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Source: *Irish Journal of Agricultural and Food Research*, Vol. 52, No. 2 (2013), pp. 229-241
Published by: TEAGASC-Agriculture and Food Development Authority
Stable URL: http://www.jstor.org/stable/23631034
Accessed: 07-12-2016 16:50 UTC

Irish Journal of Agricultural and Food Research **52:** 229–241, 2013

Farm management factors associated with the *Bacillus cereus* count in bulk tank milk

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The objective of this study was to determine the on-farm management factors that are associated with the *Bacillus cereus* count in raw bulk tank milk using a cross sectional study design. Bulk tank milk quality was monitored for *B. cereus* on 63 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 the farm visit and tested for *B. cereus*. The four most recent samples taken prior to the on-farm visit were averaged and log transformed to give the outcome variable; mean $\log_{10}$ *B. cereus* cfu/mL. On-farm data collection included recording observations and providing a questionnaire on basic hygiene, management factors and cow hygiene scoring. All independent variables were analysed individually with the outcome variable using simple linear regression and one-way ANOVA; a multivariable regression model was subsequently developed. Only significant variables were retained in the final model ($P < 0.05$). The geometric mean *B. cereus* count for all milk samples was 40 cfu/mL. The start temperature of the cleaning solution wash, dry wiping teats prior to unit application, the feeding of silage and reusing the cleaning solution more than once were all unconditionally associated ($P < 0.10$) with the *B. cereus* count in bulk tank milk but did not enter the final multivariable model. *B. cereus* count was four times greater (201 cfu/mL) when cows had been housed compared to when they were on pasture (50 cfu/mL). The allocation of fresh grass every 12 h (62 cfu/mL) resulted in a decrease in *B. cereus* count (166 cfu/mL every 24 h or greater). The testing of water for bacteriology was associated with an increase in *B. cereus* count. In conclusion, this study highlights specific management factors associated with the *B. cereus* count in bulk tank milk.

Keywords: *Bacillus cereus;* farm factors; milk quality; thermoduric bacteria

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Introduction

Bacillus cereus is a Gram positive, rod-shaped, foodborne pathogen (Christiansson 2011). When ingested in great numbers *B. cereus* can cause two types of foodborne illnesses; diarrheal and emetic type outbreak (Granum 2005). Due to its ability to form endospores *B. cereus* is resistant to pasteurisation (Granum 2005) and has been successfully isolated from pasteurised dairy products worldwide (Becker *et al.* 1994; Lin *et al.* 1998; Larsen and Jorgensen 1999). Due to the vulnerable nature of the consumers, the incidence of *B. cereus* in infant milk formulae (IMF) is of particular interest to the dairy industry. Of 92 worldwide IMF samples examined in a German study, 54% proved positive for *B. cereus*, however, counts measured were relatively low (0.3–10 cfu/g) (Becker *et al.* 1994).

Currently, the European Union (EC 1441/2007; Anon. 2007) has set a threshold *B. cereus* limit for dried infant formulae intended for infants below 6 months of age. For a product to be considered satisfactory, four out of five samples must be below 50 cfu/g while the remaining sample can be between 50 cfu/g and 500 cfu/g. While no regulatory thresholds have been put in place for raw milk, processors promote raw milk with a *B. cereus* count <10 cfu/mL for IMF manufacture.

Contamination of dairy products with *B. cereus* occurs initially at farm level where the incidence of *B. cereus* in raw milk is almost inevitable due to the organism's abundance in soil (Griffiths and Phillips 1990). Vegetative *B. cereus* cells are found in raw milk at <10 cfu/mL to a few hundred cfu/mL (Christiansson 2011). Previous studies in the Netherlands and Sweden have studied the incidence of *B. cereus* spores in bulk tank milk (BTM) where counts were in the range of <10 to 40 cfu/mL (Slaughis *et al.* 1997; Christiansson, Bertilsson and Svensson 1999; Vissers *et al.* 2007). These same studies outlined different dominant pathways of transmission of *B. cereus* that are dependent on housing and climatic conditions. When cows were housed, spores in used bedding are a major source of contamination of BTM with *B. cereus* via contaminated teat and udder surfaces (Magnusson, Christiansson, and Svensson 2007). However, when cows were out grazing, contamination of teats with soil was the main route of contamination of BTM (Christiansson *et al.* 1999). Increased growth of *B. cereus* during the summer has also been shown (Vissers *et al.* 2007); this increase was probably due to increased temperatures. Slaghuis *et al.* (1997) argued that the seasonal variation of *B. cereus* spore content in milk is influenced more by the type of housing rather than the change in seasonal temperature. In that study, when cows were housed during summer the incidence of *B. cereus* (4%) in raw milk was not as high as when cows were outside (23%).

The *B. cereus* count in milk is highly dependent on daily management practices, climate and housing conditions. Therefore, the findings from previous studies are not entirely applicable to Irish dairy farmers due to differences in climate and management practices. No farm studies on *B. cereus* in raw BTM have been carried out in Ireland. Additionally, most studies have evaluated management factors that influence the *B. cereus* spore count in milk. In Ireland, milk chosen for IMF manufacture is dependent on its *B. cereus* count pre-pasteurisation i.e. the vegetative cell count. Therefore, the objective of the study was to identify the farm management factors associated with the vegetative *B. cereus* count in BTM in Ireland.

Materials and Methods

A cross sectional study was designed to investigate a wide range of management factors that potentially influence the *B. cereus* count in BTM. Management factors relating to the cows environment, milking practices and equipment sanitation were identified and measured. For the reporting of this study, the guidelines on strengthening the reporting of observational studies in epidemiology (STROBE guidelines) were followed whenever possible (von Elm *et al.* 2008). This study was completed in collaboration with a milk processor near the Animal and Grassland Innovation and Research centre, Teagasc, Moorepark, Fermoy, Co. Cork. Microbiological analysis of all milk samples collected was carried out in the microbiology lab at the milk processing facility as part of the routine analysis at the processing facility.

Farm selection

Farms near both the research centre and the processing facility were identified by the processor from their supplier list. Only farms within a 15 mile radius of both centres could be enrolled in the study so that all milk samples collected on farm were analysed at the processing facility within 24 h. Farms were invited to take part in the study (with the option to decline) by the milk quality advisor for that area. Due to time and labour limitations, a total of 63 farms (two farms declined) were enrolled and visited during the study.

Sample collection and microbiological analysis

Routine analysis of BTM milk samples by the processors for *B. cereus* is not conducted for all suppliers enrolled. Therefore, there was no historical BTM *B. cereus* data available for the suppliers enrolled. To identify the *B. cereus* count in BTM on farms, BTM samples were collected from each farm for the two weeks preceding an on-farm visit. The last milk sample taken from each farm contained milk collected during the on-farm visit.

Bulk tank milk samples (25 mL) were collected by a trained technician and taken at the time of bulk milk collection. Samples were drawn aseptically from the top of the tank after agitation. All milk samples were immediately placed on ice and analysed within 24 h of collection.

Samples were serially diluted and surface plated on mannitol egg yolk polymyxin agar (Merck, Darmstadt, Germany). The plates were incubated at 32 °C for 48 h. Typical pink colonies surrounded by a zone of precipitation were counted. Colonies suspected as *B. cereus* were confirmed by the presence of β haemolysis on blood agar (blood agar base No. 2, Oxoid, Basingstoke, UK) plates after 24 h at 32 °C.

Data collection

Two technicians carried out the on-farm visits during milking time. To reduce the potential bias of technician, calibration of measurements took place on two farms (not enrolled in the study) prior to the study. Visits were arranged by the technicians with farmers 24 h prior to arrival.

Initially, the last paddock grazed out by the herd was scored for hygiene around the paddock entrance, the area surrounding drinking troughs and the paddock itself on a scale from 1 to 4; where 1 = <25%, 2 = 25–50%, 3 = 51–75% and 4 = >75% of the pasture was covered with faeces or mud. The roadway between the paddock and the milking parlour was hygiene scored on the same scale. Post-grazing sward height (cm) was measured using a plate meter. Explanatory variables for paddock hygiene were condition of paddock entrance, condition of paddock surrounding the drinking trough, condition of three randomly chosen blocks of the paddock and condition of the roadway.

Prior to milking, cleanliness of the dairy, holding yards and the parlour were scored on a scale from 1 to 3; where 1 = clean, 2 = slightly dirty and 3 = very dirty. The condition of the collecting yard or exit alleyway was scored after milking. These measurements provided the explanatory variables parlour hygiene, dairy hygiene and holding yard hygiene. The presence or absence of farm infrastructure was recorded. Explanatory variables that measured farm infrastructure were machine wash and bulk tank wash (automatic or manual), presence of a plate cooler, presence of automatic cluster removers (yes or no) and jetter type (candle, cup or dual).

During milking, the cows udders and legs were scored for hygiene based on the scorecard developed by Schreiner and Ruegg (2003) from 1 (free of dirt) to 4 (>30% of surface area caked with dirt). A maximum of fifty cows were scored on farms with >50 cows while all cows were scored on farms with <50 cows. The average leg and udder hygiene score for each farm was calculated to provide the explanatory variables, mean udder hygiene score and mean leg hygiene score. Whether cow's tails were recently clipped or not, or if cow's tails were docked was recorded to give the explanatory variable 'tails clipped'. Explanatory variables measured throughout the entire milking were rate of cow defecation on the platform per hour (frequency of cow defecations on the milking platform divided by the milking time), rate of cluster fall offs per hour (frequency of cluster fall offs divided by the milking time), and rate of squawks per hour (frequency of squawks divided by the milking time).

Teat preparation practices carried out prior to unit application and after milking were recorded to provide the following explanatory variables; teats stripped, dry wiped, disinfected, washed and dried prior to unit application and were teats post dipped (yes or no). Whether farmers wore gloves or not was also recorded (yes or no). Once milking was complete, all liners (up to a maximum of ten units) were scored for hygiene on a scale from 1 (free of dirt) to 4 (>30% of surface area caked with dirt). This score was averaged to provide the explanatory variable mean liner hygiene score for each farm.

After milking, the milk filter was scored for hygiene from 1 (<10% of the filter covered with sediment) to 4 (>30% of the filter covered with sediment). This was only possible if the farmer removed the filter prior to the wash cycles. The pH of the detergent solution was measured and a sample of the solution was taken to measure alkalinity. If hot water was used when running the cleaning solution wash the temperature was recorded. The sequence of cleaning washes was recorded. A tap water sample was taken to measure water hardness. Finally, bulk tank temperature was recorded 30 min after the completion of milking. Thus, equipment sanitation explanatory variables included; filter hygiene score, rinse cycles observed, pH of the detergent wash, temperature of the detergent wash (°C), alkalinity of the detergent wash (ppm), water hardness (ppm), detergent type (detergent with added steriliser or without) and the bulk tank temperature (°C) of the milk 30 min since the last addition of milk.

After wash up procedures were finished, a questionnaire was completed by each farmer. The questionnaire provided explanatory variables such as herd size (number of milking cows), whether farms were enrolled in a milk recording scheme (yes or no), frequency of fresh grass allocation (h), the number of months since the milking machine was last serviced, frequency of liner change per year, whether cows had been housed for any time within 14 days prior to the visit and was silage fed to the cows anytime within 14 days prior to the visit (yes or no). Equipment sanitation explanatory variables relevant to the 14 days prior to the on farm visit asked in the questionnaire included; reusing of the milk filter (yes or no), if detergent was left in the plant between milking (yes or no), frequency of machine and bulk tank descale washes (number of washes in the past 14 days), frequency of hot washes of the machine and bulk tank (number of washes in the past 14 days) and use of a sanitiser (yes or no). Water quality explanatory variables asked included; had water been tested for bacteriology in the last three years (yes or no), was a water softener in use (yes or no) and the source of the water supply (well, council or group scheme).

Statistical procedures

Data from the questionnaire and observations recorded during the visit were coded and entered into Microsoft Excel. The herd was the unit of analysis. Therefore, predictors that were measured at the cow level (udder and leg hygiene score) and liner hygiene scores were converted to farm-level averages.

The outcome variable was the mean $\log_{10}$ *B. cereus* count for each farm. The results from the four most recent *B. cereus* BTM samples collected prior to the visit (including the sample containing milk collected during the on-farm visit) were averaged and log transformed for normality. The detection limit for *B. cereus* was 10 cfu/mL. Where *B. cereus* were non detectable an arbitrary figure of five was used to calculate the mean.

Simple linear regression and one-way ANOVA were used to test the association between mean $\log_{10}$ *B. cereus* (cfu/mL) and each explanatory variable (Proc GLM, SAS 2011). Variables with a P-value of <0.20 were tested for co-linearity in pairs by performing the Pearson chi-square test. If co-linearity existed ($P < 0.05$) only the variable from each pair that was more strongly associated with the outcome was retained for further analysis. Additionally variables were assessed for missing values and excluded from analysis when there were $>10\%$ missing values.

Multivariable linear regression models were constructed using PROC GLM with a manual forward stepwise approach to evaluate the association of explanatory variables with the outcome variable after adjusting for each other (SAS 2011). Variables which achieved statistical significance ($P < 0.05$) in multivariable models were retained in the final model. Two way interactions of the explanatory variables in the final model were tested for significance at $P < 0.05$. Potential bias of the technician was assessed by addition of a technician-level random effect term to the model. Herd size was considered as a potential confounder and thus was forced into the final model to check whether it had a confounding effect. Outliers and influential observations were evaluated by residual diagnostics using standardised residuals, leverage and Cook's distance.

Results

Farm characteristics

Observations and sampling were completed on 63 farms and questionnaires were completed for 62 farms. The mean milking herd size was 66 cows of which 23% were first lactation cows. For most farms, cows were out on pasture, however due to periods of heavy rainfall during the study some farmers were forced to house cows occasionally ($n = 13$). The average parlour size was 10 units. The majority of farms had a herringbone milking system (n = 48), while the remaining farms had side by side milking parlours (n = 14) and one farm had an abreast milking parlour. Nineteen percent of farms had automatic cluster removers, 16% had an automatic machine washer, 70% had a plate cooler, 76% had an automatic or semi-automatic wash and 97% had a water heater in the dairy. The geometric mean *B. cereus* count for all milk samples was 40 cfu/mL (CI = 28.4 to 55.1). From a total of 252 milk samples (63 farms with 4 samples tested) tested for *B. cereus,* 95 samples were below the detection limit of 10 cfu/mL. The average milking time was 1 h. At a total of 42 farms the milk supply, composition and SCC were regularly monitored via a milk recording programme. The mean hygiene scores at herd level for udders, legs and liners are presented in Table 1. No association between hygiene scores and *B. cereus* count in the BTM was found.

Unconditional associations

A total of 17 variables were unconditionally associated ($P < 0.25$) with the geometric mean *B. cereus* (Table 2). The explanatory variable ‘cleaning solution wash start temperature’ was not offered to multivariable analysis as >10% of the observations were missing. Most farmers (n = 49) did not carry out a hot detergent wash during the evaluation period and in those instances the temperature was not measured. However, where farms did include a hot detergent wash, an increase in temperature of the cleaning solution wash was negatively associated with mean *B. cereus* count ($P < 0.10$).

A positive association was observed with feeding silage and *B. cereus* count ($P < 0.05$). Likewise, reusing the cleaning solution more than once tended to be associated with an increased *B. cereus* count ($P < 0.10$). Dry wiping teats prior to unit application ($P < 0.05$) was unconditionally associated with a decrease in *B. cereus* count. The effect of the milk filter type on *B. cereus* count depended on the position of the milk filter in the machine. The presence of a cloth filter inline or a plastic filter inline did not cause a significant difference in *B. cereus* count. However, the position of a cloth milk filter at the outlet tended to result in a higher *B. cereus* count in BTM ($P < 0.10$).

Conditional associations

Frequency of fresh grass allocation, the testing of water for bacteriology in the past 3 years and whether cows were housed within 14 days prior to the farm visit remained in the final multivariate model ($P < 0.05$) (Table 2). The *B. cereus* count was four times greater (210 cfu/mL) when cows had been housed compared to when they were on pasture (50 cfu/mL). The allocation of fresh grass every 12 h versus every 24 h or greater resulted in a decrease in *B. cereus* counts ($P < 0.01$). The testing of water for bacteriology within 3 years prior to the visit was associated with an

Table 1. Mean, standard deviation, maximum, minimum and median value for each hygiene score.

Variable	Hygiene scores				
	Mean	Standard deviation	Maximum	Minimum	Median
Udder hygiene scores	2.4	0.5	3.7	1.6	2.4
Leg hygiene scores	3.2	0.5	4.0	1.6	3.3
Liner hygiene score	2.4	0.6	3.9	1.0	2.4

Table 2. Unadjusted coefficients and geometric mean *Bacillus cereus* count (Log_{10} cfu/mL) for all explanatory variables that were individually associated with *Bacillus cereus* ($P < 0.25$).

Variable	Number of farms	Coefficient		P-value
Continuous variables				
Cleaning solution wash start temperature (° C)	14	–0.02		0.08
Categorical variables		*Bacillus cereus* count	(95% Confidence Interval)	P-value
Frequency of bulk tank descale wash (14 days prior to visit)				0.14
Never	15	28.2	(14.6–54.7)	
Once	22	27.1	(15.7–46.9)	
Twice	21	61.6	(35.2–107.7)	
Four times	3	61.0	(13.9–268.4)	
Parlour hygiene				0.22
Clean	14	51.3	(25.7–102.3)	
Slightly dirty	36	30.9	(20.0–47.9)	
Very dirty	13	58.9	(28.8–120.2)	
Milking wash				0.24
Automatic	10	25.1	(11.0–57.5)	
Manual	53	42.7	(30.2–61.7)	
Tails clipped				0.19
Yes	11	47.9	(21.9–104.7)	
No	48	41.7	(28.8–60.3)	
All tails are docked	4	12.6	(3.4–45.7)	
Post dip type				0.19
Spray	50	33.9	(23.4–50.1)	
Teat cup	6	72.4	(24.5–218.8)	
Milk filter type				0.07
Cloth sock in line	42	41.7	(28.2–63.1)	
Plastic filter in line	10	20.4	(9.1–45.7)	
Cloth sock at the milking outlet	8	87.1	(34.7–213.8)	
Frequency of fresh grass allocation				0.02
Every 12 h	45	30.9	(20.9–44.7)	
>12 h	17	70.8	(38.9–131.8)	
Type of bulk tank				0.19
Direct expansion	53	41.7	(29.5–60.3)	
Ice bank	9	22.9	(9.5–53.7)	
Cows housed 14 days prior to the visit				<0.01
Yes	13	104.7	(53.7–199.5)	
No	48	30.9	(21.9–43.7)	
Silage fed 14 days prior to the visit				0.05
Yes	25	58.9	(35.5–97.7)	
No	36	30.2	(20.0–45.7)	
Detergent stain left in plant between milkings				0.23
Yes	27	49.0	(29.5–79.4)	
No	35	32.4	(20.9–50.1)	
Detergent used more than once				0.10
Yes	21	56.2	(32.4–100.0)	
No	41	31.6	(21.4–47.9)	

(Table 2. Continued)

Variable	Number of farms	Coefficient		P-value
Chloras added to the cleaning solution wash				0.08
Yes	11	74.1	(33.9–162.2)	
No	52	34.7	(24.0–50.1)	
Water tested for bacteriology in the last 3 years				0.11
Yes	21	56.2	(31.6–100.0)	
No	40	31.6	(20.9–47.9)	
Teats dry wiped prior to unit application				0.04
Yes	4	11.0	(3.1–39.8)	
No	59	42.7	(30.9–60.3)	
Teats washed prior to unit application				0.11
Yes	18	60.3	(33.1–112.2)	
No	45	33.1	(22.9–49.0)	
Teats pre dipped prior to unit application				0.17
Yes	9	69.2	(28.8–166.0)	
No	54	36.3	(25.1–51.3)	
Teats dry at unit application				0.23
Yes	43	34.7	(23.4–51.3)	
No	20	52.5	(29.5–95.5)	

Table 3. Least squares mean *Bacillus cereus* count (Log_{10} cfu/mL) for all explanatory variables that remained in the final multivariate model.

Variable	Number of farms	*Bacillus cereus* count (Confidence Interval)		P-value
Cows housed 14 days prior to the visit				<0.01
Yes	13	209.9	(107.2–407.4)	
No	48	50.1	(33.1–74.1)	
Frequency of fresh grass allocation				<0.01
Every 12 h	45	61.6	(38.5–97.7)	
>12 h	17	166.0	(91.2–309.0)	
Water tested for bacteriology – last 3 years				0.02
Yes	21	144.5	(81.3–257.0)	
No	40	70.8	(45.7–112.2)	

increase in *B. cereus* count ($P < 0.05$). The 2-way interactions among the variables in the final model were not significant. The variable herd size and technician were forced into the final model however both were not significant. Some slightly larger residuals were observed, yet all Cook's distance values were <1, thus no observations were considered influential (Cook and Weisberg 1982).

Discussion

The objective of this study was to identify farm management factors associated with the *B. cereus* counts in BTM. Due to its ability to withstand pasteurisation and be successfully isolated from IMF it is necessary for farmers and processors alike to minimise the levels of *B. cereus* entering milk at farm level. This study involved evaluation of a large number of management variables considered to influence the *B. cereus* count in milk and were mainly related to the cow's environment, milking practices and equipment sanitation.

Strengths and limitations

The subjective nature of observations and questionnaires can often lead to misclassification of explanatory variables. To limit this, only closed categorical responses were allowed. Additionally, efforts to reduce the potential bias of technician were made prior to the study period where both technicians had to calibrate hygiene scoring on two non-study farms. As a result, the inclusion of technician into the final model had little effect on variable estimates. Additionally, herd size was considered as a potential confounder as management practices can differ due to scale (Rodrigues, Caraviello and Ruegg 2005). Therefore, this variable was forced into the final model, yet it was not significant.

Technicians and farmers were unaware of the bulk tank *B. cereus* count at the time of the visit. Furthermore, while the farmers were aware that milk samples taken were being tested for bacteriology they were unaware of what type of bacteria the samples were being tested for and thus manipulation of management practices that specifically reduce the *B. cereus* count in milk was not possible.

Due to the limited number of herds included in the study (n = 63), only variables that were strongly associated with *B. cereus* count could be evaluated in the final model. The absence of a particular variable from the final model may be due to the limited sample size. The exclusion of the 'detergent wash start temperature' variable in the final model due to the lack of observations was an example of this. In a previous study on the influence of farm management factors on bacterial counts (Elmoslemany *et al.* 2009), it was found that high temperature washes were associated with lower thermoduric counts. Thus, while it could not be included in the final model in this study a statistical tendency was seen in the unconditional association and thus high temperature detergent washes could be recommended to lower *B. cereus* counts.

Additionally due to the small sample size, the effect of some variables could not be evaluated due to a lack of variation among farms. For example, no variation was seen in udder and leg hygiene scores among *B. cereus* counts. This could be due, in part, to the fact that most cows were out on pasture during the study period and thus had very similar environments despite varying management practices. When comparing conventional and organic production systems, Ellis *et al.* (2007) concluded that farming system had no effect on cow cleanliness when cows were on pasture. However, when cows were housed, organic cows tended to be cleaner. This suggests that herd variability is more apparent when cows are housed compared to when at pasture.

Unconditional associations

The unconditional associations of most explanatory variables with the geometric mean *B. cereus* count did not enter the final model. However, some associations were biologically plausible and can be managed by farmers. These included reuse of the detergent solution more than once, feeding silage and 'dry wiping teats' prior to unit application.

B. cereus cells and spores are very adhesive to stainless steel due to their hydrophobic nature (Lindsay, Brozel and von Holy 2006; Peng, Tsai and Chou 2001). Within the milking machine, the bacteria attach to the steel surface, multiply and embed themselves in a slimy matrix composed of extracellular polymeric substances that they produce, forming a biofilm (Simões, Simões and Vieira 2010). Formation of a biofilm results in greater resistance of organisms to heat and sanitation (Frank and Koffi 1990) and acts as a potential chronic source of microbial contamination (Barnes *et al.* 1999). Caustic detergents suspend and dissolve biofilms from machine surfaces (Simões *et al.* 2010). However, when comparing wash solutions for their efficacy of removing *B. cereus* from stainless steel surfaces (*in vitro*, yet to mimic machine washing) Salustiano *et al.* (2010) found that the two main factors affecting the efficacy of removal were the concentration of the alkaline detergent and the temperature of the wash solution. Reusing detergents more than once is not recommended due to reduced cleaning efficacy of the working solution (Gleeson and O'Brien 2010). Thus, farmers that reused detergent more than once tended to have a higher *B. cereus* count in BTM.

Feeding silage contaminated with *B. cereus* spores has been previously associated with the *B. cereus* spore count BTM (transmission via contaminated faeces) (Magnusson *et al.* 2007; Vissers *et al.* 2007). During the study period (July and August 2012), greater than average amounts of rainfall were recorded at the Moorepark weather station (1.5 times of the typical rainfall; 94.8 mm was recorded for July and over double the amount of rain recorded for August; 175 mm; Met Éireann 2012). As a result, some farmers were forced to feed silage, as access to paddocks was not possible and/or grass growth had deteriorated due to very wet weather conditions. In many cases, cows were fed silage that was harvested in wet conditions and was possibly contaminated with soil, in which *B. cereus* is abundant (Christiansson 2011). Feeding silage to dairy cows with minimum soil contamination is critical to minimise transmission of *B. cereus* via faeces into BTM.

No uniform teat preparation practice was seen on the farms. While some farmers did not carry out any teat preparation prior to unit application, others partially prepared teats. The practice of dry wiping teats to remove debris prior to unit application was unconditionally associated with lowering the *B. cereus* count in BTM. As *B. cereus* is abundant in soil, removing dry soil and dirt from contaminated teats has the potential to lower the *B. cereus* count in BTM.

Conditional associations

The final multivariable model had three significant predictors, frequency of fresh grass allocation, cows housed and the frequency of water testing. The frequency of fresh grass allocation and the housing of cows directly relate to the cows environment.

As previously mentioned, excessive amounts of rain fell during the study. As a result, some farms were forced to carry out on-off grazing to protect pastures. Ellis *et al.* (2007) found that cows became dirtier in the transition from summer grazing to winter housing due to greater confinement and restriction in the cow's choice of lying area in housed systems. Similarly, Goldberg *et al.* (1992) found that milk from intensive grazing farming systems had lower TBC counts than milk from herds using confined housing. When cows are out grazing, contamination of teats with soil is the main route of contamination of BTM with *B. cereus*. The degree of contamination on teats is influenced by weather conditions, where greater spore counts are isolated when the dry matter content of soil and evapotranspiration are low (Christiansson *et al.* 1999). Therefore, in this study, the transition of cows back and forth from wet pasture to confinement would increase the likelihood of contamination and subsequent multiplication of *B. cereus* on teat surfaces. These findings disagree with the findings of Slaughis *et al.* (1997) who found that when cows were housed during the summer *B. cereus* spore counts in BTM were lower compared to keeping cows on pasture. Different management practices and the above average rainfall experienced during this study could account for these conflicting findings.

During periods of increased rainfall there is an increased risk of severe poaching of paddocks (Tuñon *et al.* 2013) and, therefore, greater risk of soil contamination of teats. In this study, allocating fresh grass less frequently was associated with increased *B. cereus* counts in BTM. Allocating fresh grass less often will increase the risk of soil exposure due to vulnerable soil conditions and increased treading of cows back and forth over the same paddock for water and to the milking parlour. Increased levels of exposed wet soil in paddocks will increase the risk of soil contamination of teats and thus increase the risk of *B. cereus* transmission in BTM. Adequate teat preparation, especially during periods of heavy rainfall to combat decreased cow hygiene (Sant'Anna and Paranhos da Costa 2011) is necessary to minimise bacterial contamination in milk.

The importance of water quality for cleaning efficiency and milk quality cannot be underestimated. Farms that had carried out a water test for bacteriology in the last 3 years had a higher geometric mean *B. cereus* count. Of the 21 farms that tested the water for bacteriology, 15 had a private well water supply. Microbial pollution of wells in Ireland is caused mainly by wastes from farmyards (silage effluent, soiled water) and septic tank effluent (Daly 2000), both of which can be a source of *B. cereus* (Bottone 2010). Farms linked to the public water supply and group schemes receive water that is regularly monitored and treated and so is less likely to be contaminated with *B. cereus*. However, private wells can be more vulnerable to contamination where the responsibility of monitoring the water lies with the owner (FSAI 2006). Thus, a possible explanation for this finding is that farmers only tested water in response to water quality issues experienced.

Conclusion

Farm management factors associated with counts of *B. cereus* in BTM relevant to dairy producers in Ireland were identified. The frequency of fresh grass allocation was negatively associated with the *B. cereus* count in BTM while housing cows and water testing were positively associated with the *B. cereus* count in BTM. 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 BTM.

Acknowledgements

Special thanks to Dairygold for their participation and cooperation in the study. Sincere gratitude is due to the farmers, Frank O'Flynn for identification of farms and Marianne O'Donnell for laboratory assistance. Aine O'Connell was in receipt of a Teagasc Walsh Fellowship.

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Received 2 October 2013