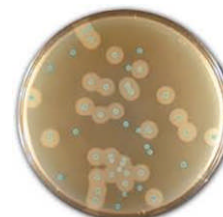


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Risk Assessment Network of Ireland

Staphylococcus aureus and *Listeria monocytogenes* in raw milk cheese



Industry Impact

The study assessed the impact of two food pathogens on the safety of raw milk cheese for the benefit of raw milk cheesemakers and the public in general. The study showed that risks associated with *Staphylococcus aureus* are low, while those associated with *Listeria monocytogenes* are more significant.

Key external stakeholders:

Raw milk cheese industry; Policymakers, Food researchers

Practical implications for stakeholders:

The study assessed the risk posed by two food pathogens (*Staphylococcus aureus* and *Listeria monocytogenes*) in raw milk cheesemaking. A range of samples (n=117), including milk, curds, whey and cheese, from 5 raw milk suppliers, and 4 raw milk cheesemakers were analysed for coagulase positive *S. aureus*. Of the isolates obtained, 17% had toxin producing ability and produced only Staphylococcal Enterotoxin C (SEC) which is generally animal rather than food associated. The other classical enterotoxins SEA, SEB or SED (food poisoning associated) were not produced. No toxin was produced in raw or pasteurised milk or in sterile reconstituted skim milk stored below 14°C for 24 h and no SEC was produced during cheesemaking. *L. monocytogenes* was found at a level of 300 colony forming units/ml in the milk of one cow with sub-clinical infection. While the numbers of naturally occurring *L. monocytogenes* increased in milk and during cheesemaking, this increase did not appear to be due to growth.

This research was carried out as part of a large national network project, *Risk Assessment Network of Ireland* which focused on the application of microbial quantitative risk assessment as a tool to underpin risk management actions. Research by Teagasc in this project focused on assessed the risk posed by two food pathogens on the safety of raw milk cheese

Main results:

- None of the *S. aureus* isolates recovered from raw milk or cheese produced the endotoxins SEA, SEB or SED, nor did they harbour the enterotoxin encoding genes *sea*, *seb*, *sed* or *see*.
- 17% of *S. aureus* isolates produced Staphylococcal enterotoxin C (SEC)
- Cheesemaking inhibited staphylococcal toxin production as did storage temperatures below 14°C
- Optimum conditions for toxin production in reconstituted skim milk were 37°C at pH 6.5
- *Listeria monocytogenes* was found in raw milk from one cow at a level of 300 cfu/ml, though there was with no evidence of infection in the animal
- Although numbers of naturally occurring *L. monocytogenes* increased in milk and during cheese making, this increase did not appear to be due to growth

Opportunity / Benefit:

The opportunity was to assess the impact of *S. aureus* and *L. monocytogenes* on the safety of raw milk cheese for the benefit of raw milk cheesemakers and the public in general. The study showed that there were different risks associated with each pathogen.

Collaborating Institutions:

University College Dublin

Teagasc project team: Dr Kieran Jordan
Dr Geraldine Duffy
Ms. Karen (Triona) Hunt

External collaborators: Prof. Francis Butler, University College Dublin

1. Project background:

Coagulase positive *Staphylococcus aureus* is a Gram-positive, facultative anaerobe that is ubiquitous in nature. Some, but not all strains produce staphylococcal enterotoxins (SEs) which are potent emetic agents causing staphylococcal food poisoning (SFP). SFP has recently been reported to have a low hospitalisation rate of 6.4%; however, due to the nature of the toxin the symptoms are rarely severe, leading to high levels of under-reporting. *S. aureus* strains produce a wide range of toxins (presently 121 SEs) of which toxins, SEA, SED and SEE are the most frequently associated with food, and are therefore most clinically relevant, while SEC is associated with animal origin. *S. aureus* is one of the major causes of bovine mastitis, and in cases of sub-clinical mastitis can easily contaminate raw milk. With no heat treatment prior to manufacture of raw milk cheese, and milk being an ideal growth medium for bacteria, numbers of *S. aureus* could potentially increase. And if subsequent growth opportunities occur during cheesemaking, it is possible that numbers could be relatively high in cheese. Approximately 10% of cheese in Europe is made from raw milk, presenting a considerable potential risk to public health.

Listeria monocytogenes is a pathogenic bacterium that can cause Listeriosis in humans and various animal species. In humans, foodborne *L. monocytogenes* causes large outbreaks of Listeriosis, with a mortality rate of 9% to 44%. In raw milk and the dairy environment, the source of *L. monocytogenes* contamination is mainly from poor silage and bedding. *L. monocytogenes* can cause bovine mastitis, and in cases of sub-clinical mastitis in cows, can go undetected if the milk remains visually unchanged, and with no clinical symptoms the contamination can normally persist even after treatment. Raw milk can thus be contaminated by direct excretion into the milk. The purpose of this study was to assess the risks associated with *S. aureus* and *L. monocytogenes* in raw milk.

2. Questions addressed by the project:

Do *S. aureus* and *L. monocytogenes* pose a threat to public health in raw milk cheese?

To address this issue, the following questions were asked:

- What is the occurrence of toxin producing *S. aureus* in raw milk?
- What are the factors that affect toxin production in milk and cheese?
- Can naturally occurring *L. monocytogenes* grow during cheese making?

3. The experimental studies:

- One hundred and seventeen samples, including milk, curds, whey and cheese, from 5 raw milk suppliers, to 4 raw milk cheesemakers in the South of Ireland, were analysed for coagulase positive *S. aureus*.
- The effect of different combinations of temperature and pH on production of SEC by *S. aureus* was investigated in batch cultures of 10% sterile reconstituted skim milk for up to 72 h. A full factorial experiment of 4 temperatures (25, 30, 37 and 40°C) and 4 pH values (5.5, 6.0, 6.5 and 7.0) was undertaken.
- Toxin production was also studied in pasteurised and unpasteurised milk at temperatures of 25, 30, 37 and 40°C with uncontrolled pH.
- During routine sampling of bulk raw milk on a dairy farm, the pathogenic bacteria *Listeria monocytogenes* was found to be a contaminant, at numbers < 100 cfu/ml. Milk samples were collected from the individual cows and analysed for *L. monocytogenes*.

4. Main results:

- The 151 isolates characterised represented up to 2 isolates from each of the 81 positive samples. The results showed 83.2% of the isolates did not contain the staphylococcal enterotoxin genes or the toxin producing capability tested for. From only one supplier, 26 isolates contained the sec genes and produced SEC. Within these 26 isolates there were only 2 PFGE types. None of the isolates from any of the 5 suppliers produced SEA, SEB or SED toxin nor did they harbour the sea, seb, sed or see genes. One SEC producing isolate showed no toxin production at 96 and 74 h in sterile 10% reconstituted skim milk at 10°C and 12°C, respectively. Some SEC was produced at 14°C and 16°C after 74 and 55 h, respectively.
- The results indicate that milk used for raw milk cheese production in Ireland poses a limited risk to public health.
- The optimum controlled conditions for SEC production were 37°C/pH6.5. At temperatures of 25, 30, 37 and 40°C with uncontrolled pH, a lower concentration of SEC was produced compared to the controlled pH conditions. In pasteurised and unpasteurised milk, no SEC production occurred at temperatures up to 30°C after 72 h.
- *L. monocytogenes* excretion (at 280 cfu/ml) from one of the 4 mammary quarters of one dairy cow out of 180 was identified
- A strain with an indistinguishable pulsed-field gel electrophoresis pattern was isolated from the bulk milk. Environmental swabs taken at the dairy environment were negative for the presence of *L. monocytogenes*. The results indicated a possible case of excretion of the *L. monocytogenes* directly into the milk.
- Although numbers of *L. monocytogenes* increased in milk, there was no evidence that growth had occurred

5. Opportunity/Benefit:

The opportunity was to assess the risk posed by two food pathogens (*S. aureus*, *L. monocytogenes*) on the safety of raw milk cheese. The study showed that there were different risks associated with each pathogen.

6. Dissemination:

The information generated from this study was disseminated by publication of the work in relevant peer reviewed journals and at meetings and workshops with farmhouse cheese makers.

Main publications:

Hunt, K., Schelin, J., Rådström, P., Butler, F., and Jordan, K. (2012). Classical enterotoxins of coagulase-positive *Staphylococcus aureus* isolates from Raw Milk and products for Raw Milk Cheese Production in Ireland. *Dairy Science and Technology*, 92, 487-499.

Hunt, K., Drummond, N., Murphy, M., Butler, F., Buckley, J., and Jordan, K. (2012). A case of subclinical mastitis resulting in bovine raw milk contamination with *Listeria monocytogenes*. *Irish Veterinary Journal*, 65: 13-18.

Hunt, K., Butler, F., and Jordan, K. (2014). Factors affecting Staphylococcal Enterotoxin C_{bovine} production in milk. *International Dairy Journal*, 39:41-46.

7. Compiled by: Kieran Jordan and Geraldine Duffy