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## Investigation of factors influencing bacterial transfer into milk on-farm



### Key external stakeholders:

Dairy processors, dairy farmers, dairy industry, milk quality advisors

### Practical implications for stakeholders:

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### Main results:

- Minimum changes in average bacterial counts of milk in Ireland were observed during the years 2007 to 2011. A trend downward in somatic cell count (SCC) occurred in 2011. This may be due to increased milk recording, introduction of bonus schemes and a national program targeting SCC reduction. The necessity for targeted advisory during early and late lactation periods to improve milk quality by changing farm management practices was highlighted.
- The farm management factors influencing the presence of *B. cereus* in milk were similar to those influencing thermophilic bacteria and the adoption of certain practices such as dry wiping of teats before cluster attachment will minimize the levels in milk.
- Hygienically produced milk can be stored for up to 96 h at 2°C or 4°C with little effect on the microbiological status of that milk, its composition or functional properties.
- Alternative cleaning protocols to chlorine based products are now available for the cleaning of milking equipment.

### Opportunity / Benefit:

The results of this research have:

- Highlighted a range of farm management factors that have negative or positive impacts on milk quality.
- Highlighted that chromogenic medium (Bacara) agar is preferable to mannitol egg yolk polymyxin agar (MYP) for the detection of presumptive *B. cereus*.
- Demonstrated that milk can be stored on farm for longer than the normal 48 h period without impacting negatively on the microbiological or processing on the quality of that milk
- 4°C is an adequate milk storage temperature, even for extended storage periods (up to 4 days).
- Shown that peracetic acid may be used as an alternative product to sodium hypochlorite (which has been associated with higher levels of TCM) for the purposes of pre-milking plant sanitation.

### Collaborating Institutions:

University of Madison–Wisconsin,

Teagasc project team:

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Dr Bernadette O' Brien  
Dr Kieran Jordan  
Aine O' Connell (WF)

External collaborators

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1. Project background:

The Food Harvest 2020 report has targeted milk production to increase by 50% by 2020. Such an expansion in herd size may have an impact on milk quality at farm level. There is no up to date national dataset available to indicate the levels of TBC, thermophilic counts or SCC in farm bulk tank milk. An observation of these bacterial levels within and over years would assist in identifying critical periods of poor milk quality. Milk producers will be required to meet more stringent specifications for total bacterial counts and specific bacteria such as thermophilic bacteria that are harmful to product and resistant to pasteurization. Therefore, it was necessary to establish the farm factors associated with specific sporeforming bacteria such as *Bacillus cereus* and the methods best suited for identification of *B. cereus* which is critical in the production of infant milk formula. The duration and temperature of storage of milk on-farm is an important issue in the overall quality of that milk. Milk may be stored for varying durations up to 3 to 4 days on Irish farms due to economic savings being made in reducing frequency of collection. Little information is available to industry on the optimum on-farm storage time and milk storage temperature to maintain good quality milk that will allow this milk to be suitable for processing. Furthermore alternate equipment cleaning protocols need to be investigated that will minimize chemical residue levels but also maintain low microbial levels in milk. This research project investigates milk quality trends in Ireland, factors influencing thermophilic bacteria in milk, equipment cleaning protocols, optimum on-farm milk storage conditions and its impact on milk for processing. This proposed research will assist the dairy industry to meet raw stringent milk quality standards necessary for dairy products being sold to international markets.

2. Questions addressed by the project:

- Are national milk quality trends improving from year to year and within years?
- What are the main farm management factors influencing levels of *Bacillus cereus* in farm bulk tank milk?
- How reliable are the test methods for the detection of *Bacillus cereus* in milk
- What is the optimum storage temperature and storage duration for milk produced on-farm?
- Is milk suitable for processing after a prolonged storage period of 96 h?
- Will non-chlorine based cleaning products maintain low bacterial counts in milk?

3. The experimental studies:

- A complete milk quality dataset was supplied by 9 milk processors and consisted of SCC and TBC obtained from January 2007 to December 2011. This dataset represented 8,002 seasonal milk production herds and 1,829 split calving herds. Herd size was calculated based on milk volume supplied. This dataset allowed for an assessment of trends in bulk tank SCC, TBC in Irish dairy herds across years and within years and to compare trends based on milk herd type and herd size.
- To establish the farm management factors associated with the *Bacillus cereus* count in bulk tank milk (BTM), bulk tank milk quality was monitored for *B. cereus* on 63 commercial dairy farms between July and August 2012. Bulk tank milk samples corresponding with processor milk collection dates were taken over a two week period prior to a farm visit and tested for *B. cereus*. On-farm data collection included recording observations and providing a questionnaire on basic hygiene, management factors and cow hygiene.
- To isolate potential *B. cereus* from a range of farm environments, samples of tap water, milking equipment rinse water, milk sediment filter, grass, soil and BTM were collected from the 63 farms. In addition, milk liners and teats were swabbed. The samples were plated on MYP agar and presumptive *B. cereus* colonies were isolated. These isolates were then plated BACARA agar and colonies identified as presumptive *B. cereus* were subsequently subjected to 16S ribosomal RNA (rRNA) sequencing. This allowed confirmation of the existence of *B. cereus* spores.
- An experiment was conducted to measure the effect of milk storage temperature and storage time (0, 24, 48, 72, 96 h) on the microbiological quality of milk, using milk from spring-calving cows and three identical bulk milk tanks. Milk production over two 6-wk periods was studied, representing summer and autumn. Each tank was set at each of the 3 temperatures (2°C, 4°C, and 6°C) on 2 occasions during each 6-wk period, resulting in 6 test periods (2 at each temperature). Bulk tank milk

samples were taken once daily at 0, 24, 48, 72, and 96 h, when there was milk from 2, 4, 6, and 8 milkings, respectively, in each tank. Milk samples were analysed for TBC, LPC, PBC, PBC-LPC, proteolytic count, lipolytic count, and for the presence of presumptive *B. cereus* and SRC.

- To measure the effect of prolonged storage on the processability of bulk tank milk, milk samples were collected daily from stored milk (0, 24, 48, 72, 96 h) produced to mimic what happens on farm, with fresh milk added twice daily. Samples were analysed for fat, protein, lactose composition, SCC, nitrogen fractions percentage total protein, NPN, non-casein nitrogen, amino acid content, Ca and P mineral contents. Furthermore, the thermal stability of bulk storage tank milk samples was measured daily including heat coagulation time (HCT) and rennet coagulation time (RCT).
- To measure the effect of using non-chlorine based cleaning products on maintaining low bacterial levels on equipment and in milk, seven milking machine cleaning routines containing varying working solutions of sodium hydroxide were randomly assigned for three-week periods to a milking facility on three research farms. Systems remained in place until all cleaning routines had completed a total testing period of 9 weeks. The cleaning routines and usage rates were generally applied as recommended by the distributors. Milk samples were collected during milking (AM and PM) from the milk line (Monday to Friday) and from BTM three times weekly. Milk line samples ( $n = 420$ ) and BTM samples ( $n = 108$ ) were analyzed for TBC and LPC. Bacterial swabs were taken during week 1 and week 3, from inside the plastic claw-piece bowl ( $n = 254$ ), inside the milk liner barrel ( $n = 126$ ) and internal stainless surface close to the receiver vessel ( $n = 126$ ). Swabs were plated using an IDF method to measure TBC.

#### • Main results:

- Annual TBC remained stable and SCC increased slightly from 2007 to 2009 and declined thereafter.
- Monthly TBC was greatest at the beginning and end of the year corresponding with cow housing period and poorer weather conditions.
- Larger herds had lower SCC and TBC across all months compared with smaller herds.
- Farm management factors associated with high *B. cereus* counts in BTM were identified
- Dry wiping of teats to remove debris was associated with lowering *B. cereus* in bulk tank milk.
- Farmers that reused detergent solution more than once tended to have a higher *B. cereus* count
- Higher counts of *B. cereus* were observed in milk from cows housed indoors on silage.
- The number of *B. cereus* s.s. organisms detected using BACARA agar differed to that identified by MYP agar.
- The use of 16S rRNA sequencing to identify isolates that grew on BACARA confirmed that the majority of isolates belonged to *B. cereus* s.l.
- Presumptive *B. cereus* s.l. were identified in all sample sources, including BTM, milk filters, tap water, final rinse water, liners and cow teats on farms.
- Bulk tank milk can be stored at 2°C or 4°C for up to 96 h with minimal deterioration of quality, provided that the milk entering the tank has minimal bacterial contamination.
- The composition and functional properties of milk differed between mid and late lactation.
- Blended milk can be stored for up to 96 h at temperatures between 2°C and 6°C with little effect on its composition or functional properties.
- Use of daily cold caustic cleaning in conjunction with daily hot acid cleaning or cleaning with a hot detergent/sanitiser twice daily product maintained the lowest TBC in milk.
- Lowest bacterial numbers on plastic surfaces were observed with the use of peracetic acid.
- Peracetic acid may be used as an alternative product to sodium hypochlorite (which has been associated with higher levels of TCM) for pre-milking plant sanitation.

#### 4. Opportunity/Benefit:

- The results of the data survey clearly demonstrate that a targeted approach is necessary during spring and autumn with regard to farm management practices to maintain good quality milk.
- The nature of the associations between 'dry wiping teats', the feeding of silage, the efficacy of the cleaning solution wash and *B. cereus* indicates that adoption of such management factors has the potential to reduce *B. cereus* count in bulk milk tanks.
- Bacara agar is a more reliable method for the detection of presumptive *B. cereus* in milk and could be adapted at processor level.
- This project highlighted that milk could be stored for longer periods on farms in situations where the initial quality of milk is of a high standard, this can have economic benefits for milk processors.
- The testing and satisfactory performance of non-chlorine based cleaning products is critical for industry targeting low residue levels in milk without impacting on the microbiological quality of that

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milk.

## 5. Dissemination:

### Main publications:

Gleeson, D., O'Brien, B. and Jordan, K.N. (2013). The effect of using non-chlorine products for cleaning and sanitising milking equipment on bacterial numbers and residues in milk. *International Journal of Dairy Technology* 10.1111/1471-0307,12037 ISSN 1471-0307 28927

O'Connell, A., McParland, S., Ruegg, P.L., O'Brien, B. and Gleeson, D. (2015). Seasonal trends in milk quality in Ireland between 2007 and 2011. *Journal of Dairy Science* 98 (6): 3778-90 ISSN 0022-0302 33830

O'Connell, P.L. Ruegg, K. Jordan, B. O'Brien, D. Gleeson. (2016). The effect of storage temperature and duration on the microbial quality of bulk tank milk. *Journal of Dairy Science* 99 (5): 3367-3374.

### Popular publications:

O'Connell, A., Jordan, K.N., O'Brien, B. and Gleeson, D. (2015). *Bacillus cereus* in bulk tank milk - importance to IMF. *Tresearch* 10 (2): 28-29 ISSN 1649-8917 33534 B1

Gleeson, D., O'Connell, A. and O'Brien, B. (2015). New cleaning protocols to minimize bacterial transfer at milking time. *Irish Dairying - Sustainable Expansion Moorepark15 Open Day 1/7/15*. Teagasc IE pages 96-97 ISSN 978-1-84170-6 33523

Gleeson, D., O'Brien, B. and Edwards, P. (2015). Implications of omitting teat preparation on bacterial levels in bulk tank milk. In: *The proceedings on Controversies & Consensus in Bovine Health, Industry & Economics*, Berlin, Germany, 27-Aug-2015, p. 69 33785 A2

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## 6. Compiled by: Dr. David Gleeson

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