporary farm demonstrated resistance to this combination.
- Ceftiofur belongs to a group known as the third generation cephalosporins, which is also a beta lactam antibiotic like Penicillin. It is of importance for use in humans and is therefore designated 3rd tier. In this study we found three farms which appeared to have resistance to Ceftiofur. However, previous determination of a minimum inhibitory concentration (MIC) by a commercial laboratory using the Sensititre platform (Thermofisher) on one of these borderline isolates, classified the clonal isolate as sensitive. Given this additional information, we can therefore conclude that the other three isolates, which had the same annular radius, would also fall within the sensitive range. Interestingly one of these farms did have the Penicillin and Lincospectin resistant isolates.

**Table 3: The number and percentage of farms which displayed resistance to the antibiotics selected for testing according to the hierarchy of defense.**

| Antibiotic | Line of Defense | Farms with R isolates | |
|---|---|---|---|
| | | Number | Percentage |
| Penicillin | 1st | 1/133 | 0.75% |
| Amoxicillin | 1st | 0/133 | 0% |
| Erythromycin | 1st | 1/133 | 0.75% |
| Oxytetracycline | 1st | 27/133 | 20.3% |
| Lincospectin (Lincomycin/Spectinomycin ) | 2nd | 1/133 | 0.75% |
| Ceftiofur | 3rd | 0/133 | 0% |

R: Resistant

### 3.2.3 Result by Farm

There were 133 farms involved in this study, of which 30 farms had one or more isolates that appeared to be resistant to one or more of the antibiotic listed above. These 30 farms included 24 archive farms, which only displayed resistance to Oxytetracycline. The resistance displayed by the six contemporary farms can be seen in Figure 3. Two of the contemporary farms had resistance to two antibiotics. These antibiotic combinations were Penicillin and Lincospectin on one farm and Oxytetracycline and erythromycin on the second farm.

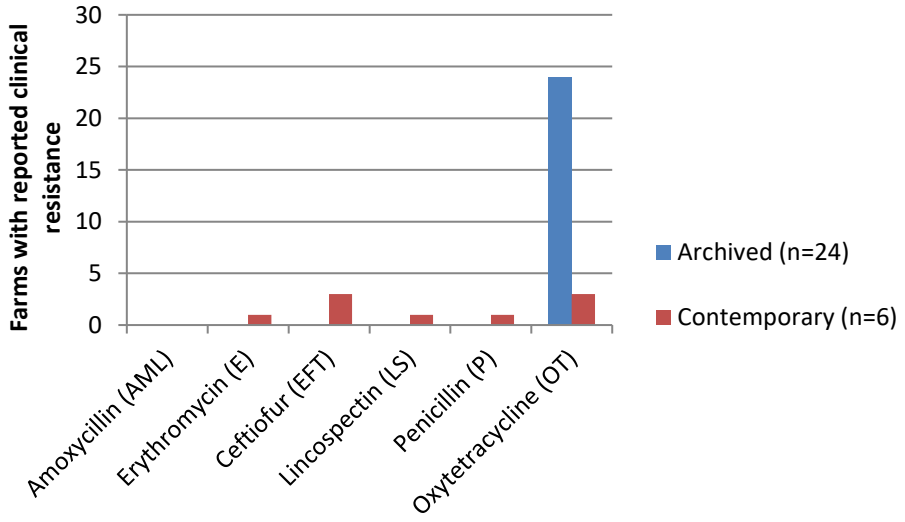



**Figure 3: Resistance of *Erysipelothrix rhusiopathiae* to antibiotics recorded as frequency per farm**

### 3.2.4 Result by Isolate

Multiple isolates taken from the same farm submission were examined. These finding are in Table 4 below expressed as per isolate and reported according to archive and contemporary.

Antibiotic sensitivity testing shows that all the strains were susceptible to Amoxicillin. A single isolate was resistant to Lincospectin a second tier antibiotic.

This was also the case for Penicillin which indicates that this first-line antibiotic remains a suitable treatment option for erysipelas. Two isolates, originating from the same farm, were resistant to either of the first generation antibiotics, Erythromycin or Penicillin. Notably, of the 30 isolates (17%) which carried a tetracycline resistance gene, 28 of these isolates (93%) also exhibited phenotypic resistance.

Whilst all care was taken, caution should be used in the interpretation of these antibiotic sensitivity results as minimum inhibitory concentrations were not determined and plate variability may occur which we attempted to minimize by purchasing Sensitest plates from a commercial supplier.

However, as the development of resistance is relative to the level and extent of selection pressure, these results should be taken into consideration during the development of holistic antibiotic treatment regimes.

**Table 4: Summary of resistance of *Erysipelothrix rhusiopathiae* isolates to antibiotics**

| Antibiotic | Line of Defense | Number R Isolates | |
|---|---|---|---|
| | | Archive | Contemporary |
| Penicillin | 1st | 0/112 | 1/66 |
| Amoxicillin | 1st | 0/112 | 0/66 |
| Erythromycin | 1st | 0/112 | 1/66 |
| Oxytetracycline | 1st | 25/112 | 3/66 |
| Lincomycin/Spectinomycin (Lincospectin) | 2nd | 0/112 | 1/66 |
| Ceftiofur | 3rd | 0/112 | 0/66 |
| **Total (%) Farms with R Isolates** | | **25 (22.3%)** | **5 (7.6%)** |

R: Resistance

### *3.3 Antimicrobial Resistance Genes*

Interrogation of the WGS for antimicrobial resistance genes found the following genes:

3.3.1 TetM_Tet

- The tetM gene encodes a tetracycline ribosomal protection protein, which prevents the antibiotic binding to receptors on the 30S ribosomal subunit of the bacteria allowing protein synthesis to continue.
- Tet represents activity against the tetracycline class of antibiotics.

3.3.2 Str_AGly and StrA_AGly

- The Str gene encodes a streptomycin resistant protein known as streptomycin adenyltransferase conferring resistance to the aminoglycosides, including streptomycin and kanamycin.
- The StrA gene is one of the Str cluster.
- AGly represents activity against the aminoglycoside class of antibiotics.

### 3.3.3 LinB_MLS

- LinB is a gene that encodes an enzyme, which catalyzes the inactivation of the antibiotic molecule.
- MLS represents activity against the macrolide-lincosamide-streptogramin group of antibiotics.

### 3.3.4 MefA_MLS

- This gene encodes an efflux pump which actively rids the bacteria of macrolides.
- MLS represents activity against the macrolide-lincosamide-streptogramin group of antibiotics.

### 3.3.5 ErmG_MLS

- Encodes a target modification on the bacterial ribosome which prevents binding at the site of action by the MLS group of antibiotics.
- MLS represents activity against the macrolide-lincosamide-streptogramin group of antibiotics.

### 3.3.6 Additional Information

It is interesting to note that bacteria have three resistant mechanisms against macrolide and lincosamide antibiotics. These are (1) modification of the target-site, (2) efflux of the antibiotic, and (3) inactivation of the antibiotic. In this study, we have identified genes responsible for all three of these mechanisms. These genes have been reported to be unequal in terms of their incidence and the clinical effects observed in disease produced by bacteria which carry them. In addition, site modification confers broad-spectrum resistance to macrolides and lincosamides whereas efflux and inactivation are only effective against some of the antibiotics in this class (Leclercq 2002).

### 3.3.7 Result by Farm

In total 133 submission sites (farms) were available for antimicrobial resistance gene identification (Figure 4). Of these only three resistance genes were found to be present on the archive farms. These were the TetM, LinB and MefA genes. Two of the archive farms had two resistance genes present. The combination of these genes was LinB/TetM and MefA/TetM. Contemporary farms had the Str, TetM, and ErmG genes. Only one contemporary farm had two resistance genes present with these being ErmG/TetM.

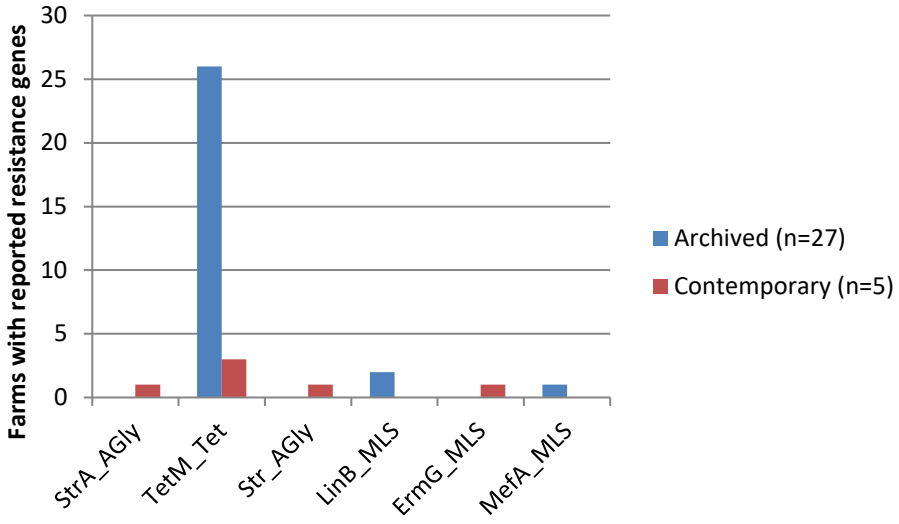



**Figure 4: Antimicrobial resistant genes identified in *Erysipelothrix rhusiopathiae* isolates recorded as frequency by farm.**

### *3.4 Virulence Genes (VGs)*

The WGS sequences were interrogated for the presence/absence of 47 *Erysipelothrix*-specific virulence genes which were identified from the literature. Ding *et al.* (2015) had noted that 15/48 isolates (31%) carried all 21 VGs involved in their study. Consistent with those results we found 50/178 isolates (28%) carried all of the 47 virulence genes of interest in this study.

VG presence/absence was also examined for trends when aligned with MLST or serotype. We were unable to identify any trends in the absence or prevalence of VGs with these two parameters. This is not to say that trends do not exist if the data were to be manipulated, for example, if the number of genes were refined. All 178 isolates (100%) carried the VGs for SpaB and SpaC, however, only 174/178 isolates (97.8%) had the SpaA gene. The four isolates which did not have the SpaA gene were also missing between 17 and 18 VGs each (as determined by blastn). As the genomes were analyzed with 95% identity and 50% coverage, these results suggest that these parameters have a lower level of discrimination for the hypervariable Spa regions to differentiate these surface protective antigens. Reanalysis can be undertaken by applying increased stringency.

Overall, 128 isolates were missing only between one and six of the 47 VGs. As a result we attempted to develop a virotype numbering system (1-24) based on the combinations of VGs present in an isolate as identified by the blastn search. This system and the breakdown of isolates into archive and contemporary are displayed graphically below (Figure 5).

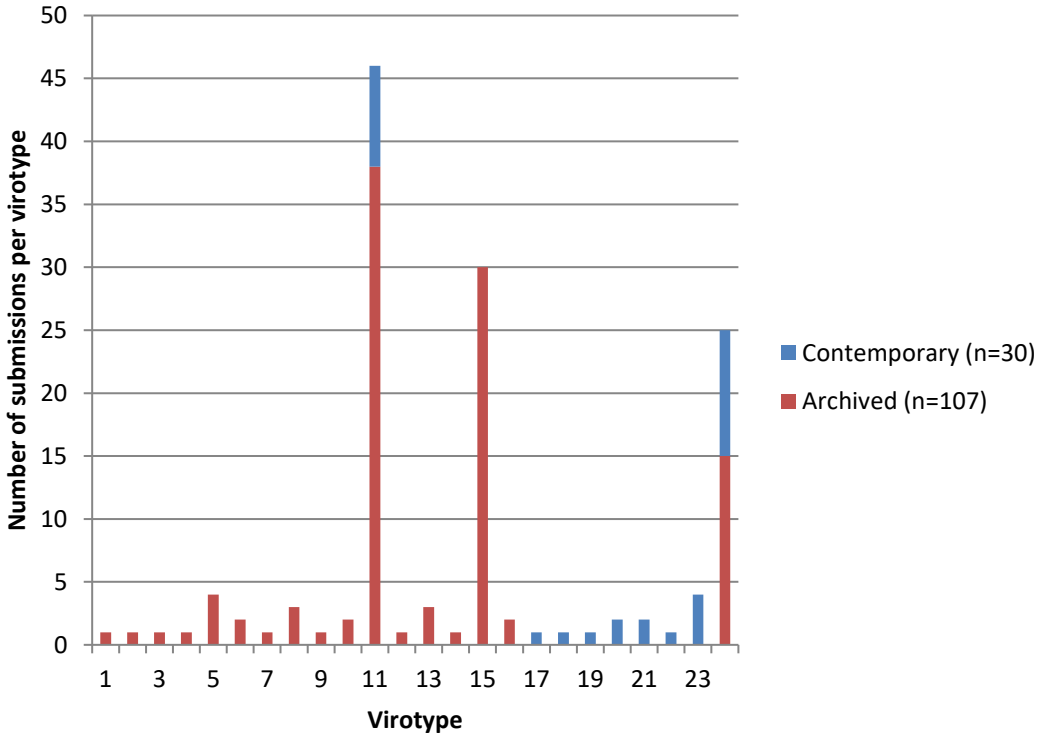



**Figure 5: Summary of the number of virulence profiles identified in E*rysipelothrix rhusiopathiae* archive and contemporary isolates.**

### *3.5 Serotype*

It was thought until recently that NSW DPI no longer had the ability to serotype *Erysipelothrix* isolates. Serotype specific anti-sera have recently been located on site but the set is not complete for common serotypes. As a result, selection of the archive isolates was biased towards serotyped isolates, including those of novel serotype, which accounted for the inclusion of a number of the international isolates as detailed in Table 5 below.

**Table 5: Serotypes of the international isolates included in this study and their country of origin**

| Serotype | Country of Origin | Host |
|---|---|---|
| 1A | Germany (East) | Pig |
| 1B | Germany (West) | Unknown |
| 2B | Germany | Fish |
| 13 | Hungary | Pig |
| 15 | Hungary | Pig |
| 17 | USA | Pig |
| 19 | USA | Pig |
| 21 | Argentina | Unknown |

The inability to serotype contemporary isolates was a disappointment given previous studies which found the main serotypes were 1a, 1b and 2 (Eamens, *et al*. 1988, Eamens, *et al*. 2006). However, extensive *E. rhusiopathiae* serotyping projects have shown that up to 97.6% of isolates can be attributed to serotypes 1a, 1b and 2b (Imada, *et al*. 2004). The value of serotyping must be questioned given 800 serotyped isolates did not correlate with the manifestation of disease (Imada, *et al*. 2004). Serotyping may not be a discriminatory tool for porcine isolates; it may be of more value to the Epi-Interface if the host range is increased. We now have the genomes from these isolates and anticipate that further analysis may elucidate a molecular approach to serotyping. At present, the correlation, if any, between serotype and disease severity is not well understood and to that end this is uninformative for the veterinarian and/or producer. Given that serotyping is unavailable in Australia this suggests that it does not play an important role in diagnosis. However, this may change as we investigate the WGS and establish a correlation between serotype and virulence.

### *3.6 Multi Locus Sequence Typing (MLST)*

#### 3.6.1 MLST system

The MLST system developed by Janssen *et al*. (2015) involved 165 isolates. These isolates covered a wide host range with only 36 from pigs. These 36 pig isolates fell into 17 of the total 72 MLSTs described in this publication. Predominately these were from European sources (25 Germany, 2 Estonia, 2 USA and 7 not applicable). In contrast, this innovation grant has produced 46 different MLSTs from 178 isolates (Figure 6). Of these 46 MLSTs, nine were equivalent to the Janssen *et al*. (2015) of which four were from Europe (1 Hungary and 3 Germany). The remaining 37 were identified as novel MLSTs of which four were of international origin (1 Argentina, 2 USA and 1 Hungary).

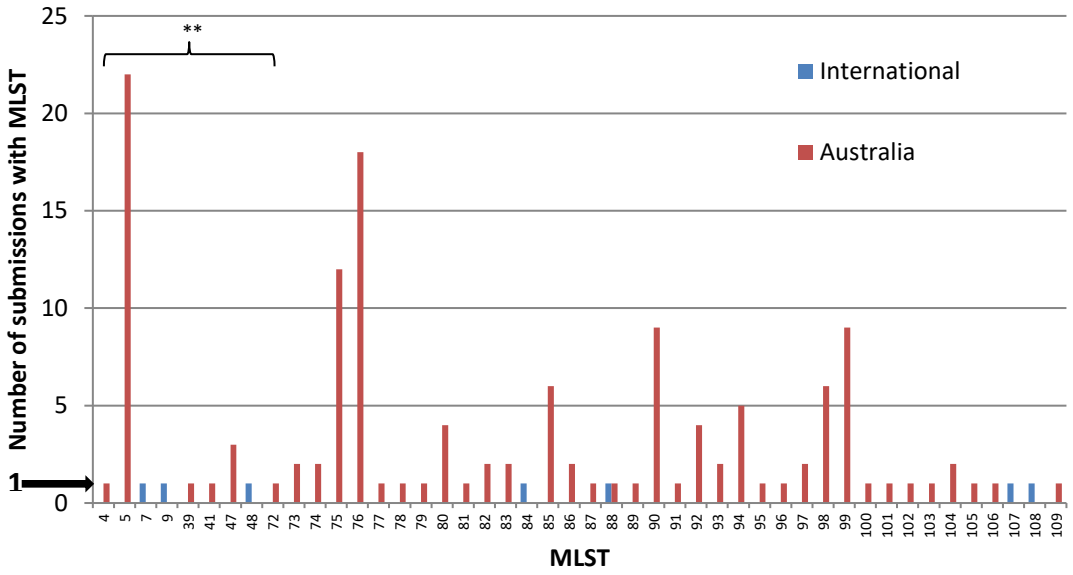



**Figure 6: Summary of the classification of *Erysipelothrix rhusiopathiae* isolates according to multi locus sequence type (MLST) **MLSTs 1-72 described by (Janssen, *et al*. 2015).**

Predominantly the isolates in this study fell into several main groupings (>10 isolates) with the majority occurring in MLSTs 5, 75 and 76. Of the 26 MLSTs represented by single isolates, seven were attributable to international isolates that had been included from the archive collection because of a novel aspect of their profile, for example serotype. The remaining 19 isolates were from Australia of which 16 were archive and three were contemporary. Longitudinal studies of single farms were not undertaken as the archive isolates were selected and had been processed prior to receipt of contemporary isolates. It is difficult to draw conclusions with respect to MLST over time due to the numbers of samples used in this study and bias potentially introduced with such small numbers.

#### 3.6.2 Additional findings

Whilst the sequence reads were being assembled we found that 26 isolates contained 1.06 – 11.0% of reads for a bacteriophage (SE-1) whose isolation from *E. rhusiopathiae* and its characterisation were first reported in 2016 in China (Yuan, *et al*. 2016).

### *3.7 The Epi-Interface Database*

The entire body of clinical, bacteriological and genomic information obtained from the 178 isolates was then imported into Access® (v6), a database management system. This centralised location enabled the metadata to be easily interrogated and trends identified.

To test the power of the Epi-Interface we posed a number of queries to the database as follows:

#### 3.7.1 What is the distribution of Australian MLSTs?

A geographical representation of the distribution of MLSTs was compiled for national and international isolates. These maps were generated using data exported from the Epi-Interface into ZeeMaps. Examples of the power of this tool are shown below, limited to Australian MLSTs for archive (Figure 7) and contemporary (Figure 8) isolates. Please note each map is limited to 100 data points.

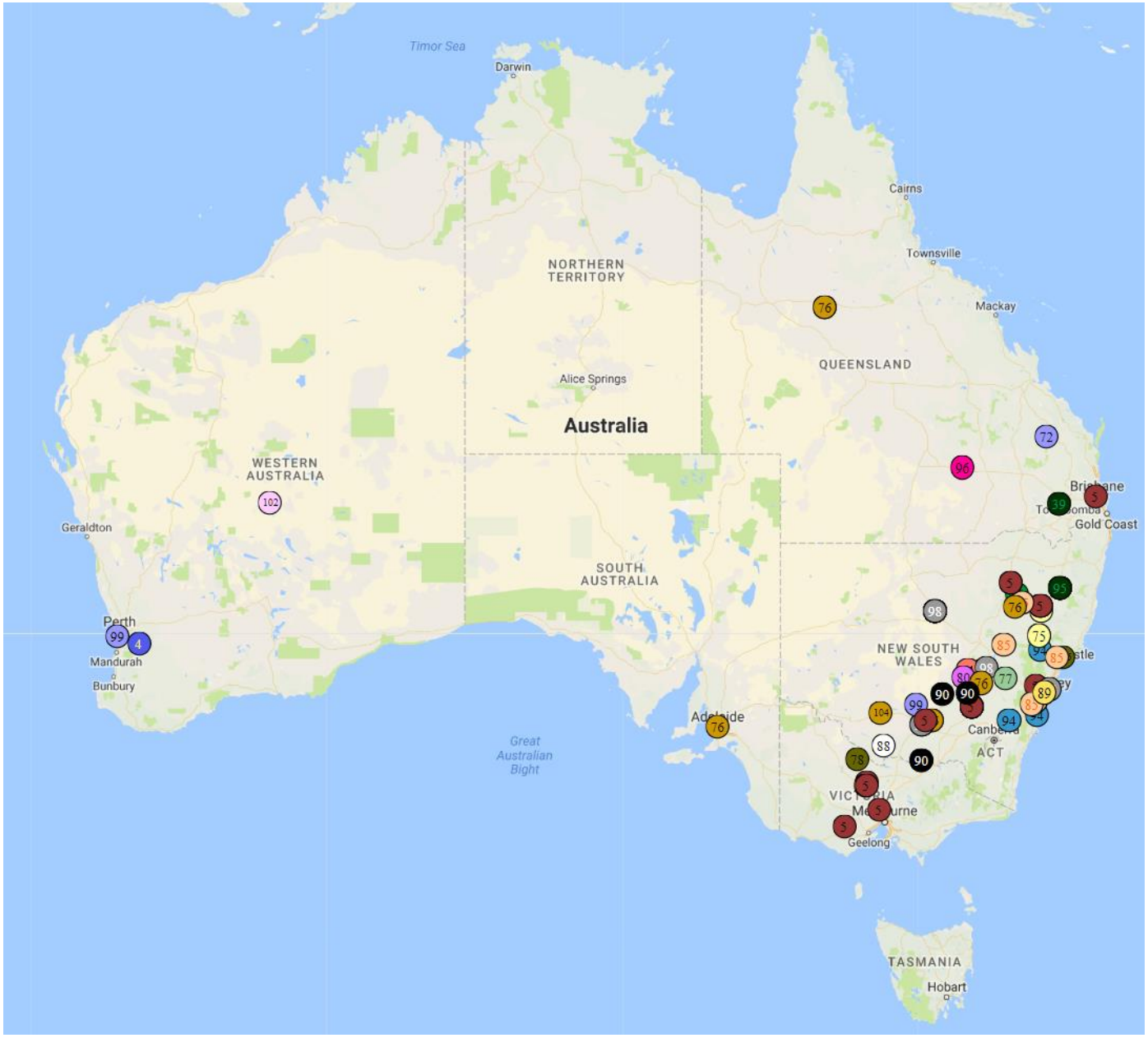



**Figure 7: Distribution of archive *Erysipelothrix rhusiopathiae* isolates. Each data point is labelled according to its' multi locus sequence type (MLST).**

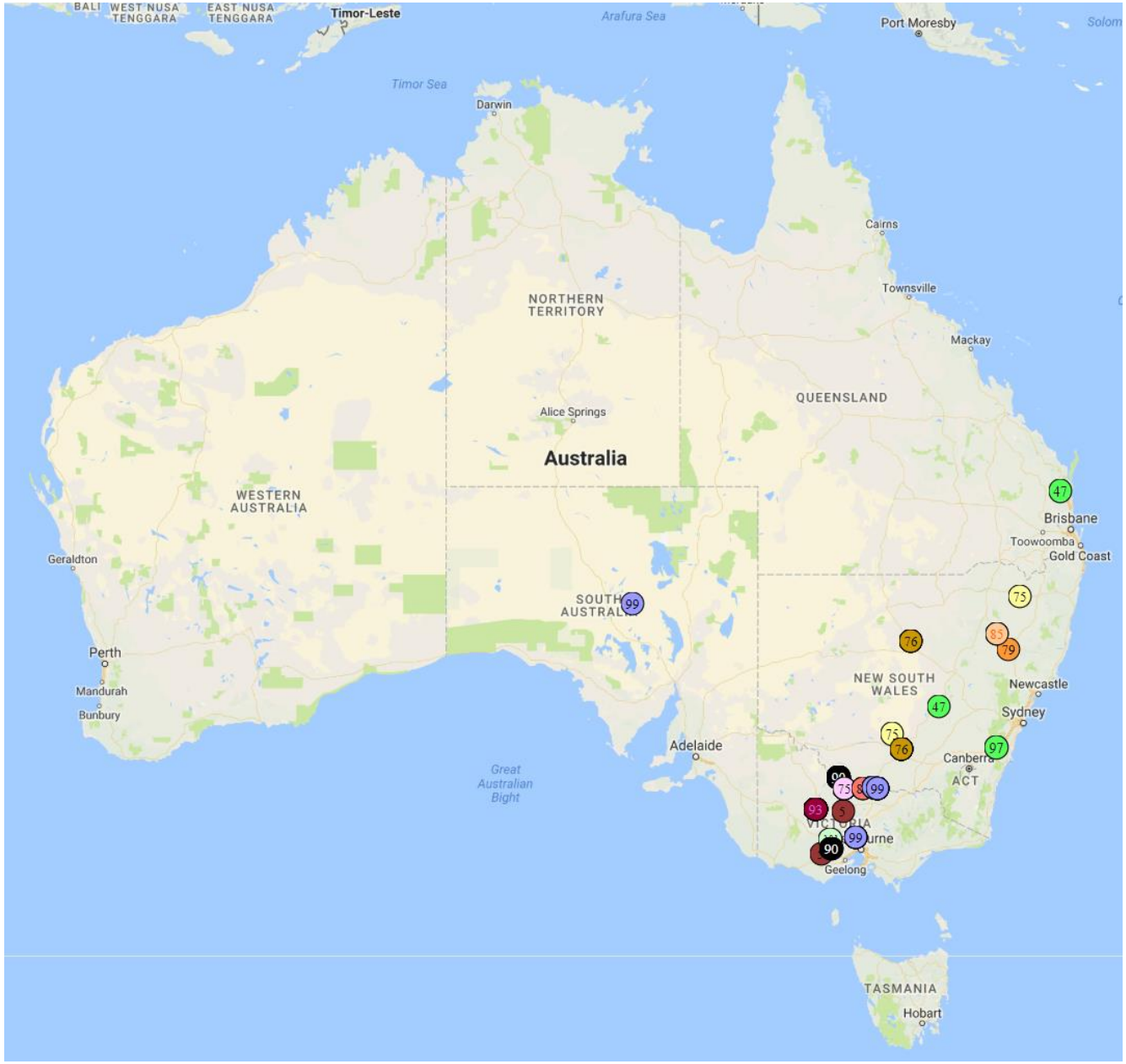



**Figure 8: Distribution of contemporary *Erysipelothrix rhusiopathiae* isolates. Each data point is labelled according to its' multi locus sequence type (MLST).**

### 3.7.2 Was there a link between serotype and MLST?

Archive isolates were selected to be representative of Serotypes 1a, 1b and 2, the main serotypes associated with erysipelas in Australian pigs, and a selection of novel serotypes from the collection. The database supplied the information on these 31 serotyped isolates in relation to MLST (Table 6). There was 17/29 (58.6%) MLSTs, which contained a single serotyped, isolate only. The greatest number of isolates (n=18) of a single serotype (2) fell into MLST 5, which was the MLST associated with vaccine breakdowns. In contrast, serotype 1A was represented in 15 different MLSTs. There were insufficient numbers of serotyped isolates to draw any other conclusions.

**Table 6: *Erysipelothrix rhusiopathiae* isolates classified according to multi locus sequence type (MLST) and serotype.**

| MSLT | Serotype | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1A** | **1B** | **2** | **2A** | **2B** | **2A/2B** | **13** | **15** | **17** | **19** | **21** | **1B/21** |
| 4 | | 1 | | | | | | | | | | |
| 5 | | | 18 | | 1 | | | | | | | |
| 7 | | | | | | | | 1 | | | | |
| 9 | | | | | 1 | | | | | | | |
| 39 | | 1 | | | | | | | | | | |
| 41 | | 1 | | | | | | | | | | |
| 48 | 1 | | | | | | | | | | | |
| 73 | | | 3 | | | | | | | | | |
| 75 | 1 | | | | | | | | | | | |
| 76 | 8 | 2 | | | | | | | | | | |
| 78 | | | 1 | | | | | | | | | |
| 80 | 2 | | | | | | | | | | | |
| 81 | 1 | | | | | | | | | | | |
| 84 | | | | | | | | | 1 | | | |
| 85 | 3 | | 1 | | | | | | | | | |
| 86 | | | | | 2 | | | | | | | |
| 88 | 1 | | | | | | 1 | | | | | |
| 89 | 1 | | | | | | | | | | | |
| 90 | 1 | 1 | | | | | | | | | 1 | |
| 92 | | | 4 | | | | | | | | | |
| 94 | 3 | 2 | | | | 1 | | | | | | |
| 96 | | | | | | | | | | | | 1 |
| 98 | 3 | | | | | | | | | | | 2 |
| 99 | 3 | | | | | | | | | | | |
| 100 | | | | | | | | | | | | 1 |
| 102 | 1 | | | | | | | | | | | |
| 104 | 1 | | | | | | | | | | | |
| 106 | 1 | | | | | | | | | | | |
| 107 | | | | | | | | | | | 1 | |
| 108 | | | | | | | | | | 1 | | |
| 109 | | | | 1 | | | | | | | | |
| **TOTAL** | **31** | **8** | **27** | **1** | **4** | **1** | **1** | **1** | **1** | **1** | **2** | **4** |

3.7.3 In an individual pig, did isolates from different organs or tissues display the same or different MLSTs?

From the limited number of animals (n=4) from which we had multi-tissues isolates only a single pig displayed two MLST types. These four isolates were from the archive collection, so the provenance of the isolates is dependent on the log book system of the time. We did however perform WGS of colonies from the tonsils (6) and spleen (3) of a single pig after various culture and enrichment regimes were applied. These isolates all belonged to the same MLST (*76*), were susceptible in the antibiotic susceptibility test and did not carry any resistance genes.

3.7.4 Were there different MLSTs present on individual farms?

We investigated submissions from six farms that had sampled from a number of pigs Table 7. These were maintained as individual pig isolates attributed to the source farm. Interrogation of the database showed that there were five farms which had different MLSTs in their pigs and the remaining farm had pigs with the same MLST.

**Table 7: Number of farms that submitted samples from multiple pigs.**

| | Archive | Contemporary | Total |
|---|---|---|---|
| Multiple MLSTs on farm | 2 | 3 | 5 |
| Single MLST on farm | 0 | 1 | 1 |
| **TOTAL** | **2** | **4** | **6** |

3.7.5 Can we attribute MLSTs to the severity of erysipelas infection i.e. acute, sub-acute or chronic?

There are three clinical forms of porcine erysipelas, a severe acute septicemic form resulting in sudden mortality; a milder, sub-acute urticarial form characterized by diamond-shaped lesions on the skin and a chronic form associated with endocarditis or arthritis (Brooke and Riley 1999). Not all isolates that were subjected to WGS had matching data with respect to the observed clinical presentation of erysipelas. We had data for 68 farms of which 52 were archive and 16 were contemporary submissions (Figure 9). Unsurprisingly, the most represented clinical assessment was acute (40% archive and 51% contemporary). This is a function of the archive collection source i.e. submissions to the NSW State Veterinary Diagnostic Laboratory system and many of the contemporary samples originating from pigs sent to abattoirs. A category in the chronic/acute contemporary submissions referred to both forms of erysipelas being observed on a farm simultaneously.

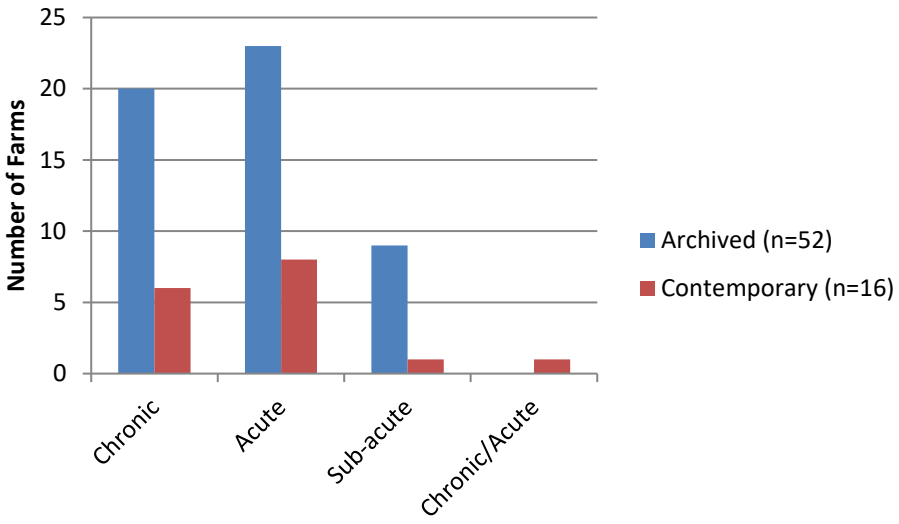
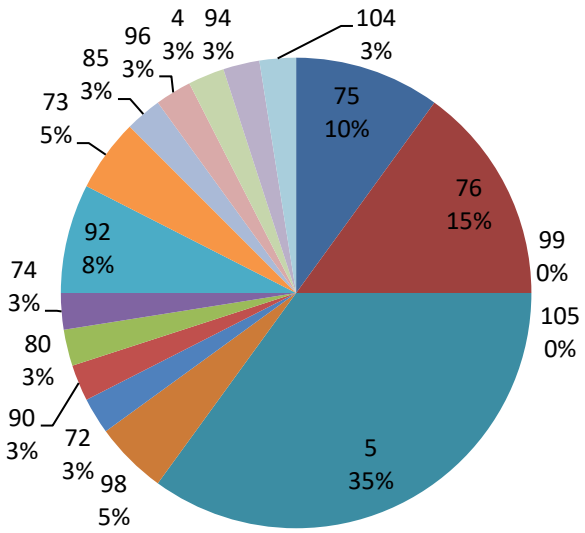



**Figure 9: Classification of erysipelas infection on farm. Note: results only reflect the number of isolates included in this study for each category and are not indicative of a change in disease severity over time.**

3.7.6 Was there a particular MLST associated with archive and contemporary suspected vaccine breakdowns?

There were a total of 45 farms with suspected vaccine breakdown. Of these, 40 farms were from the archived and five were from the contemporary collection. Submission from these 45 farms fell into 17 different MLSTs. Interestingly, the majority of archived farms associated with vaccine breakdown were grouped in MLST 5 (35%) with 14 other MLSTs represented by anything from 3 to 15% of the farms. Only four MLSTs were attributed to the contemporary farms which is a function of the small number (n=5) of farms involved in this study (Figure 10).

A



B

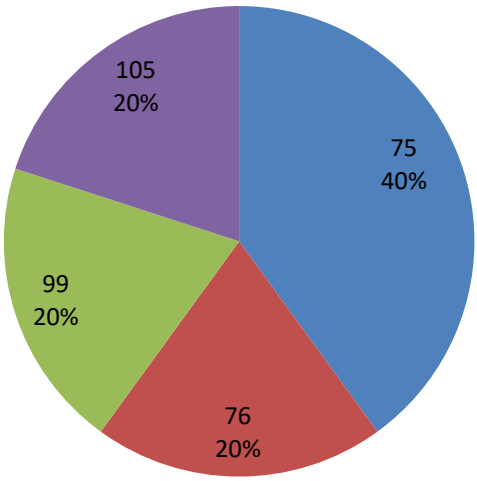



**Figure 10: *Erysipelothrix rhusiopathiae* isolates from archive (A) (n=40) and contemporary (n=5) (B) farm submissions associated with vaccine breakdown displayed according to multi locus sequence type (MLST).**

### 3.7.7 Was there any shift in MLST over time?

Graphical analysis of this data was undertaken in an attempt to highlight any major shifts that may have occurred in MLST on a decade by decade basis. As this often yielded single isolates for the decade no trend could be identified. This is the type of information which could be extracted from our database if a large number of genomes, uniformly selected across decades, were available. However, we did observe a sharp rise in MLST 5 and 75 in the 1990's, but MLST 5 was not identified in contemporary isolates whilst MLST 75 was. In general, the frequency of MLSTs (by decade) duplicates the previous analysis on suspected vaccine breakdown and reflects both sampling and isolate selection bias.

### 3.7.8 Did the anti-biotic sensitivity testing agree with the presence/absence of anti-microbial resistance (AMR) genes within an isolate?

There were 32 isolates in total that were classified resistant phenotypically by antibiotic sensitivity testing. According to the data base that was used to interrogate the genomes for AMR genes, the isolates that were phenotypically resistant to Lincospectin and Penicillin did not carry the respective resistance genes. Isolates with the AMR genes to macrolides (MefA_MLS), lincosamide (LinB_MLS) and aminoglycosides (StrA_Agly) did not exhibit any phenotypic resistance to the aforementioned antibiotics. Only two of the 30 isolates with AMR genes to Oxytetracycline (TeTM) were not phenotypically resistant. The remaining 28 isolates had the TetM resistance gene and also demonstrated phenotypic resistance.

3.7.9 A recently described phage (ST1) was identified in the WGS's of some isolates.

*3.7.9.1 Was this phage only present in contemporary isolates?*

We found 25/178 (14%) isolates contained phage, of these 13/25 (52%) were archived isolates and 12/25 (48%) were contemporary isolates (Figure 11) below.

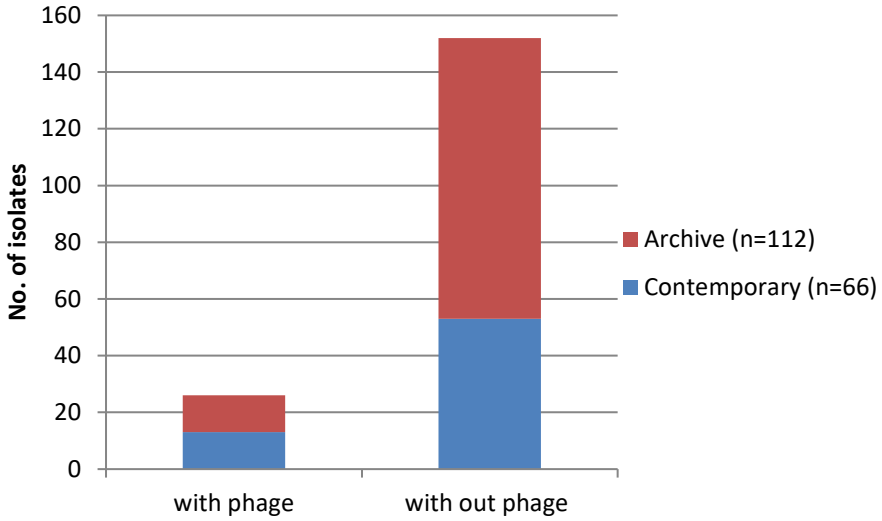



**Figure 11: Summary of *Erysipelothrix rhusiopathiae* isolates from contemporary and archive submissions containing the ST1 phage.**

*3.7.9.2 Was the bacteriophage associated with a particular MLST?*

Of the 25 isolates that contained a phage, eight had a specific MLST. For example MLST 82 had 2/2 isolates with the phage present. The remaining phages were to be found in only three additional MLSTs and in approximately 50% of the isolates in that MLST. Details of the MLSTs in which phages were present can be seen in Table 8 below.

**Table 8: *Erysipelothrix rhusiopathiae* isolates containing the ST-1 phage categorised according to multi locus sequence type (MLST).**

| MLST | Isolate with Phage/Total number of isolates assigned MLST |
|---|---|
| 7 | 1/1 |
| 78 | 1/1 |
| 79 | 1/1 |
| 81 | 1/1 |
| 82 | 2/2 |
| 84 | 1/1 |
| 94 | 3/6 |
| 98 | 3/7 |
| 99 | 12/20 |
| 106 | 1/1 |

#### *3.7.9.3 Was this phage linked to AMR genes or antibiotic sensitivity results?*

Antibiotic sensitivity results were compared with MLST and we found that one isolate resistant to Lincospectin and another resistant to Penicillin carried the phage.

### 3.7.10 Summary

One of the key outcomes of this study is an improved understanding of the genomic diversity of *E. rhusiopathiae* present within the Australian pig industry. This has been demonstrated by similar international studies, however these involved larger host ranges and/or the pig isolates were sourced from what would be considered more cosmopolitan locations than the isolates in this study. We have developed a new and dynamic Epi-Interface database for *E. rhusiopathiae* that could be a model for other diseases. Additions to this database will produce an evolving source of information.

## 4.0 Future Work

### *4.1 Development of the E. rhusiopathiae Epi-Interface – Laboratory Perspective*

This study has confirmed two key observations (1) the power and value of combining laboratory data with field data in an easy access multifactorial database, such as the *E. rhusiopathiae* Epi-Interface and (2) the complexity associated with current genomic strain typing techniques when applied to epidemiology of *E. rhusiopathiae*. However, this is not unique to *E. rhusiopathiae* and there are lessons from other studies on other pathogens that can be applied.

Traditional strain typing techniques such Restriction Fragment Length Polymorphism (RFLP) (Eamens, *et al*. 2006) and Pulsed Field Gel Electrophoresis (PFGE) (Janssen *et al*. 2015) suffer from poor discriminatory power due to the high degree of genomic rearrangement that we now know occurs in *E. rhusiopathiae* (Forde, *et al*. 2016). MLST appeared to have superior discriminatory power as demonstrated by Janssen *et al*. (2015) who used this technique to compare and contrast 165 *E. rhusiopathiae* isolates from a number of host species. Unexpectedly, in this current study we have identified a larger number of MLSTs in a more geographically discreet location from a single host species. To that end, we have shown that MLST may be well suited to epidemiology but may add little to understanding the association between MLST and erysipelas severity.

Interestingly, this characteristic is commonly observed in a number of bacteria including *Campylobacter jejuni* in which recent advances have been made through the use of whole genome MLST (wgMLST). This has more discriminatory power within individual MLSTs and is becoming more affordable (Kovanen, *et al*. 2014).

As this study elected to carry out WGS over the traditional PCR and sequencing MLST approach, we are able to transition to wgMLST. Alternatively, we may find other genes of interest within the whole genome sequences that produce more useful MLST discrimination when compared to the clinical data.

### *4.2 Development of the E. rhusiopathiae Epi-Interface - Field Perspective*

One of the limitations identified in this study was incomplete fields which diminished the *E. rhusiopathiae* Epi-Interface output. In fact any missing data

immediately eliminated that isolate from comparative queries. To that end we think it is imperative to extend and increase the amount and type of clinical data collected during disease investigations and routine health checks. We recommend a new submission form that integrates more data collection in a simple collection format (i.e. tick a box) as suggested in Figure 12. We also believe we need to work closely with field veterinarians to standardise how clinical data is collected and interpreted in order to make more meaningful use of the *E. rhusiopathiae* Epi-Interface.

There is growing evidence that environmental data is critical to the understanding of erysipelas. In a recent study, Ogawa *et al*. (2017) highlighted the importance of understanding the role of environmental factors, host factors and viral infections in outbreaks during an investigation of acute porcine erysipelas outbreaks attributable to the 1a serotype in Japan. Fortunately, the modular nature of the *E. rhusiopathiae* Epi-Interface allows for the inclusion of such environmental data.

### *4.3 Integration of the E. rhusiopathiae Epi-Interface Data into a Global Database*

The PubMLST website is an example of a MLST database that already exists, but is yet to include *E. rhusiopathiae* components (Jolley and Maiden 2010). We hope in the future to integrate the appropriate data from our studies into this database to encourage a more global approach to understanding and comparing erysipelas in pigs and other species.

| Clinical Information | | |
|---|---|---|
| WGS ID No. | | Gender |
| LIMS ID No. | | Joints affected |
| DNA Extraction No. | | Skin lesions |
| Purpose | | Clinical history |
| Project ID No | | Differential diagnosis |
| Serovar or Type | | Post-mortem |
| 1st (F7) | | Histopathology |
| 2nd (F8) | | Bacteriology |
| Source | | Management |
| Tissue | | Other disease factors/characteristics |
| Disease 1 | | Vaccination history 1 |
| Disease 2 | | Vaccination history 2 |
| Disease 3 | | Vaccination history 3 |
| Deidentification No | | Vaccination history 4 |
| Reference | | Vaccination history 5 |
| Reference M No. | | Stressors 1 |
| Reference CM No. | | Stressors 2 |
| Reference Other No. | | Stressors 3 |
| Brief Clinical Desciption | | Streptomycin |
| Interesting cases | | Chloramphenical |
| State or Country | | Erythromycin |
| Region | | Spiromycin |
| Town | | Neomycin |
| Raw Geographic location | | Penicillin G |
| Date | | Bacitracin |
| Date outbreak first noted | | Furazolidone |
| Date Samples collected | | Cloxacillin |
| Acute/Chronic 1 | | Methicillin |
| Acute/Chronic 2 | | Tetracycline |
| Amoxycillin (AML) | | Nitrofurazone |
| Erythromycin 'E' | | Linco-spectin |
| Ceftiofur (EFT) | | Sulphonamide |
| Lincospectin (LS) | | Sulpha-trimethoprim |
| Penicillin (P) | | Lincomycin |
| Oxytetracycline (OT) | | Novobiocin |
| Mortality % | | Trimethoprim |
| Affected % | | Ampicillin |
| Age | | Ceftiofur |
| Breed | | Sulphafurazole |

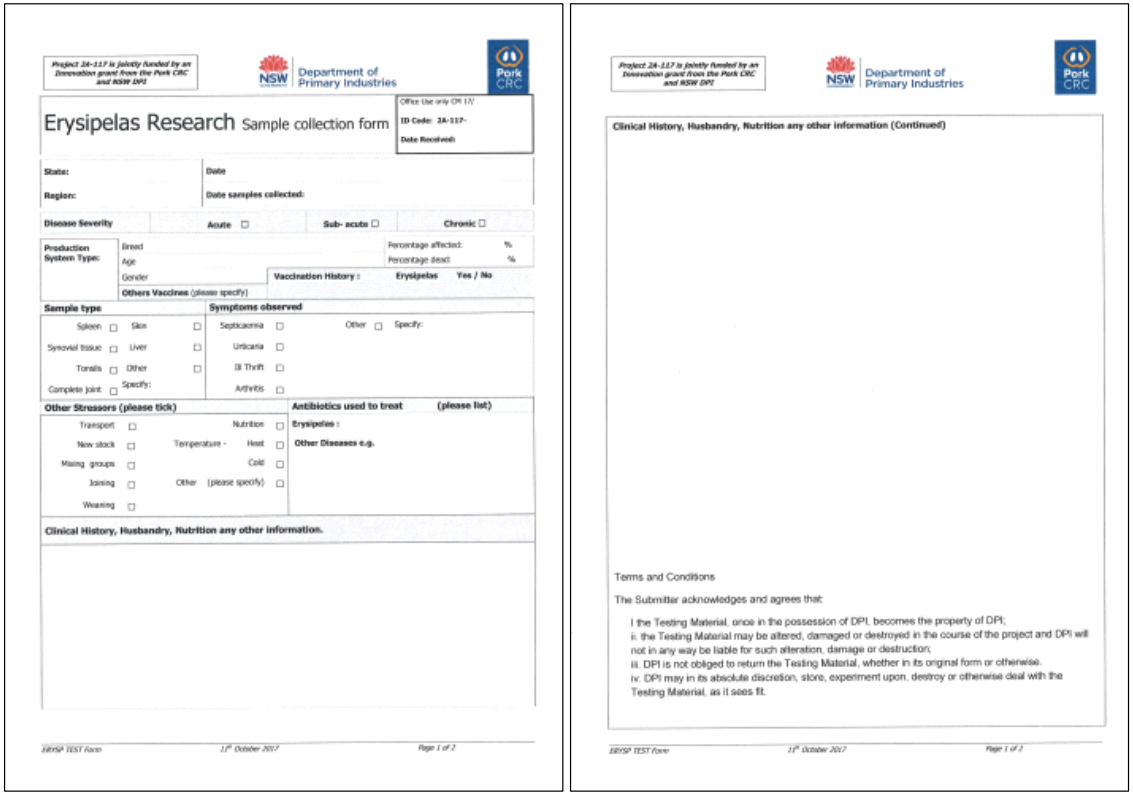



**Figure 12: Information that would need to be incorporated into the sample submission form (this is the one used in this study) to improve the clinical data collected for the *E. rhusiopathiae* Epi-Interface in the future. This would also need to include environmental data.**

## 5.0 Application of Research

This innovations grant has vastly improved the understanding of the variability of *E. rhusiopathiae* that exists within the Australian pig industry (n=168), particularly at the genomic level. It has also provided insight into the quantity and quality of information which is required, on a national basis, to develop a system capable of predictive power for improved management of porcine erysipelas.

The database has been established in Microsoft Access with portability in mind. The choice of a Microsoft based program also ensures continuity in the database and an ability to keep abreast of information technology changes. The establishment and maintenance of this database is in agreement with the NSW Biosecurity Strategy 2013-2021. Such a tool, and the information it contains, can be considered a biosecurity base-line with respect to porcine erysipelas and our understanding of E. *rhusiopathiae* in general. Whilst 55% of all pork eaten in Australia is from Australian farms 45% of the market is imported as pre-cooked cuts mainly from areas such as Europe, USA, Canada and New Zealand. International pressure to open up Australia to fresh pork imports is imposed upon the federal government from time to time. To date this has not been successful based on biosecurity grounds. The type of information within the Epi-Interface would constitute an invaluable decision making tool for national biosecurity. This foundational study has provided the Epi-Interface which can be grown into a tool for veterinarians and producers by the addition of more isolates from across Australia (for access to this Epi-Interface contact narelle.sales@dpi.nsw.gov.au or ian.marsh@dpi.nsw.gov.au)

The Epi-Interface described here is the product of an innovation grant as an example of the potential application of such an approach. We believe that the next phase would be to demonstrate the Epi-Interface to key industry representatives, and together formulate a plan to progress its' development. This would include the ongoing resources required to maintain and add to the Epi-Interface as well as identify the most appropriate means to make it accessible to other researchers and industry.

The Epi-Interface developed in this project will have cumulative commercial value as data is added to it. The genome sequences generated during this pilot study have individual commercial value for data mining in relation to diagnostic assay development, antibiotic design and vaccine development.

The presence of sequence reads for a bacteriophage (SE-1) in 14% of the studied isolates has been identified as a potential opportunity. The bacteriophage should be investigated as a new anti- *E. rhusiopathiae* agent and an alternative to antibiotics. The advantages of such an approach against *E. rhusiopathiae* have been highlighted by Yuan *et al.* (2016) who were the first to isolate, describe and sequence this bacteriophage. They are currently investigating the bacteriophages host specificity, its effectiveness in killing *E. rhusiopathiae* and if it is an alternative to antibiotics because of the increase in AMR in China. This research has not progressed to the level of disease control.

## 6.0 Conclusion

This innovation study aimed to improve the knowledge base of the Australian *E. rhusiopathiae* genome with a focus on isolates from the Australian pig industry. Ambitiously we hoped to perform MLST on 180 isolates, but discovered WGS would facilitate this in a more cost effective manner. This has been achieved with the sequencing of 168 whole genomes from Australian isolates. There were an additional eight international and two of unknown origin, whole genomes included because of a novel characteristic of the isolate. The second aim of this study has been delivered through the creation of a dynamic Epi-Interface database for *E. rhusiopathiae* which links genomic data with clinical and associated farm management information. Whilst the Epi-Interface is an evolving data and information management database it forms the basis of a system which has the capacity to facilitate the cost effective management of porcine erysipelas. Whilst there are any number of genomic and bio-informatics databases, this Epi-Interface also contains in a single location real world (on-farm) information which can be interrogated from any aspect.

## 7.0 Limitations/Risks

### *7.1 Limitations*

#### 7.1.1 Sample size

The number of isolates involved in any study is always a limiting factor. At the inception of this study we considered 180 isolates to be ambitious but attainable because of the extensive NSW DPI freeze dried archive collection housed at EMAI. Whilst the number of contemporary Australian isolates (66) was less than we would have hoped, the extensive nature of the isolates that had been archived by Graeme Eamens during major *Erysipelothrix* outbreaks in the 1980s and 1990's were considered to be extremely valuable additions to the study.

#### 7.1.2 Access to National Isolates

We attempted to access isolates from government veterinary laboratories in other states without success. This lack of success may be attributed to each states focus on maintaining and protecting their IP and commercial opportunities. Whilst this is understandable it makes a national approach to epidemiology of this disease or studies such as this difficult. In fact, the terms of Material Transfer Agreements often preclude whole genome sequencing by a third party and is contentious in respect to Intellectual Property rights.

#### 7.1.3 Availability of Complete Metadata.

The metadata associated with some of the isolates, particularly archive isolates, was limited, and for several, non-existent. Whilst our bias was towards isolates with full datasets we did include some which had little provenance, selected for their serotype. It became very apparent on interrogation of the Epi-Interface that fields which lacked information reduced the power of the analysis of the query.

## *7.2 Risks*

### 7.2.1 Lack of Differentiation

The major risk of the approach we have pursued is that there is in fact no significant and or discernible link between the differences we have detected in isolates and observed disease. This study has demonstrated the huge genomic variability that can be found in *E. rhusiopathiae*. This makes the clear identification of criteria which defines the form of the disease seen on farm a very difficult task. An example of this is the MLST system we employed. We had anticipated that the large number of MLSTs described by Janssen *et al*. (2015) would be a result of the wide host range and European origin of their isolates. Therefore, we thought we would find less MLSTs given the vast majority of our isolates were from Australia and from pigs (n=172). This was not the case with our study defining 37 additional MLSTs. However, this study has demonstrated that WGS prior to MLST interrogation is preferable to MLST *via* PCR. In the event of the failure of a single technique the entire genomes can be utilised for alternate analysis.

### 7.2.2 *E. rhusiopathiae* Inherent Risk and Limited Clinical Dataset

It is possible that *E. rhusiopathiae* may be one of a number of pathogens which cannot be separated according to a single, duplex or triplex genomic interrogation technique. Differentiation of disease may be reliant on other factors such as stress, weather, temperature, nutrition, host mediated factors, or the presence or absence of other pathogens. In fact, all or any combination of these factors may be involved which may explain why any attempts at typing pathogens like *E. rhusiopathiae* have stalled when only one or two of these factors are considered.

What we have confirmed is that employing a single genome typing technique without the collection of associated clinical and management data is not the correct approach. In fact genomic plasticity necessitates the addition of this metadata.

In this study we have had access to what would be considered considerable clinical observation data and associated laboratory findings and to a much lesser extent on-farm management data extending back decades. However, this extensive data set may or may not cover the fields required to break the code of pathogenicity and observed disease. This highlights the power of field observation and management data in the treatment, control and overall management of a disease like erysipelas.

### 7.2.3 Non–standard Nomenclature

One of the major pressure points in the development of a data base like the Epi-Interface is the standardization of nomenclature, definition of key terms and groupings or cut-offs for data sets. This has been continually refined during the development of the erysipelas Epi-Interface. These parameters will be harmonised with international conventions and future data will follow these conventions.

### 7.2.4 Database Longevity

The database has been established in Microsoft Access to ensure continuity in the database and to stay abreast with technological changes. The establishment and maintenance of this database is in agreement with the NSW Biosecurity Strategy 2013-2021.
The following supporting statement by the previous NSW DPI, Director of Research, Dr. Jeffrey Hammond, was supplied to the Pork CRC as part of the innovation grant proposal

Supporting statement:

*'NSW DPI acknowledges the importance of the Epi-Interface that has been detailed in the Pork CRC proposal 2017/017 entitled "Erysipelothrix rhusiopathiae Epi-Interface,-A new approach to the management of Porcine Erysipelas" by Sales and Marsh. I am happy to confirm that the maintenance of this database will be carried out at EMAI, with the addition of new data as it is generated.*
*Since Erysipelothrix rhusiopathiae affects a variety of livestock, wildlife, birds and humans, the curation of this database will provide a clear contribution to our NSW DPI biosecurity strategy goals for the management of biosecurity risks that affect animal industries, biodiversity and human health.'*

## 8.0 Recommendations

As a result of the outcomes in this study the following recommendations have been made:

(1) The ongoing development of this *E. rhusiopathiae* Epi-Interface.
This study has demonstrated the power of combining laboratory and clinical data in an Epi-Interface that can be used to establish epidemiological inferences. However, as with all database tools the quality of the data out is proportional to that of the data in. We believe as new genomic information is identified in association with pathogenesis, epidemiology and management of disease these can be incorporated to:

   a. Help elucidate the ongoing issue of porcine erysipelas
   b. Establish this Epi-Interface approach as a model for other pathogens of pigs and other livestock species.

   The ongoing development of this *E. rhusiopathiae* Epi-Interface to incorporate whole genome MLST (wgMLST) to better characterise the 178 whole genome sequences we currently have. Whole genome MLST is now affordable and the most highly discriminatory approach.

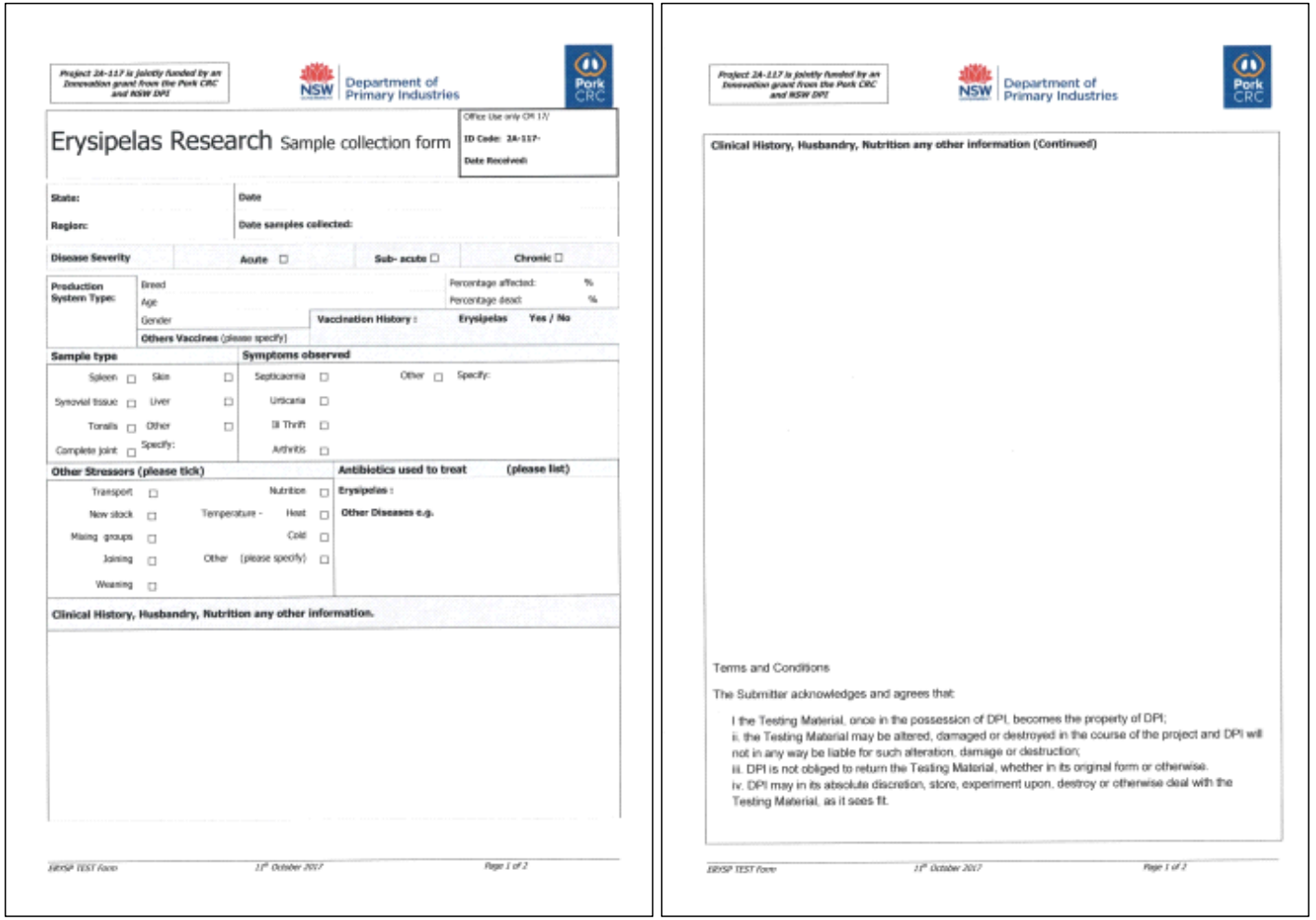
(2) Deeper investigation of the resulting whole genome sequences generated in this study.
To generate the MLSTs in this study we opted to extract this information from whole genome sequences rather than using the conventional PCR and sequencing approach on a gene by gene basis. Consequently the project has now generated a highly valuable resource, 178 complete genome sequences from a collection of Australian *E. rhusiopathiae* isolates. These complete genomes can now be interrogated for alternate genomic

signatures that can be used to better understand the pathogenesis, epidemiology and disease management options.

(3) The inclusion of more extensive and standardized clinical and environmental data.
The *E. rhusiopathiae* Epi-Interface has been constructed in modules. New modules, such as environmental data, can be easily imported into the Epi-Interface and used to interrogate existing and new isolates to fill gaps in the knowledge with respect to *E. rhusiopathiae*. This is in line with the critical mass of thought being generated around the continual integration of bacterial, microbial community, clinical and environmental data required to identity the pathway to successful management of disease.

## 9.0 Appendices

## *9.1 Appendix 1: Sample submission form submitted with contemporary isolates.*



*Project 2A-117 is jointly funded by an Innovation grant from the Pork CRC and NSW DPI*

Department of Primary Industries

Pork CRC

# Erysipelas Research Sample collection form

Office Use only CM 17/
ID Code: 2A-117-
Date Received:

State:
Region:
Date
Date samples collected:

Disease Severity — Acute ☐ Sub- acute ☐ Chronic ☐

Production System Type:
Breed
Age
Gender
Others Vaccines (please specify)
Percentage affected: %
Percentage dead: %
Vaccination History : Erysipelas Yes / No

**Sample type**

Spleen ☐ Skin ☐
Synovial tissue ☐ Liver ☐
Tonsils ☐ Other ☐
Complete joint ☐ Specify:

**Symptoms observed**

Septicaemia ☐ Other ☐ Specify:
Urticaria ☐
Ill Thrift ☐
Arthritis ☐

**Other Stressors (please tick)**

Transport ☐
New stock ☐
Mixing groups ☐
Joining ☐
Weaning ☐
Nutrition ☐
Temperature - Heat ☐
Cold ☐
Other (please specify) ☐

**Antibiotics used to treat (please list)**

Erysipelas :
Other Diseases e.g.

**Clinical History, Husbandry, Nutrition any other information.**

*ERISP TEST Form* *11th October 2017* *Page 1 of 2*

*Project 2A-117 is jointly funded by an Innovation grant from the Pork CRC and NSW DPI*

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Pork CRC

**Clinical History, Husbandry, Nutrition any other information (Continued)**

Terms and Conditions

The Submitter acknowledges and agrees that:

i. the Testing Material, once in the possession of DPI, becomes the property of DPI;
ii. the Testing Material may be altered, damaged or destroyed in the course of the project and DPI will not in any way be liable for such alteration, damage or destruction;
iii. DPI is not obliged to return the Testing Material, whether in its original form or otherwise.
iv. DPI may in its absolute discretion, store, experiment upon, destroy or otherwise deal with the Testing Material, as it sees fit.

*ERISP TEST Form* *11th October 2017* *Page 1 of 2*

## 9.2 *Appendix 2: Antibiotic disks suggested as surrogate from the CDS (*Bell *et al. 2016).*

### 12.2. Surrogate discs

**Table 12.2.a. Surrogate disc testing: *Gram Positive Organisms***

Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

| Antibiotic reported | Surrogate disc used | Disc potency (µg) |
|---|---|---|
| **Staphylococci (except *S. saprophyticus* from urine)** | | |
| Amoxycillin/Ampicillin/Penicillin V | Benzylpenicillin | 0.5 u |
| Augmentin | Oxacillin [d]/Cefoxitin [c] | 1 / 10 |
| Azithromycin/Clarithromycin/Roxithromycin | Erythromycin | 5 |
| Carbapenems | Oxacillin [d]/Cefoxitin [c] | 1 / 10 |
| *Cefovecin* [a]/*Ceftiofur* [a] /other Cephalosporins [b] | Oxacillin [d]/Cefoxitin [c] | 1 / 10 |
| Cloxacillin/ Dicloxacillin/ Flucloxacillin | Oxacillin [d]/Cefoxitin [c] | 1 / 10 |
| *Enrofloxacin* [a] */Orbifloxacin* [a] | Moxifloxacin | 2.5 |
| *Framycetin* [a] | Neomycin | 30 |
| Lincomycin | Clindamycin | 2 |
| Norfloxacin [c]/ *Ofloxacin* [a] | Ciprofloxacin | 2.5 |
| Pristinamycin | Quinupristin/Dalfopristin | 15 |
| Tetracyclines | Tetracycline | 10 |
| *Tylosin* [a] | Erythromycin | 5 |

## 9.3 *Appendix 3: Resistant and sensitive antibiotic classification as specified by the CDS (*Bell *et al. 2016).*

**Table 12.1.a. Calibrations: *Gram Positive Organisms* cont.**

Antibiotics, disc potencies, annular radii and MIC for susceptible strains, media and incubation conditions.

| Antibiotic | Disc potency (µg) | Exception to the standard interpretation [a] | MIC (mg/L) |
|---|---|---|---|
| **Streptococci & *Erysipelothrix* species (Blood Sensitest, $CO_2$, 35-37°C)[m]** | | | |
| Ampicillin [m] | 5 | 4 mm | ≤2 |
| Benzylpenicillin | 0.5 u | | ≤0.125 |
| Cefotaxime | 0.5 | 4mm | ≤0.5 |
| Cefotaxime [m] | 5 | | ≤2 |
| Ceftriaxone | 0.5 | 4mm | ≤0.5 |
| Ceftriaxone [m] | 5 | | ≤2 |
| Chloramphenicol | 30 | | ≤8 |
| Clindamycin [n] | 2 | | ≤0.5 |
| Cotrimoxazole | 25 | | ≤0.5/9.5 |
| Erythromycin | 5 | | ≤0.5 |
| *Marbofloxacin* [k] | 5 | | ≤2 |
| Moxifloxacin | 2.5 | 4 mm | ≤1 |
| Nitrofurantoin [c] | 200 | 4 mm | ≤32 |
| Quinupristin/Dalfopristin | 15 | | ≤2 |
| Rifampicin | 1 | | ≤0.5 |
| Teicoplanin | 15 | 2 mm | ≤8 |
| Tetracycline | 10 | | ≤4 |
| Tigecycline | 15 | | ≤1 |
| Vancomycin | 5 | 2 mm | ≤4 |

[a] The standard 6 mm cut-off applies where no exception has been specified.

[c] For testing urine isolates only

[k] Antibiotic calibrated for veterinary medicine.

[m] NOT for testing *Streptococcus pneumoniae* from CSF. If *Streptococcus pneumoniae* or any other *Streptococcus* species from a site other than CSF is resistant to benzylpenicillin 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg then test ampicillin 5 µg, cefotaxime 5 µg and ceftriaxone 5 µg.

[n] Isolates with inducible clindamycin resistance (iMLS$_B$ phenotype) will have a large zone to clindamycin, but should be reported as resistant. Inducible clindamycin resistance can be detected as described in section 4.7.

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