



An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

14S802 - Strain Specific Pathogenicity of Staphylococcus aureus Final Report

This project was funded under the Department of Agriculture,
Food and the Marine Competitive Funding Programme.

SUMMARY

Mastitis is a costly endemic disease for the dairy industry. It is primarily caused by bacterial infection and is the most common reason for antibiotic use in dairy cows in Ireland. *Staphylococcus aureus* is the most common mastitis pathogen in Ireland and the *S. aureus* strains that cause mastitis belong to specific bovine-adapted lineages. Current selection for mastitis-resistance is based on the host immune response, as determined by somatic cell count (SCC). However, the ability of *S. aureus* to evade and subvert the host immune response is well known, including the ability to internalise and survive within host cells. This project tested the hypothesis that bovine intramammary infection with different *S. aureus* strains results in differential activation of the host immune response. Supporting this hypothesis, significant differences between *S. aureus* lineages in their ability to internalise within bovine mammary epithelial cells were found with some strains internalising at higher levels than others. It was also found that some strains induced higher expression of cytokines and chemokines responsible for attracting immune cells and these strains induced mammary epithelial cells to produce factors that attracted somatic cells, while other strains did not. Differences in disease presentation in vivo in cows infected with different strains were also observed, indicating strain-specific virulence. Significantly higher somatic cell count and anti-*Staphylococcus* IgG and significantly lower milk yield were observed in response to infection with a more virulent strain. The infecting strain also influenced the composition of the somatic cells in milk. These results demonstrate the limitations of SCC, which is currently both the main method of mastitis detection and the principle method of selection of mastitis-resistant animals. The development of more sensitive, differential diagnostic techniques could provide valuable tools to the industry in detecting and identifying intramammary pathogens.

KEYWORDS

Mastitis, *Staphylococcus aureus* strain

ACRONYM

SSPSA

PROJECT COORDINATOR, INSTITUTION

Dr Orla Keane, Teagasc

EMAIL

orla.keane@teagasc.ie

COLLABORATORS, INSTITUTION

Dr Finola Leonard, University College Dublin

PUBLICATION DATE

18/11/2020

Section 1 - Research Approach & Results

Start Date

01 April 2015

End Date

31 October 2019

Research Programme

Research Stimulus Fund

TRL Scale

TRL 5: Technology validated in relevant environment

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€348,940.70

Total Project Expenditure

€356,537.81

Rationale for undertaking the Research

Mastitis is a threat to animal health and welfare and negatively impacts the productivity of the dairy industry. Control of intramammary pathogens relies on the implementation of control measures. Selection for mastitis resistance is performed indirectly using indicator traits genetically correlated to mastitis, such as somatic cell count (SCC). Overall, the genetic correlation, between SCC and mastitis is large and positive (~0.7). However, genetic factors governing the occurrence of mastitis caused by specific pathogens appear to differ depending on the pathogen. Of the common mastitis pathogens, the genetic correlation between SCC and pathogen-specific mastitis is lowest for *Staphylococcus aureus* (0.44). This is unexpected as correlation between SCC and mastitis caused by pathogens that have a long-term effect on SCC, such as *S. aureus*, might be expected to be the greatest. The ability of pathogens to influence the host response is known and *S. aureus* has the ability to internalise and survive in a variety of non-professional phagocytes thus evading the host immune response. Certain strains of *S. aureus* may also be more pro-inflammatory, thus resulting in more efficient somatic cell recruitment. Strain-independent variability in host-cell internalisation or host immune stimulation is a possible explanation for the low genetic correlation between SCC and *S. aureus* mastitis. This study examined the ability of the infecting *S. aureus* strain to influence the host response and SCC.

Methodology

The *Staphylococcus aureus* isolates associated with bovine mastitis in Ireland predominantly belong to 4 main lineages, CC71, CC97, CC151 and ST136. In order to test their strain-specific virulence in vitro a model system using immortalised and primary cultured bovine mammary epithelial cells was established. These cells were then infected with 3 different strains from each of the lineages and the ability of the *S. aureus* strains to (i) survive in bovine mammary epithelial cells (ii) to evade the immune response by adhering to and internalising within the bovine mammary epithelial cells (iii) to survive killing by bovine neutrophils (iv) to stimulate expression of pro-inflammatory cytokines and chemokine gene expression and (v) to express signalling molecules that would attract somatic cells were quantified. Based on the in vitro results 2 strains that were highly divergent for the virulence traits assessed were chosen and used to infect 14 intramammary pathogen-free, low somatic cell count (SCC) first lactation cows (7 infected with one strain and 7 infected with the other strain). The course of infection was monitored over 30 days with milk yield, SCC, bacterial load, anti-*S. aureus* immunoglobulin levels and cytokine levels in milk determined. The composition of the milk somatic cells was also assessed. Somatic cells were also purified at various time points, RNA extracted and global gene expression quantified using high-throughput transcriptomics. The genome sequence of key bacterial strains was also determined in order to identify key bacterial virulence factors.

Project Results

In this project strain-specific virulence of *Staphylococcus aureus* was demonstrated with the bacterial strain influencing the host immune response and the subsequent outcome of infection. Initial in vitro studies demonstrated that *S. aureus* strains induced expression of immune signalling cytokine and chemokine genes in a strain-specific manner and functional neutrophil chemotaxis assays confirmed that the genotype of the infecting strain determined somatic cell recruitment. Differences between strains of the various lineages in their virulence were further evident in their ability to internalise within mammary epithelial cells, their effect on mammary epithelial cell morphology and viability and their ability to survive killing by bovine neutrophils. Subsequent in vivo studies demonstrated that 2 *S.*

aureus strains belonging to different lineages (CC97 and CC151) caused mastitis of different severity. Infection with a strain belonging to CC151 resulted in clinical signs of infection, significantly higher somatic cell count (SCC) and lower milk yield than infection with a strain belonging to CC97. Evaluation of selected markers of the innate and adaptive immune response demonstrated higher IgG antibody concentration in serum and milk, higher IgA concentration in milk and higher IL-8 and IL-1 β in milk in response to infection with a CC151 strain compared to a CC97 strain. Analysis of global somatic cell gene expression demonstrated differences in the immune response to the different strains. An initial immune response to both strains was evident but this was sustained in response to the strain belonging to CC97 only, indicating a difference in disease progression or a response to different virulence strategies employed by the two strains. The expression of genetic markers of various immune cells also differed in response to each strain, indicating differing immune cell proportions among somatic cells recruited in response to each strain.

S. aureus mastitis is a global problem with substantial economic costs and negative impact on animal health and welfare. Current mastitis control methods such as disease detection, culling of persistently infected animals and genetic selection for mastitis resistance are primarily based on the host response to

infection, determined by milk SCC. Therefore the ability of *S. aureus* strains to influence this key trait impacts diagnosis, treatment and control of this disease. If the severity of disease can be predicted based on bacterial genotype, this may enable better prediction of disease outcome and therefore inform decisions about treatment or culling of infected animals. The low average SCC in cows infected with a CC97 strain revealed a potential challenge in detecting infection with this strain, thus a need for better detection options for *S. aureus* intramammary infection exists. Lower SCC limits may also need to be used to classify healthy animals. In summary, each *S. aureus* lineage has a unique set of characteristics that influences the outcome of infection.

Section 2 - Research Outputs

Summary of Benefits / Improvements of Project Findings

This project has demonstrated for the first time that the signs and severity of *Staphylococcus aureus* intramammary infection is dependent on the infecting strain. This creates opportunities to develop diagnostic tools which identify the infecting pathogen to the strain level. This information would enable prediction about the likely severity and outcome of disease in addition to the likelihood of antibiotic cure. This strain-specific information could therefore be used to inform treatment and culling decisions. In addition to use as a diagnostic method, somatic cell count is currently used as a phenotypic tool for the identification and selection of mastitis resistant animals. A strainspecific marker would have the potential to be used as a more robust phenotype for the selection of mastitis-resistant animals. This project also developed a number of resources that are available to the research community and can be used in future projects. Genome sequences of a number of bovine-adapted *S. aureus* isolates have been made publicly available and can be utilised for the identification of key virulence factors that are responsible for strain-specific virulence and as bacterial strain-specific markers. This project has also resulted in 5 peer-reviewed publications to-date with one more in preparation which will be of benefit to the scientific community.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	0	1	1
Post Doctorates	1	0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	5	<p>Niedziela DA, Murphy MP, Grant J, Keane OM, Leonard FC. Clinical presentation and immune characteristics in first-lactation Holstein-Friesian cows following intramammary infection with genotypically distinct <i>Staphylococcus aureus</i> strains. <i>J. Dairy Sci.</i> 2020 103:8453–8466.</p> <p>Murphy MP, Niedziela DA, Leonard FC, Keane OM. The in vitro host cell immune response to bovine-adapted <i>Staphylococcus aureus</i> varies according to bacterial lineage. <i>Sci Rep.</i> 2019 Apr 16;9(1):6134.</p> <p>Keane OM. Symposium review: Intramammary infections-Major pathogens and strain-associated complexity. <i>J Dairy Sci.</i> 2019 May;102(5):4713-4726.</p> <p>Cormican P, Keane OM. Complete Genome Sequences of Sequence Type 71 (ST71) and ST97 <i>Staphylococcus aureus</i> Isolates from Bovine Milk. <i>Microbiol. Resourc. Announc.</i> 2018 7 (5): e00954-18</p> <p>Murphy MP, Niedziela DA, Keane OM. EHS matrix incubated in media containing penicillin retains sufficient concentrations of antibiotic to inhibit growth of susceptible microorganisms. <i>J Microbiol Methods.</i> 2017 Aug;139:103-106. One further scientific paper in preparation:</p> <p>Niedziela DA, Cormican P, Leonard FC, Keane OM. Bovine milk somatic cell transcriptomic response to <i>Staphylococcus aureus</i> is dependent on strain genotype.</p>
PhD Theses	1	Dagmara A. Niedziela. Strain-specific virulence of <i>Staphylococcus aureus</i> . PhD. 2019. UCD.

- D. Niedziela. Immune response to bovine-adapted *Staphylococcus aureus* is Papers dependent on bacterial genotype. Microbiology Society Annual Conference 2019, Belfast, Northern Ireland. April 2019.
- D. Niedziela, M. P. Murphy, P. Cormican, F.C. Leonard, O.M. Keane. Immune response to bovine-adapted *Staphylococcus aureus* is dependent on bacterial genotype. UCD College of Health and Agricultural Sciences (CHAS) Inaugural Graduate Research Student symposium, Dublin, Ireland. March 2019 (best presentation prize).
- D. Niedziela, M. P. Murphy, P. Cormican, F.C. Leonard, O.M. Keane. Transcriptomic response of dairy cows to *Staphylococcus aureus* intramammary infection is dependent on strain genotype. 9th UCD Computational and Molecular Biology symposium, Dublin, Ireland. November 2018.
- D. Niedziela. Immune response of *Bos taurus* to two genotypically distinct mastitis causing *Staphylococcus aureus* strains. 48th meeting of the Irish branch of Association for Veterinary Teaching and Research Work (AVTRW), Backweston, Celbridge, Co. Kildare, Ireland. October 2018.
- O.M. Keane, D. Niedziela, M. P. Murphy, F.C. Leonard. *Staphylococcus aureus* lineage influences the bovine immune response to intramammary infection. American Dairy Science Association Annual Meeting, Knoxville, Tennessee, USA, June 2018. (invited presentation).
- D.A. Niedziela. Lineage and strain specific differences in the in vitro and in vivo virulence of bovine adapted *Staphylococcus aureus*. Microbiology Society Annual Conference 2018, Birmingham, UK. April 2018.
- D.A. Niedziela. Clinical presentation, somatic cell count and cytokine secretion in response to intramammary infection of Holstein Friesian heifers with isolates from two *Staphylococcus aureus* lineages. British Society of Animal Science (BSAS) Annual Meeting 2018, Dublin, Ireland. April 2018.
- D. Niedziela, P. Cormican, F.C. Leonard, O.M. Keane. Transcriptomic response of *Bos taurus* to two genotypically distinct *Staphylococcus aureus* strains. 8th International Symposium on Animal Functional Genomics (ISAFG), Adelaide, Australia. November 2018 (best poster prize).
- D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. Lineage and strain specific differences in the in vitro and in vivo pathogenicity of bovine adapted *Staphylococcus aureus*. 8th meeting of Dublin Academy of Pathogenomics and Infection Biology (DAPI), Dublin, Ireland. January 2018.
- D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. Lineage and strain specific differences in the in vitro and in vivo pathogenicity of bovine adapted *Staphylococcus aureus*. 8th UCD Computational and Molecular Biology symposium, Dublin, Ireland. November 2017.
- D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. In vivo infection with two bovine adapted *Staphylococcus aureus* suggests strain specific disease outcome. 47th meeting of the Irish branch of Association for Veterinary Teaching and Research Work (AVTRW), AFBI, Hillsborough, UK. October 2017 (best presentation prize).
- M. P. Murphy. Lineage associated differences in host cell interactions with bovine mastitis-associated *Staphylococcus aureus*. *Staphylococcus* Great Britain and Ireland, Swansea, UK, September 2017.
- O.M. Keane, D.A. Niedziela, M.P. Murphy, F.C. Leonard. *Staphylococcus aureus* genotype modulates the in vitro immune response of bovine mammary epithelial cells. 36th International Society for Animal Genetics (ISAG) conference, Dublin, Ireland. July 2017.
- D.A. Niedziela, M.P. Murphy, O.M. Keane. Bovine-adapted lineages of *Staphylococcus aureus* induce a strain-specific pro-inflammatory immune response. 7th meeting of Dublin Academy of Pathogenomics & Infection Biology (DAPI), Dublin, Ireland. January 2017.
- D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. Lineage specific differences in host cell internalisation and immune response to bovine adapted *Staphylococcus aureus*. 46th meeting of the Irish branch of Association for Veterinary Teaching and Research Work (AVTRW), University College Dublin, Ireland. October 2016 (best presentation prize).

Intellectual Property

N/A

Summary of other Project Outputs

N/A

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	The results of this project have a potential future impact on mastitis control procedures in commercial herds, mastitis detection and genetic selection for mastitis resistance. The results demonstrated that the infecting <i>Staphylococcus aureus</i> strain influences the cow immune response, including the somatic cell response. Both strain-specific and strain-independent biomarkers of infection were identified in milk. The validation of strain-specific biomarkers would allow the development of commercial strainspecific diagnostic tools. This would allow the identification of cows infected with virulent strains or those infected with less virulent strains which have a minimal impact on SCC and are currently difficult to detect. Cows could then be targeted for treatment or culling as appropriate. The development of strain-independent diagnostics would also enable the development of novel health phenotypes which could be used to select animals resistant to all strains of <i>S. aureus</i> infection. These impacts are subject to further research being completed to confirm and broaden the findings of this project.

Dissemination Activities

Activity	Details
Seminars at which results were presented	Current Research seminar, University College Dublin, Ireland. February 2017. Current Research seminar, University College Dublin, Ireland. April 2016.
Other	Technology Update highlighting the findings and key technologies from this project for agricultural advisers and their farmer clients.
Other	Presentation of project and results at science week public events at Teagasc Grange.

Knowledge Transfer Activities

N/A

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€136,000.00	Molecular characterization of adhesin-receptor interactions in bovine-adapted <i>Staphylococcus aureus</i> . Teagasc RMIS award.
Other	€181,175.30	The impact of bovine-specific <i>Staphylococcus aureus</i> genetic variability on mastitis control and milk processing. Irish Research Council CAROLINE award co-funded by Marie Skłodowska-Curie Actions.

Future Strategies

This project demonstrated for the first time that *Staphylococcus aureus* displays strain-specific virulence. This has already been used to leverage 2 additional grants as described above. The first of these grants will identify (i) adhesins expressed on the surface of *S. aureus* from the major bovine-adapted lineages and (ii) immunogenic surface proteins from each lineage that could be utilized as vaccine antigens or strain-specific diagnostic markers.

The second project is examining the association of *S. aureus* strain with udder health traits on 20 Irish dairy farms and includes assessing the effect of strain on antimicrobial resistance and ability of the strain to persist after dry cow therapy.

In addition to these on-going projects, collaborations have been established with researchers in Spain and the UK on the development of novel diagnostics for pathogens of livestock which has led to the submission of a grant pre-proposal to a European funding agency.

Project Publications

Publications in peer reviewed scientific journals

1. Niedziela DA, Murphy MP, Grant J, Keane OM, Leonard FC. Clinical presentation and immune characteristics in first-lactation Holstein-Friesian cows following intramammary infection with genotypically distinct *Staphylococcus aureus* strains. *J. Dairy Sci.* 2020 103:8453– 8466.
2. Murphy MP, Niedziela DA, Leonard FC, Keane OM. The in vitro host cell immune response to bovine-adapted *Staphylococcus aureus* varies according to bacterial lineage. *Sci Rep.* 2019 Apr 16;9(1):6134.
3. Keane OM. Symposium review: Intramammary infections-Major pathogens and strain-associated complexity. *J Dairy Sci.* 2019 May;102(5):4713-4726.
4. Cormican P, Keane OM. Complete Genome Sequences of Sequence Type 71 (ST71) and ST97 *Staphylococcus aureus* Isolates from Bovine Milk. *Microbiol. Resour. Announc.* 2018 7 (5): e00954-18.
5. Murphy MP, Niedziela DA, Keane OM. EHS matrix incubated in media containing penicillin retains sufficient concentrations of antibiotic to inhibit growth of susceptible microorganisms. *J Microbiol Methods.* 2017 Aug;139:103-106.

One further publication is in preparation: Niedziela DA, Cormican P, Leonard FC, Keane OM. Bovine milk somatic cell transcriptomic response to *Staphylococcus aureus* is dependent on strain genotype. In preparation.

PhD theses

1. Dagmara A. Niedziela. Strain-specific virulence of *Staphylococcus aureus*. PhD. 2019. UCD.

Peer reviewed conference papers

1. D. Niedziela. Immune response to bovine-adapted *Staphylococcus aureus* is dependent on bacterial genotype. Microbiology Society Annual Conference 2019, Belfast, Northern Ireland. April 2019.
2. D. Niedziela, M. P. Murphy, P. Cormican, F.C. Leonard, O.M. Keane. Immune response to bovine-adapted *Staphylococcus aureus* is dependent on bacterial genotype. UCD College of Health and Agricultural Sciences (CHAS) Inaugural Graduate Research Student symposium, Dublin, Ireland. March 2019 (best presentation prize).
3. D. Niedziela, M. P. Murphy, P. Cormican, F.C. Leonard, O.M. Keane. Transcriptomic response of dairy cows to *Staphylococcus aureus* intramammary infection is dependent on strain genotype. 9th UCD Computational and Molecular Biology symposium, Dublin, Ireland. November 2018.
4. D. Niedziela. Immune response of *Bos taurus* to two genotypically distinct mastitis causing *Staphylococcus aureus* strains. 48th meeting of the Irish branch of Association for Veterinary Teaching and Research Work (AVTRW), Backweston, Celbridge, Co. Kildare, Ireland. October 2018.
5. O.M. Keane, D. Niedziela, M. P. Murphy, F.C. Leonard. *Staphylococcus aureus* lineage influences the bovine immune response to intramammary infection. American Dairy Science Association Annual Meeting, Knoxville, Tennessee, USA, June 2018. (invited presentation).
6. D.A. Niedziela. Lineage and strain specific differences in the in vitro and in vivo virulence of bovine adapted *Staphylococcus aureus*. Microbiology Society Annual Conference 2018, Birmingham, UK. April 2018.
7. D.A. Niedziela. Clinical presentation, somatic cell count and cytokine secretion in response to intramammary infection of Holstein Friesian heifers with isolates from two *Staphylococcus aureus* lineages. British Society of Animal Science (BSAS) Annual Meeting 2018, Dublin, Ireland. April 2018.
8. D. Niedziela, P. Cormican, F.C. Leonard, O.M. Keane. Transcriptomic response of *Bos taurus* to two genotypically distinct *Staphylococcus aureus* strains. 8th International Symposium on Animal Functional Genomics (ISAFG), Adelaide, Australia. November 2018 (best poster prize).
9. D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. Lineage and strain specific differences in the in vitro and in vivo pathogenicity of bovine adapted *Staphylococcus aureus*. 8th meeting of Dublin Academy of Pathogenomics and Infection Biology (DAPI), Dublin, Ireland. January 2018.
10. D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. Lineage and strain specific differences in the in vitro and in vivo pathogenicity of bovine adapted *Staphylococcus aureus*. 8th UCD Computational and Molecular Biology symposium, Dublin, Ireland. November 2017.
11. D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. In vivo infection with two bovine adapted *Staphylococcus aureus* suggests strain specific disease outcome. 47th meeting of the Irish branch of Association for Veterinary Teaching and Research Work (AVTRW), AFBI, Hillsborough, UK. October 2017 (best presentation prize).
12. M. P. Murphy. Lineage associated differences in host cell interactions with bovine mastitis-associated *Staphylococcus aureus*. *Staphylococcus Great Britain and Ireland*, Swansea, UK, September 2017.
13. O.M. Keane, D.A. Niedziela, M.P. Murphy, F.C. Leonard. *Staphylococcus aureus* genotype modulates the in vitro immune response of bovine mammary epithelial cells. 36th International Society for Animal Genetics (ISAG) conference, Dublin, Ireland. July 2017.
14. D.A. Niedziela, M.P. Murphy, O.M. Keane. Bovine-adapted lineages of *Staphylococcus aureus* induce a strain-specific pro-inflammatory immune response. 7th meeting of Dublin Academy of Pathogenomics & Infection Biology (DAPI), Dublin, Ireland. January 2017.
15. D.A. Niedziela, M.P. Murphy, O.M. Keane, F.C. Leonard. Lineage specific differences in host cell internalisation and immune response to bovine adapted *Staphylococcus aureus*. 46th meeting of the Irish branch of Association for Veterinary Teaching and Research Work (AVTRW), University College Dublin, Ireland. October 2016 (best presentation prize).