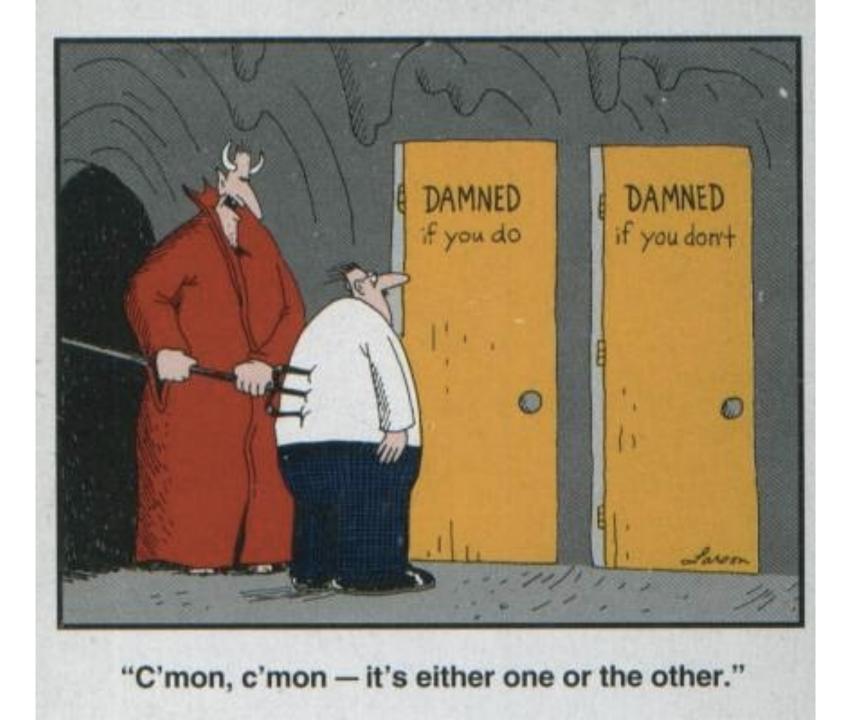
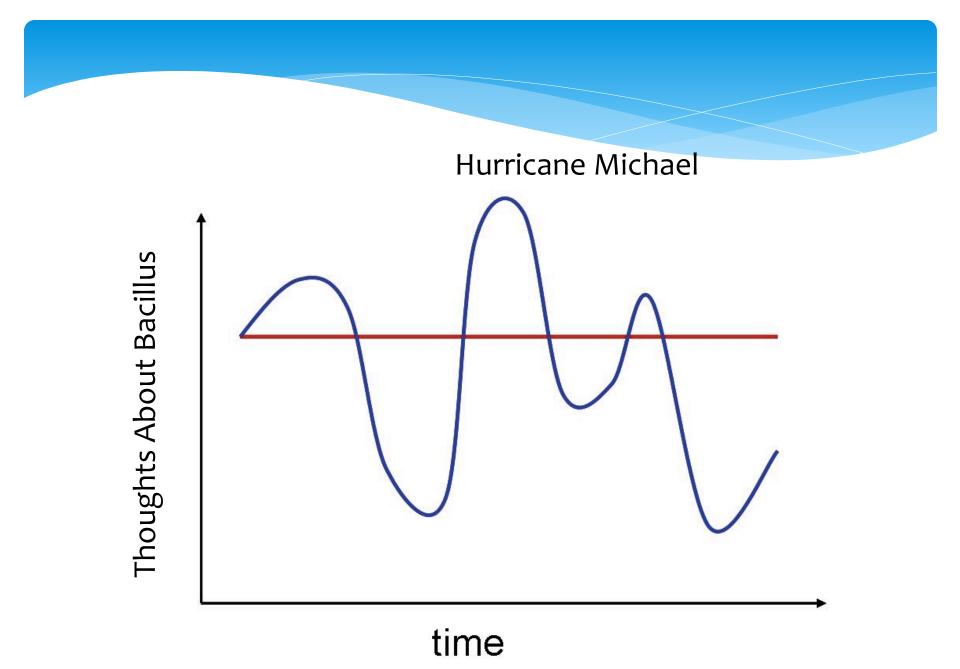


# **Bacillus Detection Project**

Michelle G. Brenner, MD, IBCLC Medical Director The King's Daughters Milk Bank Norfolk, VA





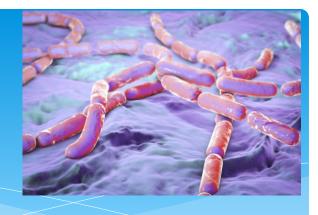


### What is Bacillus?

- \* Gram-positive rod
- \* Widely distributed in nature: soil, dust & vegetation
- \* B. cereus cells & spores are in every raw agricultural commodity, e.g. herbs, spices, vegetables, milk, meat etc.
- \* However, B. cereus forms spores to ensure its survival through all stages of food processing allowing it to multiply

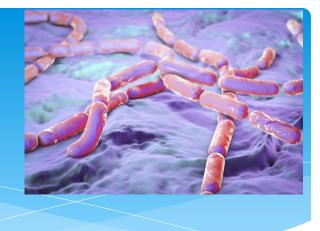






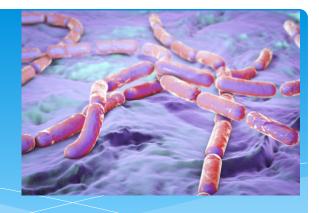
- Spores are capable of overcoming extreme conditions: drought, freeze, heat, disinfectants, desiccation, ionization, radiation, UV Light
- \* Can be airborne and remain as surface contaminants in labs
- Spores are like plant seeds because they "sprout" under the right conditions

#### A Toxin-Producer



- \* 5 different enterotoxins
- \* 1 emetic toxin
- Extensively reported in starchy foods: vegetables, puddings, sauces, dairy, cereals, infant cereals, fried and cooked rice
- \* B. cereus typically found in infant formula have fewer enterotoxin genes

## It's Everywhere!



\* B. cereus infections are often seasonal, with a peak in summer, corresponding to high ambient temperatures

#### \* Found all over hospitals:

- Respiratory equipment, ventilation bags
- Air conditioning systems
- \* Linen from laundry services outside the hospital
- \* TPN
- \* Caregivers----spores are resistant to hand sanitizers



- \* 100% of 205 samples were contamination by aerobic spore formers
- \* Milk based infant food with fruit, vegetables, honey, rice and infant milk powder.....

Toxicology Reports Microbiological evaluation of infant foods quality and molecular detection of Bacillus cereus toxins relating genes Sadek et al. 2018



WEBMD HEALTH NEWS

#### Recall: Baby Cough Syrup for Deadly Bacteria Risk

By Aaron Gould Sheinin

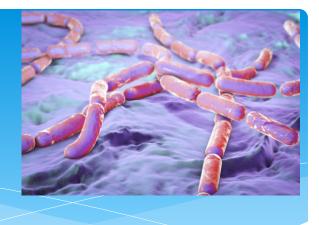




- \* B. cereus spores in raw (cow's) milk were the major source of B. cereus in pasteurized milk
- Post-pasteurization contamination was a minor source of B. cereus in pasteurized milk

Lina S, Schrafta H, Odumerub JA, GriffithsA MW (1998) Identification of contamination sources of Bacillus cereus in pasteurized milk. Int J Food Microbiol 43:159–117

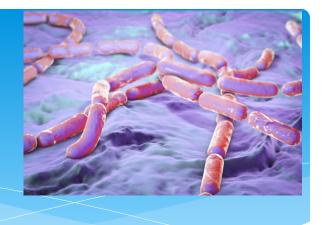
#### Pasteurization kills...



\* Pasteurization kills the **vegetative** cells

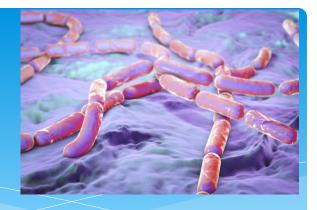
 But..... provides a suitable environment for the subsequent germination and growth of Bacillus spores (Andersson and others 1995).

### Bacillus & Illness



- Besides food poisoning , B. cereus induces local and systemic infections
- \* Severe infections in immunocompromised individuals
  - Preterm infants: bacteremia, pneumonia, meningoencephalitis, hemorrhagic brain lesions, endocarditis, and endophthalmitis

#### How common is it?



- \* Case reports of individual cases or single hospital centers
- \* 2008-2012: France, 9 hospitals, reported 39 cases.
  No studies demonstrated the same B. cereus strain could be recovered from both the patient and the hospital environment.
- \* 2016: 3 NICU cases in France: no link between the strains isolated from the infants and the 1 batch of donor milk that had sub-threshold levels of Bacillus



#### Research Article

#### Banked Human Milk and Quantitative Risk Assessment of Bacillus cereus Infection in Premature Infants: A Simulation Study

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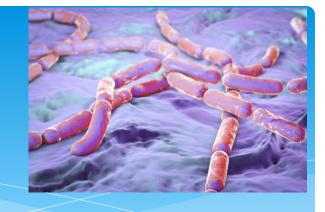
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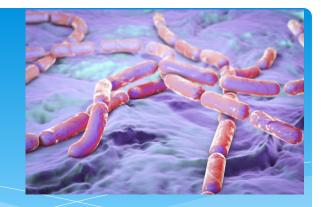
*Background*. Banked human milk (BHM) offers potential health benefits to premature babies. BHM is pasteurized to mitigate infectious risks, but pasteurization is ineffective against sporulating bacteria such as *Bacillus cereus*. Sepsis related to *Bacillus cereus* in premature infants is severe and can often be fatal. Even if a causal link has never been established, BHM has been suggested as a potential source of infection in premature infants. *Objective*. Our aim was to estimate the potential risk of *Bacillus cereus* infection in preterm infants caused by the ingestion of contaminated pasteurized BHM using different post-pasteurization release criteria (i.e., 9 sampling of 100 microliters versus the HMBANA guideline of 1 sampling of 100 microliters per pool). *Methods*. In the absence of scientific evidence regarding the risk of *Bacillus cereus* infection by the ingestion of BHM in premature infants, risk assessment using Monte Carlo simulation with the exponential dose-response model was performed. Three scenarios of infectious risk (annual incidence rate of 0.01%, 0.13%, and 0.2%) with 18 variations of the *B. cereus* virulent dose (from 0.5 CFU/ml to 200 CFU/ml) were simulated. *Results*. The mean risk differential between the two methods of post-pasteurization bacteriological control for realistic infectious doses of 30 to 200 CFU/ml ranges from 0.036 to 0.0054, 0.47 to 0.070, and 0.72 to 0.11 per million servings, for each of the three scenarios. *Conclusion*. Simulation highlights the very small risk of *Bacillus cereus* infection following the ingestion of pasteurized BHM, even in the worst case scenarios, and suggests that a 100-microliter sample for post-pasteurization culture is sufficient.



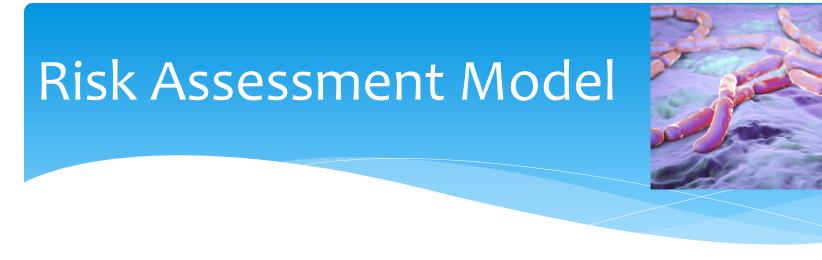
"To our knowledge, the risk is purely theoretical since there has never been a single case of Bacillus cereus infection in a preterm infant proven to be caused by BHM ingestion."

> Canadian Journal of Infectious Diseases and Medical Microbiology 2019 Lewin et al. Banked Human Milk and Quantitative Risk Assessment of Bacillus cereus Infection in Premature Infants: A Simulation Study

#### Methods



- Risk assessment Monte Carlo simulation with an exponential dose-response model
- Three scenarios of infectious risk (annual incidence rate of 0.01%, 0.13%, and 0.2%) with 18 variations of the B. cereus virulent dose (from 0.5 CFU/ml to 200 CFU/ml) were simulated.
- \* The mean risk differential between the two methods of postpasteurization bacteriological control for realistic infectious doses of 30 to 200 CFU/ml ranges from 0.036 to 0.0054, 0.47 to 0.070, and 0.72 to 0.11 per million servings, for each of the three scenarios.

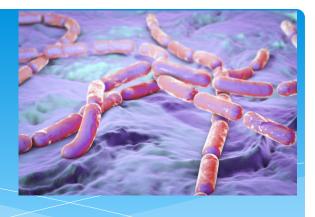


"Our simulation results suggest that, if it exists at all, the risk of Bacillus cereus infection following the ingestion of pasteurized banked milk is extremely small."



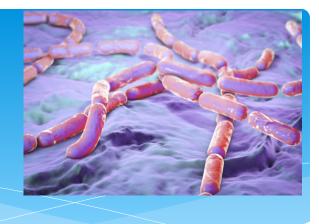
"Our analysis also shows that even in worst-case scenarios, a 100-microliter sample for postpasteurization culture is amply sufficient to mitigate this risk; a larger sampling volume would only lead to a higher rate of disqualification for this important health-care resource, without having any significant positive impact on safety. "

### **Theories on Bacillus**



- \* High volume donors
- \* Blocked duct donors
- \* Summer weather conditions
- \* Poor pump hygiene
- \* Bra/Clothing
- \* Milk storage issues

# Selective Chromogenic Medium



- \* Detection and enumeration of B. cereus
  - \* No growth of background flora
  - \* Results in 24 hours
- \* Ideas to test:
  - \* New donors prior to pooling
  - Pre-pasteurization & post-pasteurization to estimate risk
  - \* Donor pump kit parts

