http://dx.doi.org/10.1590/1678-4162-12798

Arq. Bras. Med. Vet. Zootec., v.75, n.1, p.14-26, 2023

Epidemiology of mastitis and interactions of environmental factors on udder health in the compost barn system

[*Epidemiologia da mastite e interações de fatores ambientais na saúde do úbere no sistema de Compost Barn*]

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ABSTRACT

This study aimed to describe the epidemiological indexes of mastitis, milk quality and udder hygiene in the *Compost Barn* system, as well as to search for associations between isolated pathogens from milk with compost characteristics. Three dairy herds participated in the study, and the samples were collected during different periods on each farm. Individual milk samples were collected in duplicate for SCC analysis and microbiological culture. Environmental pathogens caused most cases of clinical mastitis on farm 2, and contagious pathogens caused the most cases on farm 1. Bed moisture was not associated with the incidence of environmental pathogens. Most of the animals remained in good udder hygiene during the study. Poor udder hygiene contributed to the increased incidence of environmental pathogens in one of the farms. A higher number of animals with a hygiene score of ≥ 2 were observed during the warmer and rainfall periods. There was no association between hygiene scores and somatic cell counts. The results suggest that pathogens isolated from milk in animals confined in *Compost Barn* under tropical climate are like other confinement systems adopted elsewhere. The year period influenced the udder hygiene score, reinforcing the importance of bed management throughout the year.

Keywords: mastitis, composting, milk quality

RESUMO

O objetivo deste estudo foi descrever os índices epidemiológicos da mastite, da qualidade do leite e da higiene do úbere em animais confinados no sistema Compost Barn, *bem como buscar associações entre patógenos isolados do leite e características do composto. Três rebanhos leiteiros participaram do estudo e as amostras foram coletadas em diferentes períodos em cada fazenda. Amostras individuais de leite foram coletadas em duplicata para análise de CCS e cultura microbiológica. Patógenos ambientais causaram a maioria dos casos de mastite clínica na fazenda 2, e na fazenda 1 a maioria dos casos foi em decorrência de patógenos contagiosos. Amostras de material da cama foram coletadas em duas fazendas para análise da densidade bacteriana e da umidade. A umidade da cama permaneceu dentro da faixa de controle durante o estudo e não foi associada à incidência de patógenos ambientais. A maioria dos animais permaneceu com boa higiene de úbere durante o estudo. A falta de higiene do úbere contribuiu para o aumento da incidência de patógenos ambientais em uma das fazendas. Durante o período mais quente e mais chuvoso, um número maior de animais com escore de sujidade ≥ 2 foi observado em todos os rebanhos. Não houve associação entre escore de sujidade e contagem de células somáticas em qualquer uma das três fazendas. Os resultados indicam que o perfil de patógenos isolados do leite em animais confinados em* Compost Barn *sob clima tropical é semelhante a outros sistemas de confinamento adotados em outros lugares. O período do ano influenciou o escore de higiene do úbere, o que reforça a importância do manejo da cama ao longo do ano.*

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Submitted: June 21, 2022. Accepted: October 25, 2022.

Palavras-chave: mastite, compostagem, qualidade do leite

INTRODUCTION

The compost bedded pack barn (CBP) is a dairy confinement system that emerged in the state of Virginia (USA). It is currently used in many countries worldwide. The barn is based on a covered area without partitions and consists of an organic bed usually formed by sawing or shaving, which through the incorporation of the feces and urine of the animals, produces an organic semi-compound, which can be stored for up to 24 months (Eckelkamp *et al*., 2016). The semi-compound requires periodic addition of bedding material and should be revolved two to three times a day, usually during milking, to promote aerobic composting (Janni *et al*., 2006; Petzen, 2009; Bewley *et al*., 2012). Efficient bed management provides a dry and comfortable surface for the animals (Barberg *et al*., 2007b; Black *et al*., 2013). To obtain efficient composting, bed temperature and humidity should be adequate. The internal temperature of the CBP in the depth of 15 to 30 cm should be between 44 to 65°C, and the humidity should vary between 40 to 60% (Bewley *et al*., 2012).

Beds of organic matter present a high concentration of environmental bacteria that cause mastitis compared to beds of inorganic materials (Hogan *et al*., 1989). The high concentration of environmental bacteria in bed is directly related to the incidence of clinical mastitis, and increased bacteria count at the end of the teats (Hogan and Smith, 1997; Zdanowicz *et al*., 2004). Schreiner and Ruegg (2003) observed that animals with poor udder hygiene (scores 3 and 4) were 1.5 times more likely to get pathogens isolated from milk samples than animals with good udder hygiene (scores 1 and 2).

A more recent study by Favero *et al.* (2015b) found a high concentration of total bacteria in the CBP bed (>8.0 $\log_{10}$ CFU/g). Environmental pathogens' high pressure of infection when there is no adequate bed management emphasizes the importance of hygiene procedures during milking (Hogan *et al.*, 1989).

Studies conducted to investigate the incidence of mastitis in CBP herds have shown contradictory results. Astiz *et al*. (2014) conducted a study with dry cows housed in CBP. They observed that at the beginning of lactation, the animals showed promising results as a reduction in the incidence of mastitis and a tendency to reduce SCC and increased milk production in the first 100 days postpartum. However and Svennesen (2013) demonstrated in her study that after the fifth experimental month, animals handled in the CBP system had an increase of 60,000 to 80,000 somatic cells/mL compared to animals housed in the freestall system.

Few studies were conducted in Brazil on the confinement of dairy cattle in the CBP system. The main objective of this longitudinal study was to evaluate the epidemiology of subclinical and clinical mastitis and milk quality and correlate bed characteristics with udder hygiene in animals confined in the CBP system.

MATERIALS AND METHODS

Three dairy herds in the state of Minas Gerais that implemented the CBP system were evaluated, two herds in the Southern region and one in the Southeast region of the state. The criteria for inclusion of the dairy herds were adopting the CBP system as the only type of confinement for lactating cows and commitment to the study protocols.

The dairy herds from the South of Minas Gerais were visited from October 2014 until February 2016 (farm 1) and February 2015 until February 2016 (farm 2). The study period at farm 3 was from May 2016 to February 2017.

The year was divided into two periods: from November to April (period 1 or raining period) and from May to October (period 2 or dry period). The data on total precipitation, relative humidity, and average temperature were obtained from the meteorological stations of the National Institute of Meteorology.

Farm 1 had 81±9 lactating Holstein cows, milked three times daily. The milking was done with mechanical milking in a concrete room with a spine system, and the milk was stored in a bulk tank milk (BTM). The total milk/day production was approximately 2,713kg, with an average production of 33.5kg per cow. The CBP had been installed eight months before and had a shed with an area of 80 x 12m (average of 11m^2

per cow) consisting of a sawdust bed tilled two times a day at the moment of milking with a deep cultivator and rototiller. The addition of new bedding (sawdust) was done periodically according to the veterinarian's evaluation. The barn had 11 fans in all extensions (longitudinal direction), and the access of the animals to the food and water fountains was done inside the shed. The hygiene routine during milking consisted of the accomplishment of pre-dipping, strip test (the first streams of milk examined during the preparation of the udder for milking), drying the teats with individual paper towels, and using 1% iodine solution on teats after milking (post dipping disinfection).

Farm 2 had 158±10 lactating Holstein cows, milked three times daily. The milking was done with mechanical milking in a spine system, and the milk was stored in a BTM. The total milk/day production was approximately 3,575kg, with an average production of 34.7kg per cow. The CBP had been implemented in the month that the study started and had a barn with 90 x 14m (average of $9m^2$ per cow) consisting of a sawdust bed. The bed was revolved twice a day with a deep cultivator and rototiller and the addition of a new bed periodically, according to the veterinarian's evaluation. The barn had 13 fans throughout its longitudinal extension, and the access of the animals to the food and water was done from inside the shed. The routine of milking hygiene consisted of pre-dipping, strip test, drying the teats with individual paper towels and using 1% iodine solution in post dipping disinfection.

Farm 3 had 147±12 lactating Holstein cows, milked twice daily in a spine system with mechanical milking. The total milk/day production was approximately 4,993kg, with an average production of 35.41kg per cow. The CBP was installed in September 2015, in substitution for freestall, and the sampling began eight months after its installation, with a barn of 28.5 x $90m^2$ (average of 14 m^2 per cow) consisting of a bed of sawdust. The bed was tilled twice a day with a deep cultivator and rototiller. The shed had fans disposed of longitudinally, and the access of the animals to the food and water was through the feed alley (concrete 3,5m x 90m), besides having a system of a sprinkling of water for cooling the animals. The hygiene of the milking routine consisted of the accomplishment of pre-dipping, cleaning of the teats with a chlorine solution, strip test, and use of iodine solution to 1 % post dipping disinfection.

During the study period, 13 visits to farm 1 were made, nine to farm 2, and five to farm 3.

Individual composite samples of milk from all lactating cows (individual mammary quarters) were collected, aseptically, in duplicate for subsequent somatic cell count (SCC) and microbiological culture. Samples were also collected from the bulk tank milk (BTM) to analyze SCC and total bacteria count (TBC).

The cases of clinical mastitis were identified based on the guidelines of Hogan *et al*. (1989). A clinical case of mastitis was recorded when milk abnormalities (flakes, clots, and color change) were observed during the dark bottom mug's strep test. A sample was collected and frozen before antibiotic therapy. The farmer registered these cases during the study period on each farm. The lag time for a new clinical case in the same mammary quarter was 15 days.

In the experimental period, the udder hygiene of all lactating cows was evaluated before milking. The evaluation was carried out according to Schreiner and Ruegg (2003) based on a 4-point scale, where 1 was considered clean (no dirt), 2 was considered as little dirty (2 to 10% dirtiness), 3 was considered moderately dirty (10 to 30% of dirtiness) and score 4 was considered very dirty (more than 30% of dirtiness). The evaluation was performed by a single researcher throughout the study period.

The individual samples of milk for microbiological culture were processed in the Laboratory of Milk Microbiology at Embrapa Dairy Cattle, according to the recommendations of the National Mastitis Council (Hogan et al., 1999). Intramammary infections were identified by the presence of three or more colonies of the same type in microbiological culture, except for *S. aureus*, where only one colony was required. Growth considered insignificant (less than three colonies) was considered negative. The growth of three or more different colonies was considered as sample contamination.

The analysis for SCC and TBC was carried out at the Laboratory of Milk Quality at Embrapa Dairy Cattle. Flow cytometry was used using Bentley 75 Somacount 300 electronic equipment, with determination in cells/mL or cells/mL^{-1}, according to the International Dairy Federation (IDF, 2006).

The collection of bed samples was carried out on farms 1 and 2. Ten collections were carried out at farm 1, and five collections were carried out at farm 2. At farm 3 it was not possible to collect bed samples due to financial constraints. The bed samples were collected from the surface of the compost to about 5 cm depth in the first layer, 20 cm depth for the second stratum, and 30cm depth for the third. Each CBP was divided into 12 evenly distributed sections. The samples were transported in an isothermal box under refrigeration to the Federal University of Juiz de Fora and Embrapa Dairy Cattle laboratory.

For the determination of the microbial community, i.e., the number of bacteria, the samples were placed in sterile vials and taken to the laboratory. Two grams of sawdust from each of the sampling points were added to sterile 0.9% saline and taken to the ultrasonic bath for one minute. Then, these samples were serially diluted in 0.9% sterile saline solution and 100μL of each sample was seeded with tryptone soybean agar (TSA) plates. After 24 hours in a bacteriological oven at 35ºC, the colony-forming units (CFU) were counted with a stereoscopic microscope. The average bacterial density of samples collected in nine points of the CBP was made: feeding line, drinker line, and average line. These evaluations were carried out in the Laboratory of Ecology and Molecular Microbiology of Microorganisms of the Federal University of Juiz de Fora. Moisture analysis was performed at Embrapa Dairy Cattle at the Food Analysis Laboratory (LAA).

The SCC results, together with the microbiological culture results, were used to estimate the presence of intramammary infection, and the indicative limit of subclinical infection was ≥ 200,000 cells/mL. The incidence of clinical mastitis was determined by the number of new mastitis cases over a period of one month (milk alterations and positive microbiological culture results). The incidence rate of subclinical mastitis was determined as the number of new cases divided by the animal-time at risk (100/cows month) (Dohoo *et al*., 2010). The prevalence of subclinical mastitis was defined as the number of monthly cases divided by the number of animals at risk that month. The mastitis pathogens isolated during the study were also grouped into environmental (coliforms, environmental streptococci, *Prototheca spp*, *Trueperella pyogenes*, and *Pseudomonas spp*), contagious (*Staphylococcus aureus*, *Streptococcus agalactiae*, and *Corynebacterium spp*) and opportunistic (coagulase-negative Staphylococci) (Santos and Fonseca, 2007).

The Chi-square test (χ 2) for categorical variables was used to evaluate the associations.

RESULTS AND DISCUSSION

The results of this study present information on the epidemiology of mastitis and its consequences on the quality of milk in the CBP system in tropical conditions. Contagious pathogens were the most prevalent isolates of clinical mastitis on farm 1 (43.8%), of which all isolates were *S. agalactiae*. Environmental pathogens were the most frequent cases of isolation of clinical mastitis at farm 2 (71%), with *E. coli* being the most prevalent environmental pathogen (44.7%). Contagious pathogens contributed only 5.3% of total clinical cases. Coagulase-negative *Staphylococci* (CNS) pathogens were isolated in 15.6% and 5.3% of clinical cases for farms 1 and 2, respectively. There were no isolates of *Klebsiella pneumonia*. In Farm 3, only the number of clinical mastitis cases were retrieved; however, no milk samples were taken from farmers, which made it impossible to report microbiological culture results for this farm (Table 1).

Table 1. Results of microbiological culture (clinical mastitis) from isolates recovered from milk samples collected on farms 1, 2, and 3, located in Minas Gerais, Brazil

Etiological agent	Farm 1		Farm 2	
	N	%	N	%
Escherichia coli	-	-	17	44.7
Pseudomonas spp	-	-	1	2.5
Staphylococcus aureus	-	-	2	5.3
Coagulase-negative *Staphylococci*	5	15.6	2	5.3
Streptococcus agalactiae	14	43.8	-	-
Streptococcus spp	-	-	6	15.8
Streptococcus uberis	1	3.1	3	7.9
No growth	11	34.4	5	13.2
Contaminated	1	3.1	2	5.3
Total	32	100.0	38	100.0

*Farm 3 did not save the milk samples from clinical mastitis. N= number of isolates recovered from milk samples.

The farms participating in the study also presented a different pattern of clinical and subclinical mammary gland infection. In farm 1, a higher number of clinical and subclinical mastitis cases caused by contagious and opportunistic pathogens (33.5% and 29.0% of total cases, respectively) were observed. *Streptococcus agalactiae* was the most isolated pathogen, totalizing 31.6%, followed by coagulase-negative *Staphylococci* (CNS) with 29.0%. On farms 1 and 2 the highest number of cases was due to CNS, accounting for 30.5% and 30.0% of the cases, respectively. Environmental pathogens were isolated in 5.3%, 6.7%, and 16.8% of the total cases for farms 1, 2, and 3, respectively (Table 2).

Table 2. Results of microbiological culture (subclinical mastitis) from isolates recovered from milk samples collected on farms 1, 2, and 3, located in Minas Gerais, Brazil

Etiological agent	Farm 1		Farm 2		Farm 3	
	N	%	N	%	N	%
Corynebacterium sp.	2	0.2	33	4.1	45	16.6
Enterococcos sp.	7	0.7	5	0.6	1	0.4
Escherichia coli	6	0.6	2	0.2	1	0.4
Protheus sp.	1	0.1	-	-	-	-
Prototheca sp.	-	-	-	-	1	0.4
Pseudomonas sp.	-	-	-	-	1	0.4
Staphylococcus aureus	17	1.8	27	3.4	-	-
Coagulase-negative *Staphylococci*	287	30.0	245	30.5	51	18.7
Coagulase positive *Staphylococci*	15	1.6	17	2.1	-	-
Streptococcus agalactiae	312	32.6	-	-	1	0.4
Streptococcus equinus	10	1.0	4	0.5	7	2.6
Streptococcus sp.	18	1.9	32	4.0	7	2.6
Streptococcus uberis	11	1.1	11	1.4	23	8.5
Trueperella pyogenes	-	-	-	-	3	1.1
No growth	269	28.1	342	42.8	107	39.4
Contaminated	3	0.3	83	10.3	23	8.5
Total	958	100.0	802	100.0	271	100.0

N= number of isolates recovered from milk samples.

The distribution of pathogens isolated from clinical and subclinical mastitis cases was similar to those reported in Brazil and other countries with other production systems like free stall (Jobim *et al*., 2010; Oliveira; Ruegg, 2014; Favero *et al*., 2015a).

In agreement with the authors mentioned above, the absence of growth was a frequent result in the milk samples collected. In the present study, the proportion of subclinical (all three farms) and clinical cases (farms 1 and 2) were 36.8% and 23.8 %, respectively. The most isolated

pathogens in all cases of clinical mastitis in farms 1 and 2 were *S. agalactiae* with 43,8% and *E. coli* with 44,7% (Table 1).

The pathogens isolated from cases of subclinical mastitis were characterized by the high prevalence of *S. agalactiae* on farm 1 and CNS on farms 2 and 3. Farm 3 presented a strictly controlled management of contagious microorganisms. All fresh cows were submitted to three consecutive microbiological examinations, with an interval of one week, to detect *S. aureus* and *S. agalactiae*. Cows with positive tests were discarded from the herd. Currently, in properties where primary mastitis agents are controlled, opportunistic agents have become a concern as causes of intramammary infection in dairy cows (Pyörälä; Taponen, 2009).

In Brazilian herds, it is still common to find a high prevalence of contagious mastitis pathogens (Bueno *et al*., 2008; Favero *et al*., 2015a). Oliveira *et al*. (2013), in a study conducted in the states of Minas Gerais, found a high prevalence of *S. aureus* (90%) and *S. agalactiae* (40%) in the 112 dairy herds, which corroborates with the results found in this study. It is important to emphasize that this may be associated mainly with management deficiency during milking, which facilitates the dissemination of these microorganisms.

The mean prevalence and incidence of subclinical mastitis during the study period were 60.6% and 15.2% for farm 1, 31.1% and 13.6% for farm 2, and 39.2% and 13.8% for farm 3, respectively. The mean incidence of clinical mastitis was 5.2%, 2.4%, and 5.1% for farms 1, 2, and 3, respectively (Table 3).

Table 3. Descriptive statistics for mastitis epidemiologic indexes, bulk tank milk, and udder cleanliness scores on farms 1, 2, and 3

Variable	Farm 1					Farm 2					Farm 3				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Incidence of subclinical mastitis (%) [a]	12	15.2	4.0	10.2	22.2	8	13.6	6.5	6.7	26.2	4	13.8	6.1	5.7	20.2
Prevalence of subclinical mastitis (%) [b]	13	60.6	9.7	44.7	83.8	9	31.1	6.4	22.2	46.3	5	39.2	9.3	29.7	49.3
Incidence of clinical mastitis (%) [c]	17	5.2	2.1	2.4	9.9	13	2.4	1.0	0.0	4.1	10	5.1	2.5	3.3	11.3
Bulk milk concentration of total bacteria (log10 CFU\mL)	13	4.5	0.50	3.8	5.6	9	4.3	0.3	3.9	4.8	5	4.3	0.5	3.8	5.2
Udder cleanliness score (herd weighted mean) [d]	13	1.8	0.2	1.4	2.2	9	2.0	0.2	1.8	2.2	5	1.5	0.1	1.4	1.6

N= number of months in which milk samples were collected; SD= standard deviation; Min= minimum; Max= maximum.

[a] Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from <200.000 to ≥200.00 cells/mL on two consecutive test days. The number of animals was adjusted to 100 cows/month.

[b] Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200.000 cells/mL at a given test day.

[c] Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during one month, and the number of animals was adjusted to 100 cows/month.

[d] Cleanliness scoring was performed before milking within the milk parlour area, sampling 100% of the lactating animals. Results were reported as weighted mean scores, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

Subclinical mastitis prevalence on farm 1 increased during the study period and peaked at 83.8% in October 2015 (Figure 1). It is essential to note that this farm had a high prevalence of *S. agalactiae.* Similar results were found by Souza *et al.* (2009), where the highest SCC increase in the 24 herds analyzed was related to *S. agalactiae* infection. Thus, we can suggest that CBP does not influence the infection profile by contagious pathogens since these pathogens are restricted to mammary glands, and the transmission is more related to hygiene procedures during milking and entry of infected animals into the herd. The incidence of clinical mastitis peaked at 9.9% and 8.6% in December 2014 and February 2015, respectively. Over the study period, the incidence of clinical mastitis varied from 2.4% to 9.9%. According to Figure 1, it was possible to observe a higher percentage of animals with SCC above 200,000 cells/mL, especially during the months of higher precipitation in 2016, where this percentage increased. A study performed by Machado *et al*. (2000) corroborates the results found in the present study, indicating that SCC may increase in the hottest months of the year due to lower immune resistance and an increase in the incidence of intramammary infections due to heat stress in these periods of the year.

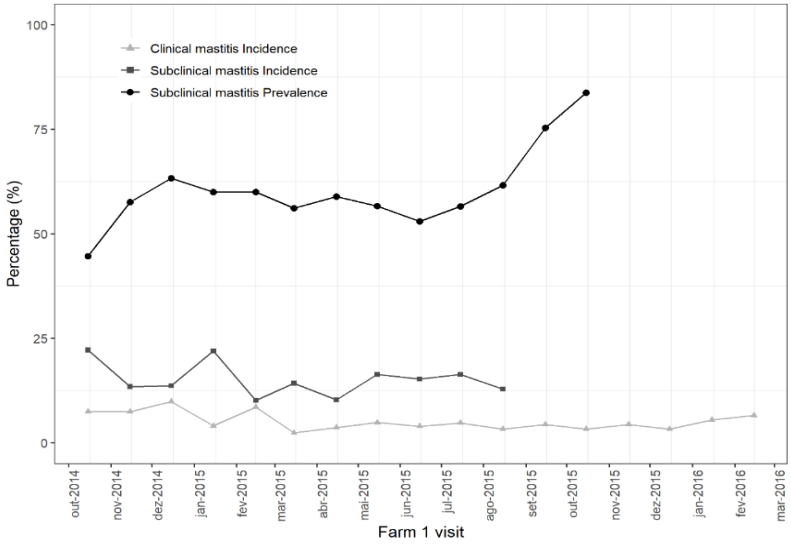

Figure 1. Epidemiology of mastitis on farm 1.

For farm 2, it was possible to observe a peak in the incidence of subclinical mastitis in January 2016, reaching 26.2%. Except for February 2015 (prevalence = 46.3%), no trends were observed in the prevalence of subclinical mastitis, ranging from 22.2% to 32.6% (Figure 2). In general, it was possible to observe a higher percentage of animals with SCC below 200,000 cells/mL throughout the study period. The low SCC may be due to a lower percentage of animals with infections by contagious pathogens, especially *S. agalactiae*, which was not isolated from herd 2. It is possible to infer that the increase in the percentage of animals with SCC above 200,000 cells/mL in the summer may be related to the increase in heat stress, which increases the susceptibility of the animal to infections, favoring the incidence of mastitis during these warmer months (Harmon, 1994). In the CBP system, there may be some heat stress periods, and in this particular property, the fans were turned on and off manually, and there was no thermostat (16ºC), as in farm 1.

For farm 3, subclinical mastitis peaked at 49.3% in June and July. After this period, it was possible to observe a decreasing trend in the incidence and prevalence of subclinical mastitis (Figure 3). The animal density at Farm 3 was $14m^2$ per cow in the CBP. It was the lowest density among the farms evaluated. Additionally, this farm had an effective protocol for controlling contagious pathogens (animals were discarded at the beginning of lactation if positive for *S. aureus* and *S. agalactiae*) and excellent hygiene scores (most animals with udder hygiene score 1).

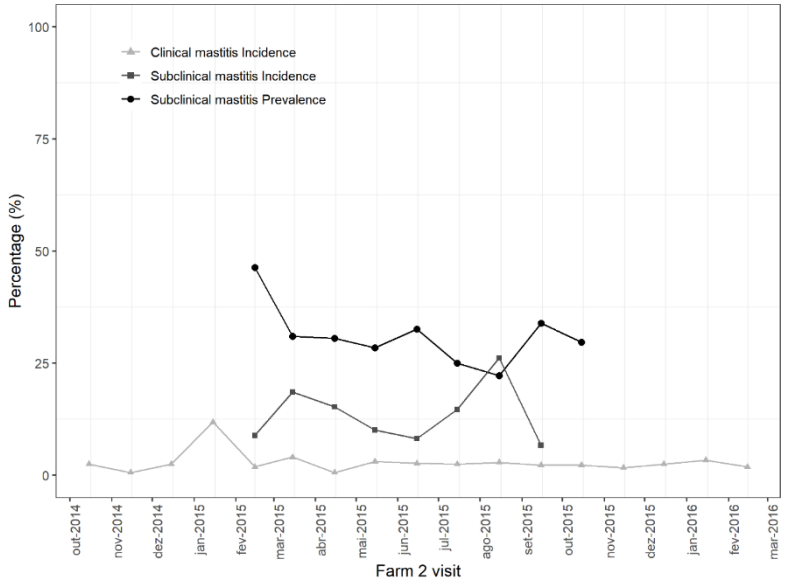

Figure 2. Epidemiology of mastitis on farm 2.

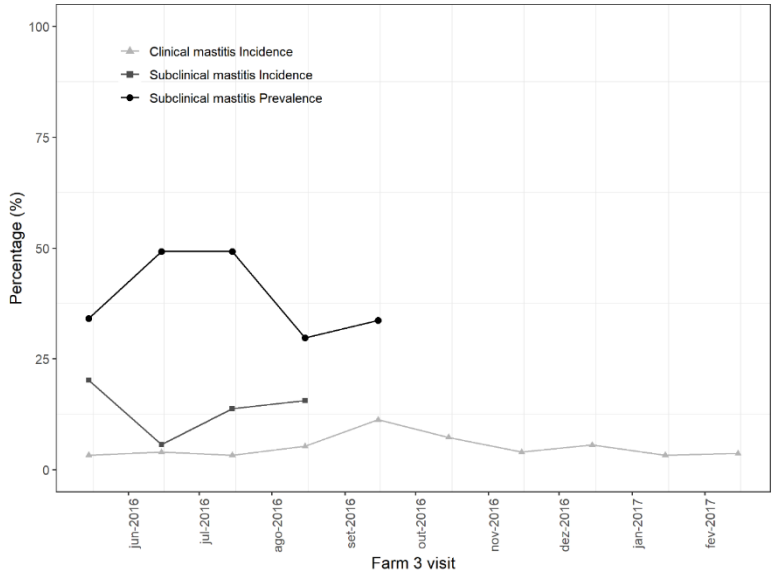

Figure 3. Epidemiology of mastitis on farm 3.

For farms 2 and 3, the BTM concentration of total bacteria ranged from 3.78 to 5.15 $\log_{10}$ CFU/mL. It remained below the Brazilian legal limit (5.48 $\log_{10}$ CFU/mL at the time of data collection) during the study period (Figure 4). For farm 1, the BTM concentration of total bacteria varied during the study and reached a peak of 5.56 $\log_{10}$ CFU/mL in November 2015, exceeding the official regulatory limit.

The somatic cell count of the BTM in farm 1 presented a significant fluctuation throughout the study, where it was possible to observe the count of nine months above the official regulatory limit of 400,000 cells/mL established by the Brazilian Government at that time (Figure 5). It is important to remember that this farm presented a high prevalence of clinical and subclinical mastitis caused by *S. agalactiae*, a pathogen responsible for the most substantial increase in SCC in Brazilian herds (Souza *et al*., 2009). For farms 2 and 3, the SCC remained within the official regulatory limits for most of the months during the study period.

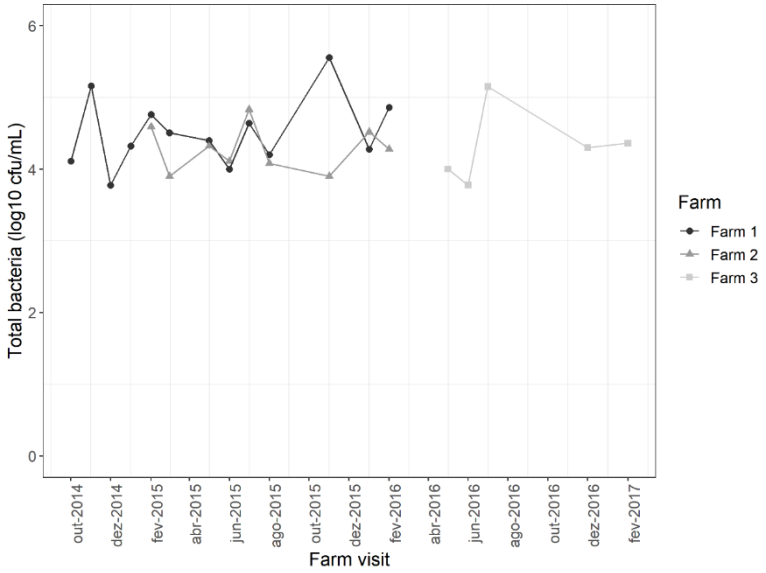

Figure 4. Concentration of total bacteria on bulk milk (log_{10} CFU/mL).

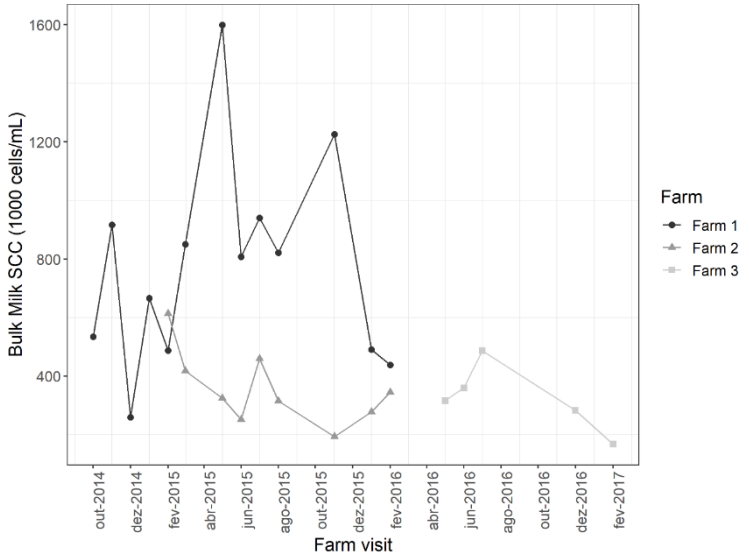

Figure 5. Somatic cell count on bulk milk tank (x1,000 cells/mL).

No correlations were found between the concentration of total bacteria on BTM and hygiene score for farm 1 (p=0.58), farm 2 (*p*=0.71), and farm 3 (*p*=0.74). The BTM SCC also did not correlate with the hygiene score in the three farms (*p*=0.78, 0.43, and 0.15). This result could be a consequence of good hygiene scores on the three properties, indicating the udder remained clean most of the time. It is worth mentioning that farm 1 had a high infection rate for *S. agalactiae*, and farms 2 and 3 had a high level of CNS infection, which may influence CFU and SCC.

The variation in bed moisture during the year was not associated with environmental streptococci and CNS incidence at farm 1 (p = 0.25 and p = 0.73) and farm 2 (p = 0.96 and p= 0.47). The clinical mastitis did not correlate with the bed moisture variation in farms 1 and 2 (p = 0.405 and p = 0.943, respectively). Rather than what was found in this study, Favero *et al.*

(2015a) reported a correlation between bed moisture with the increased incidence of clinical mastitis caused by environmental pathogens. Controlling bed moisture can be difficult (Lobeck *et al.,* 2011) because it depends on the management of the compost and also on climatic influences. High animal density, lack of ventilation, and inadequate aeration of the bed can lead to a significant increase in moisture (Janni *et al*., 2006; Black *et al*., 2014). It was possible to observe a variation in the mean of the bed humidity between farms 1 and 2 (Tab. 4). Farm 2 presented higher average moisture than farm 1 but was still within North America's standard values (40 to 60%) (Black *et al.*, 2013). Bed moisture during the year was not associated with the incidence of environmental streptococci and CNS nor with the incidence of cases of clinical mastitis in farms 1 and 2.

Table 4. Variation of averages of bed moisture during the period from October 2014 to February 2016 on farm 1 and farm 2

	Farm	N	Mean	Standard deviation	Standard error	*p-value*
Moisture	1	10	40,44[a]	6,3169	1,9976	0,001
	2	5	53,22[b]	3,9499	1,7665	

Lowercase letters differ from each other with $p \leq 0.05$.

The average bacterial density was 7.1 $\log_{10}$ UFC/g for farm 1 and 7.9 $\log_{10}$ UFC/g for farm 2, which corroborates with other authors who also found approximate values in the concentration of bacteria on the bed surface (Barberg *et al*., 2007a; Black *et al*., 2014; Endres and Barberg, 2007; Favero *et al*., 2015b). Farm 1 worked with an average area per animal of 11m^2 and farm 2 of 9m^2. The increase in the number of cases of *E. coli* in farm 2 may be associated with the higher bed moisture and the high number of pathogens on the bed surface, which may contribute to the increase of intramammary infection pressure if there is a transfer of these pathogens from the skin to the teats (Hogan *et al.*, 1989). Thus, due to the high concentration of bacteria in the compost bed, researchers have recommended adopting strict hygiene procedures before and after milking and adequate bed management. These good management practices can contribute to keeping the bed continuously dry and prevents the adherence of dirt in animals (Barberg *et al*., 2007a; Barberg *et al*., 2007b; Black *et al*., 2013).

On all farms, most cows remained clean (score 1) or slightly dirty (score 2) during the study period (Figure 6). The mean hygiene score was 1.8, 1.9, and 1.5 on farms 1, 2, and 3, respectively. Although Schreiner and Ruegg (2003) reported an association between hygiene scores and SCC in the free-stall confinement system, in this study, there was no association between them in any of the three farms (p = 0.539, p = 0.784, and p = 0.593, respectively). Probably, the non-association was due to the higher number of animals classified with a score of 1 (clean). Also, in farm 1, the increase in SCC occurred due to the high prevalence of *S. agalactiae* and was not associated with environmental pathogens.

The year period influenced the hygiene score of the animals only on farm 3 (Table 5). On this farm, it was possible to observe more cows with scores 2, 3, and 4 during period 1 (November to April) than in period 2 (May to October). The association of the period of the year with the hygiene score was an interesting result in this study. Despite being a closed system of confinement, in the period of higher precipitation, more animals were found with a hygiene score ≥2, indicating the need for special attention in the wetter months of the year. It is noteworthy that Favero *et al*. (2015a) did not find an association between the hygiene score and the year period, observing the same confinement system under Brazilian tropical conditions.

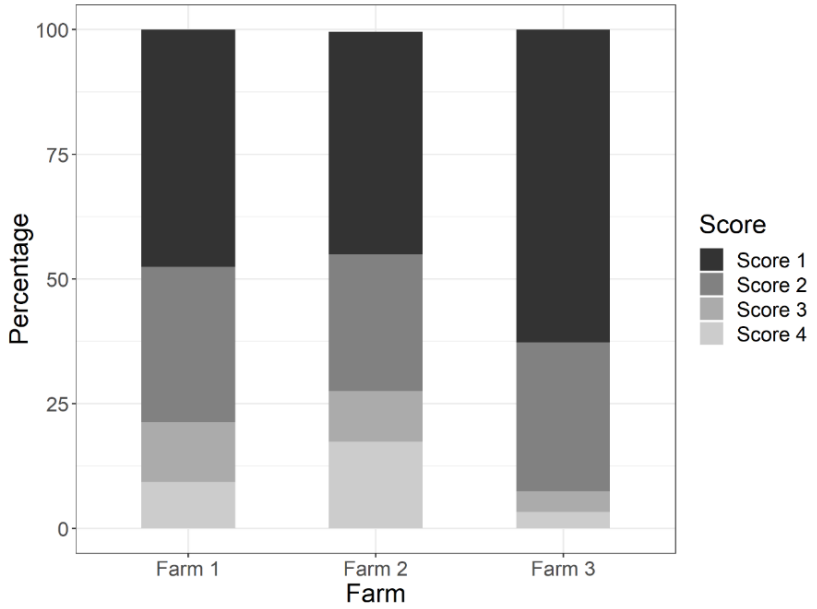

Figure 6. Hygiene score based on a four points scale.

Table 5. Association between the period of the year and hygiene score on farms 1, 2 and 3.

	Farm 1					Farm 2					Farm 3				
	Score					Score					Score				
	1		2 to 4			1		2 to 4			1		2 to 4		
Period	N	%	N	%	*p*-value	N	%	N	%	*p*-value	N	%	N	%	*p*-value
1	146	37.2	140	32.3[a]	0.14	186	43.2	245	56.8[a]	0.20	184	66.9	91	33.1[a]	0.04
2	247	62.8	294	67.7[a]		227	47.4	252	52.6[a]		127	57.7	93	42.3[b]	

Lowercase letters differ from each other with $p \leq 0.05$. Chi-square test

Udder hygiene has also been associated with the quality of milk produced (Schreiner; Ruegg, 2003). The results found in this study corroborate studies conducted in North America, where animals managed in the CBP system presented good udder hygiene conditions when compared to the well-managed freestall with sand bed (Lobeck *et al.*, 2011). In the study conducted by Black *et al*. (2013), the animals presented a mean of 2.2 in the udder hygiene score (1 = clean and 4 = very dirty) in a CBP group in Kentucky. Similar studies conducted on animals confined in the CBP system in Minnesota showed a mean score of 2.6 (Barberg *et al*., 2007a, 2007b) and 3.1 (Shane *et al.*, 2010). Several researchers reported the difficulty of maintaining animal hygiene during harsh winters and in humid and high rainfall climates. In Brazil, where variations between the seasons are not extreme, it is possible to keep the animals with adequate hygienic conditions all year in the CBP system if it is well managed.

CONCLUSION

The epidemiology of mastitis and the profile of the pathogens isolated in the CBP are similar to other confinement systems when well managed. The period of the year influenced the udder hygiene score; even the system being closed confinement, the bed (temperature and humidity) management, and ventilation are essential parameters that shall be controlled. The hygiene score was related to the incidence of environmental streptococci and might be an additional tool to control these pathogens in the routine of farms that use the CBP. Because it is a new confinement system in tropical regions, more studies are needed about the epidemiology of mastitis and its association with the organic bed that characterizes the system.

ACKNOWLEDGMENTS

We are thankful to Marcos Aurélio Souto Silva, laboratory technician of Milk Microbiology Lab of Embrapa Dairy Cattle, Alessandro Del'Duca for bed CFU analyses and the farmers who cooperate with the study. This study was supported by the Brasilian Agricultural Research Corporation, Conselho Nacional de Desenvolvimento Científico e Tecnológico–-CNPq, Fundação de Amparo à Pesquisa do Estado de Minas Gerais – Fapemig.

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