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## Uncorrected silo management increases the risk of contamination of the milk production chain with *Clostridium* spp. and *Paenibacillus* spp.

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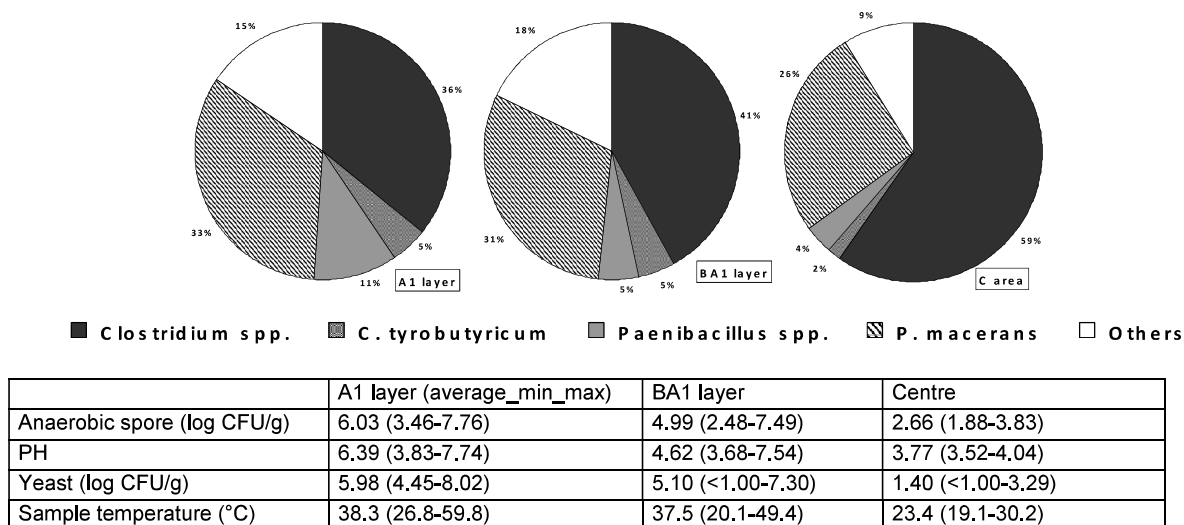
**Keywords:** anaerobic sporeformer, aerobic deterioration, maize silage, milk, TMR

**Introduction** The occurrence of *Paenibacillus* and *Clostridium* spores in silage is of great concern for dairy producers, because their spores can survive milk and cheese processing, and some strains are capable of subsequently germinating and causing damage to conserved milk and dairy products (te Giffel et al. 2002). Anaerobic spore-formers can multiply in silage (Borreani et al. 2013), and, at critical levels of contamination, their control becomes very difficult during the process of making hard cheeses (Vissers et al. 2007). The objectives of this study were to verify the role of the aerobic deterioration of maize silage on the proliferation of *Paenibacillus* and *Clostridium* spp. spores, and to evaluate the efficacy of different management strategies on reducing aerobic deterioration and on mitigating these undesirable bacterial outbreaks.

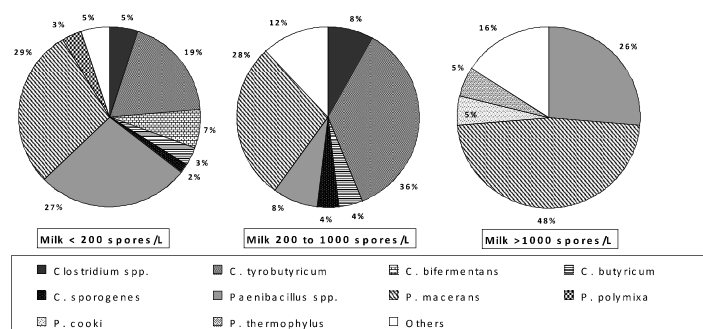
**Material and Methods** A survey was carried out, over a 2 year period, on 15 dairy farms in northern Italy, which produce milk destined for the production of Grana Padano cheese. A questionnaire, containing information on the silo management practices, the amount of silage consumed daily, the filling and packing methods, the silo cover, the typology of weigh down the silo cover and the feeding rates, was filled in on each farm. Samples of maize silage, total mixed ration (TMR), faeces (mixed sample of 5 milking cows), bedding, water and bulk tank milk were collected on each farm. Three silage sampling areas were chosen in each silo: centre (C), and two peripheral areas, close to the sealant film (A1, first 15 cm—visibly spoiled) and below A1 (BA1, 15 to 30 cm immediately below the A1 layer). Each silage and TMR sample was analysed to establish their chemical, microbial and fermentative characteristics. Faeces, milk and water were also collected and analysed for anaerobic spore-former enumeration. Anaerobic spores were counted after pasteurisation of the serial dilutions at 80°C for 10', and this was followed by the streak plate technique on RCM agar, 0.005% neutral red, and 200 ppm of d-cycloserine, incubated anaerobically for 7 d at 35°C. Five to ten bacterial colonies per sample, representative of distinct morphologies, were chosen for isolation and identification. Bacterial identification was performed by sequencing the V1-V3 region of the 16S rRNA gene, according to Borreani et al. (2013).

**Results and Discussion** Spoiled maize silage was confirmed to be a source of anaerobic spores, with *Paenibacillus* and *Clostridium* being present at higher levels than 5 log cfu/g of silage in more than 80% of the samples (Figure 1). *Paenibacillus* (4 species) and *Clostridium* (10 species) represented 29% and 61% of all the maize silage spores, respectively. The frequently dominant clostridia species were: *C. tyrobutyricum*, *C. aerotolerans*, *C. aminovalericum*, *C. amygdalium*, *C. celerecrescens*, *C. jejuense*, *C. sporogenes*, *C. xylanoliticum*; while the *Paenibacillus* species were *P. macerans*, *P. thermophylus*, and *P. cookii*. The zones immediately below the visible spoiled silage (BA1), even though no evident spoiling was visible, also showed spore content levels of up to 7.49 log colony-forming units (cfu)/g. This aspect determines the potential capability of this masked spoiled area to become the main source of spores for dairy cow TMR. The TMR spore counts were as follows: 30% of samples below 3 log cfu/g (47% *Clostridium* spp., 11% *C. tyrobutyricum*, 13% *P. thermophylus*, 3% *P. macerans*); 50% from 3.00 to 4.99 log cfu/g (51% *Clostridium* spp., 9% *C. tyrobutyricum*, 9% *P. thermophylus*, 4% *P. macerans*); and 20% greater than 5 log cfu/g (46% *Clostridium* spp., 3% *C. tyrobutyricum*, 13% *P. thermophylus*, 3% *P. macerans*). The dominant anaerobic sporeformers of the bulk milk are reported in Figure 2. Among the spore-forming species, clostridia represented 36, 52 and 0% in the milk samples with <200, from 200 to 1000 and >1000 spores/L, respectively; with 5 species frequently identified: *C. beijerinckii*, *C. bifermentans*, *C. butyricum*, *C. sporogenes*, *C. tyrobutyricum*, and *C. aminovalerianicum*. The *Paenibacillus* species

represented 59, 36 and 84% in the milk with <200, from 200 to 1000 and >1000 spores/L, respectively; *P. macerans*, *P. cooki*, *P. thermophilus*, and *P. polymixa* were the most frequently identified species.



**Figure 1.** Distribution of the anaerobic spore-forming bacteria isolates in different areas of the maize silage, mean spore and yeast counts, pH and temperature of the sampling area of 49 commercial farms in northern Italy.



**Figure 2.** Distribution of the anaerobic spore-forming bacteria in the bulk milk of 49 commercial farms in northern Italy.

**Conclusion** The adoption of good silage management practices that are able to reduce spoiled zones in a silo could greatly decrease the risk of spore contamination with *Clostridium* spp. and *Paenibacillus* spp. in the successive phases of the milk production chain.

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