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Predictive Modeling of *Bacillus cereus* Spores in Farm Tank Milk During Grazing and Housing Periods

M. M. M. Vissers,*[1] M. C. Te Giffel,* F. Driehuis,* P. De Jong,† and J. M. G. Lankveld‡
*Department of Health and Safety, and
†Department of Processing, NIZO Food Research, PO Box 20, 6710 BA Ede, the Netherlands
‡Department of Dairy Science, Wageningen University and Research Centre, PO Box 8129, 6700 EV Wageningen, the Netherlands

ABSTRACT

The shelf life of pasteurized dairy products depends partly on the concentration of *Bacillus cereus* spores in raw milk. Based on a translation of contamination pathways into chains of unit-operations, 2 simulation models were developed to quantitatively identify factors that have the greatest effect on the spore concentration in milk. In addition, the models can be used to determine the reduction in concentration that could be achieved via measures at the farm level. One model predicts the concentration when soil is the source of spores, most relevant during grazing of cows. The other model predicts the concentration when feed is the main source of spores, most relevant during housing of cows. It was estimated that when teats are contaminated with soil, 33% of the farm tank milk (FTM) contains more than 3 $\log_{10}$ spores/L of milk. When feed is the main source, this is only 2%. Based on the predicted spore concentrations in FTM, we calculated that the average spore concentration in raw milk stored at the dairy processor during the grazing period is 3.5 $\log_{10}$ spores/L of milk and during the housing period is 2.1 $\log_{10}$ spores/L. It was estimated that during the grazing period a 99% reduction could be achieved if all farms minimize the soil contamination of teats and teat cleaning is optimized. During housing, reduction of the concentration by 60% should be feasible by ensuring spore concentrations in feed below 3 $\log_{10}$ spores/g and a pH of the ration offered to the cows below 5. Implementation of these measures at the farm level ensures that the concentration of *B. cereus* spores in raw milk never exceeds 3 $\log_{10}$ spores/L.

Key words: *Bacillus cereus*, farm management, raw milk, modeling

INTRODUCTION

Growth of *Bacillus cereus* often limits the shelf life of pasteurized dairy products kept at refrigeration temperatures. *Bacillus cereus* in dairy products originates, at least partly, from the farm environment. The spore-forming properties of *B. cereus* enable the organism to survive pasteurization processes. In addition, recontamination during milk processing via (improperly cleaned) pasteurization equipment and during filling of the product can occur (Te Giffel et al., 1996; Lin et al., 1998; Svensson et al., 2000).

To prevent spoilage of pasteurized dairy products, *B. cereus* should be controlled by a chain management approach. It is important to reduce the concentration of spores of *B. cereus* in raw milk at the farm level or by bactofugation and to prevent recontamination and growth of *B. cereus* during processing. Of these aspects, least is known about the measures needed at the farm level to achieve a significant reduction and the quantitative effects of potential control measures at the farm level.

Raw milk in the silo tank at the site of dairy processors is a collection of a large number of farm tank milk (**FTM**) deliveries (up to 50 in the Netherlands). The concentration of *B. cereus* spores in the raw milk processed is the weighted average of the spore concentrations in the different FTM deliveries. Concentrations of *B. cereus* spores in FTM range from <2 $\log_{10}$ to 4.3 $\log_{10}$ spores/L. The highest concentrations occur at the end of summer and in early autumn (Te Giffel et al., 1995; Slaghuis et al., 1997). Raw milk before processing should always contain less than 3 $\log_{10}$ spores/L (Walstra et al., 2005).

Farm tank milk is contaminated with spores of *B. cereus* via the exterior of the cow's teats and through improperly cleaned milking equipment (Griffiths and Phillips, 1990; Saran, 1995). A further increase in spore concentration could occur due to growth and sporulation of *B. cereus* during storage of the milk in the farm tank. Contamination via the exterior of teats occurs when teats are contaminated with dirt. During the grazing period, dirt attached to the teats mainly consists of soil. During housing, attached dirt predominantly consists of feces and bedding material (Christiansson et al., 1999). Dirt attached to the exterior of teats rinses off during milking. Subsequently, spores

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[1]Corresponding author: marc.vissers@nizo.nl

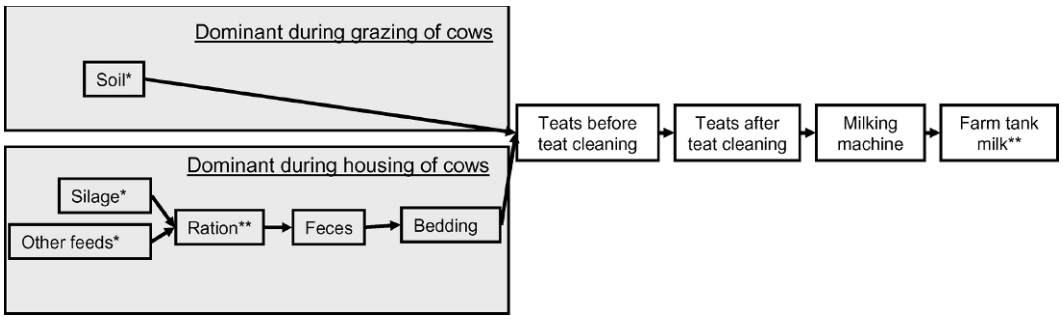

Figure 1. Contamination pathways for *Bacillus cereus* spores in farm tank milk; * = source of contamination, ** = growth can occur within this carrier.

present in rinsed-off dirt contaminate the milk. The concentration of *B. cereus* spores transmitted to milk depends on the amount of dirt rinsed off the teats and the spore concentration in attached dirt.

Various measures, ranging from providing feeds of high (microbial) quality to thorough cleaning of teats before milking, potentially reduce the concentration of *B. cereus* spores in FTM. For efficient farm management it is important to identify the most effective measures to reduce the concentration of *B. cereus* spores in FTM. Based on the contamination pathway, Vissers et al. (2006) developed a simulation model to identify a strategy to control the concentration of butyric acid bacteria spores in FTM. The model contained interpretable and measurable variables and accounted for effects of uncontrollable variables. A control strategy was defined using Monte Carlo simulations.

In this study, a similar approach was applied to the contamination of FTM with *B. cereus* spores. The objectives of this study were to identify quantitatively factors that affect the concentration of *B. cereus* spores in FTM and to determine the reduction of the concentration that can be achieved via measures at the farm level. Based on this information a control strategy can be defined.

MATERIALS AND METHODS

Model Development

Model Assumptions. Based on the 2 contamination pathways (Figure 1), 2 simulation models were developed following the approach of Vissers et al. (2006). In the first model, soil was the only source of *B. cereus* and teats were only contaminated with soil (the soil-based model). During the grazing period teats are predominantly contaminated with soil (Christiansson et al., 1999) and therefore the soil-based model mostly corresponds to the contamination process during the grazing period. In the second model, feed is the only source and teats are solely contaminated with bedding (feed-based model). The feed-based model reflects the housing period. The following assumptions apply to the models developed: 1) Growth of *B. cereus* is possible in the silage-based ration provided to cows and in milk stored in the farm tank. Based on data of Te Giffel et al. (1995) and Slaghuis et al. (1997), no growth in bedding material was assumed; 2) When *B. cereus* can grow in the ration offered to the cows and in FTM the spore concentration increases at the same rate as the vegetative cells; 3) Lag time is assumed to be equal to 1 divided by the growth rate (μ). This is a common assumption in predictive microbiology (Zwietering et al., 1996); 5) The maximum attainable spore concentration in the feed ration ($C_{ration,\infty}$, see Table 1) is assumed to be 5 $\log_{10}$/g. This value is slightly above the maximum concentration of *B. cereus* spores measured in animal feeds (Te Giffel, et al., 1995; Slaghuis et al., 1997; Christiansson et al., 1999); 6) The composition of the silage-based ration provided to cows is constant for the period of 6 milkings and is refreshed at constant intervals. Residence time in the gastrointestinal tract can be neglected when the composition of the ration is constant; 7) The cows are milked twice a day at a constant interval of 12 h; 8) The milking equipment is cleaned properly and no contamination of the milk via the milking equipment occurs; 9) The concentration of *B. cereus* spores in the milk entering the farm tank [$C_{milking}$ (spores/L)] is equal for all milkings collected in the farm tank; and 10) FTM is collected after 6 milkings. This is common practice in the Netherlands.

Model Structure. To have a measure of the hygiene status of pasture, cattle housing, and milking parlor, the herd is divided into 3 cow groups (slightly, moderately, and highly contaminated cows). The proportions of slightly, moderately, and highly contaminated cows represent the hygiene status. First, the concentration of *B. cereus* spores in the milk of each cow group [$C_{cowgroup\_y}$ (spores/L)] is calculated using the equations in Table