

Project number: 6742
Funding source: DAFM

Date: Jan 2020
Project dates: Mar 2015 – Oct 2019

Strain-specific pathogenicity of *Staphylococcus aureus*



Key external stakeholders:

Veterinarians, Dairy farmers, Animal health pharmaceutical companies.

Practical implications for stakeholders:

- *Staphylococcus aureus* strains associated with intramammary infection and mastitis in Ireland belong to 4 main genetic lineages (groups).
- Examination of the genetic make-up of strains from different lineages shows that they differ with respect to factors associated with the ability to cause disease
- When one strain from each of the 2 most common lineages CC97 and CC151 was used to infect first lactation cows, there were major differences in the severity of the resultant mastitis between the two strains:
 - The CC151 strain caused severe clinical mastitis with a high somatic cell count (SCC), a significant drop in milk yield, high levels of inflammatory markers in milk and high levels of anti-*S. aureus* antibodies in serum and milk.
 - The CC97 strain caused subclinical mastitis, a moderate SCC increase, no significant drop in milk yield and low levels of inflammatory markers in milk and low levels of anti-*S. aureus* antibodies in serum and milk.
- These findings suggest that the clinical manifestation of *S. aureus* mastitis may vary depending on the strains circulating in the herd. The lack of obvious clinical signs associated with some strains could lead to failure to detect infection and widespread circulation of such strains, especially in herds that do not participate in regular milk recording.

Main results:

- Strains of *Staphylococcus aureus* associated with clinical mastitis in Ireland were genotyped and found to belong to 4 main bovine-adapted genetic lineages, CC71, CC97, CC151 and ST136.
- Strains from each lineage encode different virulence genes and express different virulence factors *in vitro* suggesting that the ability to cause disease depends on the bacterial lineage.
- First lactation cows, 7 per treatment, were infected with either *S. aureus* CC97 or *S. aureus* CC151 and the infection was monitored for 30 days.
- Cows infected with *S. aureus* CC151 developed clinical mastitis in the first week post-infection, characterized by high SCC, a significant drop in milk yield, high levels of inflammatory markers in the milk and high levels of anti-*S. aureus* antibodies in serum and milk. In contrast, cows infected with *S. aureus* CC97 had subclinical mastitis with moderate SCC, no significant drop in milk yield, low levels of inflammatory markers in milk and low levels of anti-*S. aureus* antibodies in milk and serum.
- The outcome of *S. aureus* intramammary infection depends on the genotype of the infecting strain of *S. aureus*.

Opportunity / Benefit:

This study demonstrates the influence of *S. aureus* strain on the outcome of intramammary infection. *S. aureus* is one of the main causes of mastitis in Ireland and worldwide. Two distinct strains of *S. aureus* were used to infect first-lactation Holstein-Friesian cows and one strain induced predominantly clinical mastitis,

while the other induced mild and subclinical mastitis. Information regarding the infecting *S. aureus* strain/genotype may influence the ability to detect infection and treatment and culling decisions in the future.

Collaborating Institutions: UCD

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1. Project background:

Mastitis is an inflammation of the mammary gland that may be clinical or subclinical and of infectious or non-infectious origin. Both clinical and subclinical mastitis have been associated with significant economic losses. *S. aureus* is the most common pathogen associated with mastitis in Ireland, isolated from 23% and 21% of milk samples from clinical and subclinical cases respectively. Mastitis caused by *S. aureus* is often characterised as mild, chronic and persistent. The reoccurrence of *S. aureus* infections after antibiotic treatment or recovery from infection makes this pathogen difficult to eradicate in dairy herds. Strains of *S. aureus* can be genotyped and related genotypes grouped into lineages and it has been proposed that the infecting strain/lineage of *S. aureus* influences the progression and outcome of *S. aureus* mastitis.

2. Questions addressed by the project:

- What genetic lineages of *S. aureus* are associated with intramammary infection of dairy cows in Ireland?
- Do these genetic lineages differ in the virulence genes that they carry and in the virulence traits that they can express?
- Do strains from the different genetic lineages have different abilities to cause intramammary infection in cows?

3. The experimental studies:

Three separate studies were carried out:

1. *S. aureus* strains (n = 126) recovered from milk of cows with clinical mastitis were genotyped using multi-locus sequence typing and microarray genotyping. The strains predominantly belonged to 4 bovine-adapted genetic lineages, CC71, CC97, CC151 and ST136.
2. The virulence genes present in each strain were identified. There were significant differences between strains in the virulence genes they carried, with CC151 strains carrying many toxin genes. The strains differed in their ability to elicit an immune response and to internalise within bovine mammary epithelial cells *in vitro*.
3. The ability of a representative strain from each of CC97 and CC151 to cause intramammary infection was investigated by challenging 14 low SCC, bacteriologically negative first lactation cows in mid-lactation; 7 cows were challenged with each strain.

• Main results:

- Irish clinical mastitis *S. aureus* strains predominantly belong to 4 main lineages: CC71, CC97, CC151 and ST136.
- Strains from these lineages differ genotypically, with CC151 encoding many toxin genes.
- Strains from these lineages also differ phenotypically with different abilities to elicit an immune response and internalise within bovine mammary epithelial cells where they can evade both the immune system and antibiotic therapy.
- A representative strain from CC97 and CC151 were used to infect first-lactation cows. CC151 caused severe clinical mastitis while CC97 caused subclinical mastitis.
- Infection with *S. aureus* CC151 resulted in a large increase in SCC, a significant drop in milk yield, high levels of inflammatory markers in milk and high levels of anti-*S. aureus* antibodies in milk and serum.
- Infection with *S. aureus* CC97 resulted in a small increase in SCC, no significant drop in milk yield,

low levels of inflammatory markers in milk and low levels of anti-*S. aureus* antibodies in milk and serum.

4. Opportunity/Benefit:

This study demonstrates the influence of *S. aureus* strain on the outcome of intramammary infection. *S. aureus* is one of the main causes of mastitis in Ireland and worldwide. Two distinct strains of *S. aureus* were used to infect first-lactation Holstein-Friesian cows and one strain induced predominantly clinical mastitis, while the other induced mild and subclinical mastitis. Information regarding the infecting *S. aureus* strain/genotype may influence treatment and culling decisions in the future.

5. Dissemination:

- Murphy MP, Niedziela DA, Leonard FC, Keane OM. (2019). The *in vitro* host cell immune response to bovine-adapted *Staphylococcus aureus* varies according to bacterial lineage. *Scientific Reports* 9:6134.
- Murphy MP, Niedziela DA, Keane OM. (2017). EHS matrix incubated in media containing penicillin retains sufficient concentrations of antibiotic to inhibit growth of susceptible microorganisms. *J Microbiol Methods*; 139:103-6.
- Niedziela DA. (2019). Strain-specific virulence of *Staphylococcus aureus*. PhD thesis. UCD.
- Niedziela DA, Murphy MP, Keane OM and Leonard FC. (2019). Immune response to bovine-adapted *Staphylococcus aureus* is dependent on bacterial genotype. *Microbiology Society Annual Conference 2019*, Belfast, Northern Ireland.
- Niedziela DA, Murphy MP, Keane OM and Leonard FC. (2018). Immune response of *Bos taurus* to two genotypically distinct mastitis causing *Staphylococcus aureus* strains. 9th UCD Computational and Molecular Biology symposium, Dublin, Ireland.
- Niedziela DA, Murphy MP, Keane OM and Leonard FC. (2018). 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, Cellbridge, Co. Kildare, Ireland. October 2018.
- Niedziela DA, Murphy MP, Keane OM and Leonard FC. (2018). Lineage and strain specific differences in the *in vitro* and *in vivo* pathogenicity of bovine adapted *Staphylococcus aureus*. *Microbiology Society Annual Conference 2018*, Birmingham, UK.
- Niedziela DA, Murphy MP, Keane OM and Leonard FC. (2018). 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.
- Niedziela DA, Murphy MP, Keane OM and Leonard FC. (2018). Transcriptomic response of *Bos taurus* to two genotypically distinct *Staphylococcus aureus* strains. 8th International Symposium on Animal Functional Genomics (ISAFG), Adelaide, Australia.
- Keane OM, Niedziela MP, Murphy MP. (2017). Lineage-specific expression of virulence traits and the strain-specific host immune response to bovine-adapted *Staphylococcus aureus*
- 6th International Society for Animal Genetics (ISAG) conference, Dublin, Ireland. July 2017.

6. Compiled by: Dr Orla Keane