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Factors associated with mastitis epidemiologic indexes, animal hygiene, and bulk milk bacterial concentrations in dairy herds housed on compost bedding

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ABSTRACT

The primary objective of this study was to identify compost bedding characteristics associated with mastitis epidemiologic indexes, cow cleanliness, and concentration of selected bacterial populations found in bulk tank milk. Secondary objectives were to monitor the occurrence of environmental mastitis outbreaks, and to describe the profile of pathogens isolated from mastitis cases of cows housed in the CBP system. Three dairies were visited monthly during 1 year. On each visit day, milk samples were collected from the bulk tank and from a sample of mammary quarters for microbiological examination. Milk samples were collected from all cases of clinical mastitis. Flank, leg, udder, and teat cleanliness were assessed using a score chart based on a 4-point scale (1=clean to 4=very dirty). Bedding samples were collected to estimate concentrations of total bacteria, streptococci, and coliforms, moisture, organic matter, carbon–nitrogen ratio, pH, and density. Mixed models were used to identify factors associated with incidence and prevalence of mastitis, and cow cleanliness. Except for farm A, on which contagious pathogens caused most cases, *Escherichia coli,* coagulase-negative staphylococci, and environmental streptococci were the most frequent pathogens isolated from clinical mastitis cases. *Corynebacterium bovis* was the most frequent pathogen isolated from subclinical cases of farms B (17.6) and C (26.0%). Environmental pathogens were isolated from 17.2%, 10.1%, and 14.8% of all subclinical cases of farms, A, B, and C, respectively. No outbreaks of environmental mastitis were observed during the course of the study. Bedding moisture, carbon–nitrogen ratio, pH, and dry density were unconditionally associated with the incidence of environmental clinical mastitis. Nonetheless, bedding moisture remained as a sole predictor in the final model. The odds of a case of environmental clinical mastitis increased 5.7% for each one-unit increase in bedding moisture. The odds of a new case of subclinical mastitis, and of a cow having SCC $\geq$ 200,000 cells/mL increased 32% and 16% for each one-unit increase in leg cleanliness score, respectively. Overall means for udder, teat, flank, and leg hygiene scores were less than 2.1 for all farms and did not vary among seasons of the year. Bedding wet density was positively associated with all cleanliness scores and bulk milk concentration of total bacteria. Results suggest that managing bedding to remain dry and loose will result in cleaner animals with decreased risk of mastitis.

1. Introduction

The compost bedded pack system (**CBP**) has been increasingly used worldwide to confine dairy cows. The CPB is characterized by a process of microbiological decomposition of an organic substrate (such as wood shavings or sawdust), to which feces and urine are constantly added by cows. Bedding is tilled twice a day to incorporate animal waste, facilitate aerobic composting, and provide a comfortable and dry surface to cows (Barberg et al., 2007a, 2007b; Janni et al., 2007; Black et al., 2013).

Because compost bedding is mostly organic, one of the potential hazards for udder health is the concentration of environmental mastitis pathogens. Coliforms (such as *Escherichia coli* and *Klebsiella* spp) and environmental streptococci (such as *Streptococcus uberis* and *Streptococcus dysgalactiae*) are the most prevalent pathogens causing clinical mastitis on farms that have successfully controlled contagious mastitis (Jobim et al., 2010; Lago et al., 2011; Oliveira and Ruegg, 2014). Environmental streptococci are also one of the main causes of subclinical mastitis in herds worldwide (Jobim et al., 2010; Oliveira and Ruegg, 2014).

Barberg et al. (2007a, 2007b) reported that mean bedding concentration of total bacteria was 9.1×10^6 cfu/cc for a group of 12 CBP

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in Minnesota. Lobeck et al. (2012) reported bedding concentrations of 1.4×10^3, 280, and 3×10^6 cfu/mL for coliforms, *Klebsiella* spp, and environmental streptococci, respectively. Bedding concentration of environmental streptococci was not different between CBP and sand-bedded freestalls, but concentrations of coliforms and *Klebsiella* spp in CBP bedding were 47 and 14 times greater than those observed in naturally ventilated sand-bedded freestalls, respectively (Lobeck et al., 2012). Other environmental pathogens that might be present in compost bedding, such as *Nocardia* spp, *Pseudomonas* spp, and *Prototheca* spp, have been associated with herd outbreaks of clinical and subclinical mastitis, and are capable of causing chronic, untreatable mastitis (Janosi et al., 2001; Condas et al., 2013). The occurrence of such pathogens in herds housed on compost bedding has not been studied.

In this context, little research has been conducted to describe the profile of pathogens causing clinical and subclinical mastitis, to assess the risk of intramammary infections (**IMI**) caused by environmental pathogens, and to characterize the quality of milk produced in CBP systems. Two studies were conducted to describe longitudinal changes in bulk tank milk SCC from herds that shifted from other systems to the CBP. Barberg et al. (2007a, 2007b) observed SCC reduction in 5 of 7 herds by comparing mean monthly dairy herd improvement (**DHI**) somatic cell count (**SCC**) before (2 years) and after (1 year) the change. Likewise, Black et al. (2013) reported that mean SCC of 8 herds decreased from 411,000 cells/ml (12-month mean prior to the change) to 305,000 (first year) and 275,000 cells/ml (second year) after the change. Nonetheless, a causal relationship between the use of CBP and bulk tank milk SCC should not be established solely on these data because control groups were not used for comparison.

Results of experimental studies performed to investigate the effect of housing cows in the CBP on the occurrence of mastitis have been contradictory. Astiz et al. (2014) reported that cows maintained on compost bedding during the dry period had reduced incidence of clinical mastitis in the subsequent lactation, as compared to cows housed on straw bedding. In contrast, Svennesen et al. (2014) reported a herd SCC increase of 72.000 cells/mL for animals randomly allocated to a CBP of high moisture content (65–70%, bedded with chopped roots, wood shavings and garden organic residues), as compared to a group of cows that remained in a sand-bedded freestall. These conflicting results suggest that compost bedding characteristics play a major role in minimizing the risk of IMI.

Among bedding-related indicators of animal health, cow cleanliness has been universally used as an indicator of udder health. Results of cross-sectional studies conducted at the herd level have consistently demonstrated that herds in which most cows were scored “clean” were more likely to have less bulk tank milk SCC than herds in which most cows were scored “dirty” (Barkema et al., 1998; Ellis et al., 2007; Dufour et al., 2011). Although results of North American studies (Barberg et al. 2007a, 2007b; Shane et al., 2010; Black et al., 2013) have demonstrated that cows housed in the CBP are maintained in good hygienic conditions (comparable to those found in well managed sand-bedded freestalls), researchers reported difficulties maintaining clean cows during humid and rainy weather (Lobeck et al., 2011). In those studies, visual observations suggested that cow cleanliness was dependent on bedding moisture (especially during winter) and density of the CBP, but these associations have not been scientifically demonstrated. Identification of CBP bedding factors associated with animal hygiene is integral for developing management practices towards maintaining clean cows and minimizing the risk of mastitis.

The primary objective of this longitudinal study was to identify compost bedding characteristics associated with mastitis epidemiologic indexes, cow cleanliness, and concentration of selected bacterial populations found in bulk tank milk. Secondary objectives were to monitor the occurrence of environmental mastitis outbreaks, and to describe the profile of pathogens isolated from mastitis cases of cows housed in the CBP system.

2. Materials and methods

2.1. Farm selection and sampling strategy

At the beginning of the study, all known CBP dairies in Sao Paulo state, Brazil ($N=4$) were contacted and agreed to participate in the study. Of these dairies, one was not used for the study due to the distance from the university (>300 km). Other inclusion criteria were adoption of compost bedding as sole system to confine lactating cows, participation in a monthly DHI testing program, and willingness to comply with the study protocol.

Initially farms were visited to explain the study protocol and provide training for collection of bedding and milk samples. Farms were then visited monthly between May 2013 and June 2014 for data collection and sampling.

2.2. Farm and animal characteristics

Farm and animal characteristics are given in detail elsewhere (Favero et al., 2015). In brief, Farm A had 33 lactating Holsteins that were milked twice a day. The CBP was approximately 6 months old at the beginning of the study and consisted of an area of 290 m^2 (11 m^2/cow) bedded with peanut shells. Seventy m^3 of new bedding were added monthly to the CBP. Bedding was tilled twice a day between milkings using a deep cultivator. Fans were installed throughout the barn over the bedding area. Cows were machine-milked on the concrete feeding alley and milk was stored within a bulk tank. The milking routine consisted of examination of the first milk streams on a strip cup, pre-milking teat disinfection with a chlorine-based solution, drying of teats with single paper towels, and use of a barrier post-milking teat dip (1% iodine).

Farm B had 53 lactating Holsteins that were milked twice a day. The CBP was approximately 2 months old at the beginning of the study and was bedded with sawdust. The CBP area on farm B was 1000 m^2 (19 m^2/cow) and 34 m^3 of new bedding were added monthly. Bedding was tilled twice a day between milkings using a deep cultivator, and a rototiller was used occasionally to loose the material and decrease particle size. Bedding was entirely replaced once, during the last month of the study. No fans were installed over the bedding area. Cows were machine-milked in a Herringbone pit parlor and milk was stored in a bulk tank. The milking routine consisted of examination of the first milk streams on a streak cup, pre-milking teat disinfection with sodium hypochlorite, drying of teats with single paper towels, and use of a barrier acid lactic-based post-milking teat dip.

Farm C had 145 Simmental lactating cows that remained on a bedding area of 1580 m^2 (12 m^2/cow). The CBP was approximately 2 months old at the beginning of the study. Wood shavings were used as bedding material and 38 m^3 of new bedding were added monthly to the CBP. Bedding was tilled twice a day between milkings using a deep cultivator and a rototiller was used twice a week to further decrease particle size. Bedding was entirely replaced once, during the first month of the study. Fans were installed over the bedding area at the 7th month of the study. Cows were machine-milked in a Herringbone pit parlor and milk was stored in a bulk tank. The milking routine consisted of examination of the first milk streams on a streak cup, pre-milking teat disinfection with iodine 0.5%, drying of teats with single paper towels, and use of a lactic acid-based post-milking teat dip.

Although the study was observational, farmers asked for milk

quality advice during the study. Therefore, at the beginning of the study, the authors recommended management practices based on the NMC's 10-point mastitis control plan (NMC; Verona, WI). Farms complied with proposed changes at different levels, according to their interest in improving milk quality.

2.3. Milk sampling

On each visit day, cows whose most recent DHI composite SCC was >200.000 cells/mL were tested with the California Mastitis Test (**CMT**) for identification of possibly infected quarters. Aseptic milk samples were collected from a random sample of CMT-positive quarters (1 quarter per cow) for microbiological examination. Sampling of 50%, 50%, and 30% of the high SCC cows was attempted on farms A (<100 cows), *B* (<100 cows), and *C*(>100 cows), respectively. The number of cows sampled was calculated based on herd size, to provide a representative sample of mastitis pathogens, without disrupting milking routines.

Bulk tank milk samples were collected on each visit day using sterile uterine infusion pipettes attached to 60-mL syringes, after milk was agitated for at least 5 min. All milk samples were refrigerated and frozen on the same day.

Aseptic quarter milk samples were also collected and frozen by trained farm personnel before treatment (or at detection for cases that were not treated) from all cases of clinical mastitis that occurred during the study. Clinical mastitis was identified using a strip cup and was defined as the presence of milk abnormalities such as flakes, pus, and changes in color. Severity of the cases was recorded as mild, moderate, or severe, according to the scale proposed by Wenz et al. (2001).

2.4. Cow cleanliness assessment

On each visit day, cleanliness of the flank, leg, and udder was assessed before milking (within the CBP area) by use of a score chart based on a 4-point scale ranging from 1 (clean) to 4 (very dirty) (Canadian Bovine Mastitis Research Network; Montreal, Canada). All lactating cows were scored on farms A and B (<100 cows), and 50% of the cows were scored on farm C (>100 cows), according to chart instructions.

On each visit day, teat swabs were collected during milking (before any milking procedure was performed) to assess teat cleanliness and estimate the population of total bacteria, coliforms, and streptococci on teat skin. Eight sterile gauze pads were placed into a sterile 50-mL plastic container and 25 mL of 0.1% peptone water were added to moisten swabs and preserve bacteria. To collect swabs, teats were scrubbed with 1 circular movement around the teat barrel, finishing with a pinch of the teat end. For all farms, swabs were collected from a random sample of 30 cows (1 teat per cow), alternating the swabbed teat between cows in a clockwise manner. Swabs were returned to sterile containers and refrigerated until processing. Teat cleanliness was assessed by use of a score card based on a 4-point scale (GEA Farm Technologies, Inc.; Naperville, IL, USA). For each visit, results of each cleanliness score type were reported as herd weighted mean score (weight = 1, 2, 3, or 4).

2.5. Bedding sampling

Bedding samples were collected biweekly by trained farm personnel, as described by Barberg et al. (2007a, 2007b). For the present study, a subset of monthly bedding samples collected on the visit days was used for analysis. In brief, the bedding area of each farm was divided into 12 equal squares, from which 1 sample was collected from the superficial and deep (20 cm) layers. All samples from each layer were mixed to create a composite sample for each layer, which were used to determine bedding bacterial concentrations. The composite samples of each layer were then mixed to create a composite sample that was used to determine bedding physical–chemical characteristics (moisture (%), organic matter (%), carbon–nitrogen ratio, pH, and wet and dry densities (kg/m^3)).

2.6. Microbiological examination of milk, bedding, and teat swabs

Bedding samples were processed in the Sao Paulo State University's Mastitis Research Laboratory. Microbiological analyses of bedding was performed by adding 90 mL of 0.1% peptone water to 10 g of bedding (Zdanowicz et al., 2004). Samples were mixed for 1 minute and let settle for 2 min. One-hundred μL of diluted samples (10^{-2}–10^{-5}) were spread onto blood agar, MacConkey, and Edward's medium and incubated for 24 h to determine the concentration (log10 cfu/g of bedding) of total bacteria, coliforms and streptococci, respectively. Bedding samples were sent to the Sao Paulo State University's Soil Science Laboratory for determination of physical–chemical characteristics.

Milk samples from clinical and subclinical cases were processed in the Sao Paulo State University's Mastitis Research Laboratory and cultivated according to the NMC recommendations (NMC, 1999). In brief, 10 μL of each milk sample were streaked onto blood agar and McConkey plates. Plates were incubated at 37 °C and read at 24, 48, and 72 h. Mastitis pathogens were diagnosed based on morphology (Gram staining) and biochemical reactions. *Staphylococcus aureus* was differentiated from other staphylococci by means of mannitol and tube coagulase reactions. *Streptococcus* spp were identified with the Christie–Atkins–Munch–Petersen (CAMP) test, esculin, and bile-esculin reactions. Gram-negative bacteria were identified by growth on McConkeky agar, lactose production, and reactions on MIO (motility–indole–ornithine), citrate, and TSI (triple sugar iron) agar slants.

An intramammary infection was defined as the presence of 3 or more colonies of the same type. Non-significant growth (<3 colonies of the same type) was considered negative for analysis and samples were contaminated when there were 3 or more colony types on plates. Bulk tank milk concentrations of total bacteria, coliforms, and streptococci were estimated by inoculating 100 μL of milk (undiluted to 10^{-4}) onto Blood agar, McConkey, and Edward's medium, respectively. Plates were incubated at 37 °C and read at 24 h. Results were expressed as log10 cfu/mL.

Upon arrival to the laboratory, teat swabs were placed into a sterile plastic container and weighted. Twice as much peptone water was added to the plastic bag, which was stomached for 2 min. One mL of the solution was then used to create serial dilutions (10^{-1}–10^{-3}). Concentrations of total bacteria, coliforms, and streptococci were estimated by inoculating 100 μL of the swab solutions onto Blood agar, McConkey, and Edward's medium, respectively, as previously described. Results were expressed as log10 cfu/mL of solution.

2.7. Statistical analysis

2.7.1. Definitions

Monthly DHI SCC was used to estimate the presence of IMI at the cow level (suspect infection). The SCC threshold used by the DHI association to identify a suspect subclinical infection was 200,000 cells/mL. Prevalence of subclinical mastitis was defined as the percentage of cows with SCC >200,000 cells/mL at a given test day. Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from <200,000 to ≥200,000 cells/mL on 2 consecutive test days, divided by the number of cows whose SCC was <200,000 cells/mL on the previous test day.

Table 1
Results of microbiological examination of milk, by farm and mastitis type.

Mastitis type	Result	Farm B		Farm A		Farm C	
		N	%	*N*	%	*N*	%
Clinical	*Bacillus* spp					1	1.4
	Citrobacter spp	1	1.7			2	2.8
	Coagulase-negative staphylococci	7	11.7	1	2.2	6	8.5
	Corynebacterium bovis	3	5.0	4	8.7	7	9.9
	Enterobacter spp	2	3.3	1	2.2	1	1.4
	Enterococcus spp			2	4.3	1	1.4
	Escherichia coli	5	8.3			12	16.9
	Gram-negative rods	3	5.0			3	4.2
	Klebsiella spp	3	5.0	4	3.1	1	1.4
	Yeast	3	5.0				
	Serratia spp	1	1.7				
	Staphylococcus aureus					5	7.0
	Streptococcus agalactiae	1	1.7	20	43.5		
	Streptococcus dysgalactiae	2	3.3				
	Streptococcus spp			1	2.2	3	4.2
	Streptococcus uberis	1	1.7				
	Trueperella pyogenes	1	1.7				
	No growth	25	41.7	10	21.7	24	33.8
	Contaminated	2	3.3	7	15.2	5	7.0
	Total	60	100	50	103	71	100
Subclinical	*Citrobacter* spp	2	0.7				
	Coagulase-negative staphylococci	30	10.8	9	7.0	23	7.6
	Corynebacterium bovis	49	17.6	16	12.5	79	26.0
	Enterobacter spp	1	0.4				
	Enterococcus spp	3	1.1	1	0.8	10	3.3
	Escherichia coli					2	0.7
	Gram-negative rods	3	1.1				
	Klebsiella spp	3	1.1	4	3.1	4	1.3
	Yeast	1	0.4			4	1.3
	Prototheca spp					1	0.3
	Pseudomonas spp	2	0.7	1	0.8		
	Serratia spp	1	0.4			1	0.3
	Staphylococcus aureus	3	1.1			36	11.8
	Streptococcus agalactiae	6	2.2	29	22.7		
	Streptococcus dysgalactiae	4	1.4	1	0.8	7	2.3
	Streptococcus spp	8	2.9	11	8.6	13	4.3
	Streptococcus uberis			4	3.1	3	1.0
	No growth	160	57.6	42	32.8	106	34.9
	Contaminated	2	0.7	10	7.8	15	4.9
	Total	278	100	128	100	304	100

Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd on the DHI test day. For cows that experienced repeated episodes of clinical mastitis (regardless of the quarter), only cases that occurred after 14 days from a previous case were considered new.

Mastitis pathogens isolated during the study (Table 1) were also grouped as environmental (coliforms, *Bacillus* spp, Lactose-negative Gram-negative rods, environmental streptococci, yeast, *Prototheca* spp, *Trueperella pyogenes*, and *Pseudomonas* spp), contagious (*Streptococcus agalactiae*, *S. aureus*, and *Corynebacterium bovis*), and opportunistic (Coagulase-negative staphylococci).

Bedding age was defined as the time interval between the last bedding total replacement and a given visit day. Bedding age was categorized into 1 (≤ 4), 2 (5–8), and 3 (≥ 9 months old).

2.7.2. Analytical procedures

Initially the distribution of variables was analyzed to assess normality. All bacterial counts (bedding, milk, and teat swabs) were not normally distributed and therefore transformed to a log10 scale for analysis. Descriptive statistics were produced to generate reference values for the variables studied. Explanatory variables were mastitis epidemiologic indexes (incidence of clinical mastitis, incidence of environmental clinical mastitis, incidence of subclinical mastitis, and prevalence of subclinical mastitis) and cow cleanliness scores (udder, teat, flank, and leg). Explanatory variables for mastitis epidemiologic indexes were teat, udder, leg, and flank cleanliness, season (summer, fall, winter, and spring), bedding age, and all bedding characteristics presented in Table 2. Explanatory variables for cleanliness scores were season, bedding age, and all bedding characteristics presented in Table 2. Preliminarily, bivariate analysis was used to identify unconditional associations between each explanatory variable and study outcomes. Variables associated with a given outcome at a significance level of 0.15, and interaction terms between farm and explanatory variables were included in a stepwise model selection procedure to select final models.

All epidemiologic indexes were initially modeled as binomial outcomes by use of logistic regression, according to the following structure (Littell et al., 2006; PROC GLIMMIX, SAS Institute, 2011):

$$\text{logit}\,(Y) = \alpha + \beta_i(X_i) + \delta_j$$

where Y was prevalence or incidence of mastitis, α was the intercept, β_i (X_i) was the ith coefficient for the ith explanatory variable, and δ_j was a random term to model repeated measurements within the jth farm. When overdispersion was detected, a negative binomial distribution was used for modeling (Palta, 2003), according to the following structure:

$$\log(Y) = \alpha + \beta_i(X_i) + \text{offset} + \delta_j$$

were Y was the number of new mastitis cases in a one-month period, or number of cases at a given test day, α was the intercept, β_i (X_i) was the ith coefficient for the ith explanatory variable, δ_j was a

Table 2
Descriptive statistics for bedding characteristics, by farm.

Bedding variable	Farm A					Farm B					Farm C				
	N	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max
Concentration of total bacteria[a] (log10 cfu/g)	9	8.74	0.47	8.14	9.38	12	8.50	0.31	8.01	8.89	12	8.98	0.41	8.34	9.78
Concentration of coliforms[a] (log10 cfu/g)	9	6.58	0.58	5.64	7.57	12	6.16	0.63	5.34	7.18	12	6.77	0.86	5.45	8.20
Concentration of streptococci[a] (log10 cfu/g)	9	6.70	0.99	4.30	7.75	12	5.83	0.81	4.48	7.32	12	6.85	0.50	6.18	7.80
Organic matter[b] (%)	9	40.56	5.50	31.00	47.00	11	31.18	3.12	27.00	37.00	12	31.92	7.18	18.00	41.00
Carbon–nitrogen ratio[b]	9	25.56	2.60	21.00	30.00	12	27.17	10.15	15.00	43.00	12	21.83	6.51	15.00	33.00
Moisture[b] (%)	9	36.78	3.11	30.00	40.00	12	40.75	6.02	32.00	50.00	12	37.08	9.40	26.00	58.00
pH[b]	9	9.04	0.42	8.50	9.60	12	8.81	0.52	7.70	9.60	12	8.85	0.38	8.20	9.40
Wet density[b] (kg/m^3)	9	372.67	46.07	320.00	460.00	12	526.17	39.64	470.00	596.00	12	477.33	103.12	368.00	690.00
Dry density[b] (kg/m^3)	9	234.67	21.50	198.00	276.00	12	313.17	48.08	257.00	402.00	12	298.42	91.21	160.00	496.00

[a] Estimated from samples collected from the bedding surface.
[b] Estimated from composite samples of the bedding superficial and deep (20 cm) layers.

random term to model repeated measurements within the *j*th farm, and offset was the log of the number of cows included in the denominator of the incidence or prevalence calculations. For continuous outcomes, linear mixed models (Littell et al., 2006) were construced with PROC MIXED (SAS Institute, 2011) to identify predictors for each cleanliness score. Farm was considered a random effect to model the correlation between repeated observations within the same farm. Statistical analyses were performed at a significance level of 0.05.

3. Results

3.1. Missing data

The study was interrupted 3 months before the attempted endpoint for farm A, due to difficulties in complying with the study protocol. On the 7th visit to farm C, teat swabbing and teat hygiene scoring could not be performed.

3.2. Mastitis epidemiology

Mean prevalence and incidence of subclinical mastitis (based on DHI SCC) during the study period were 40.9% and 20.6% for farm A, 45.7% and 10.1% for farm B, and 41.1% and 23% for farm C, respectively (Table 3). Mean incidence of environmental clinical mastitis was 3.0%, 6.4%, and 2.3% for farms A, B, and C, respectively, and was not different among seasons of the year ($P=0.68$). No interaction was found between season and farm ($P=0.12$).

Subclinical mastitis incidence peaked at 44% during the fifth month and prevalence reached 53% on farm A at the 8th month of the study (Fig. 1). Except for the first month of the study (incidence = 7%), farm A's incidence of environmental clinical mastitis ranged from 0% to 3% during the study period.

For farm B, no apparent increasing or decreasing trends were observed for the incidence of subclinical mastitis, which ranged from 10% to 26% during the study (Fig. 1). Prevalence of subclinical mastitis increased steadly from the 4th (36%) to the 9th month of the study (60%), and decreased to 40% at the end of the period. Incidence of environmental clinical mastitis ranged from 2% to 12% during the study (Fig. 1).

For farm C, decreasing trends were observed for the prevalence and incidence of subclinical mastitis during the study period (Fig. 1). Similar decreasing trends were observed for the incidences of clinical (all pathogens) and environmental clinical mastitis. None of the herds experienced outbreaks of environmental clinical mastitis during the course of the study.

Bedding moisture, carbon–nitrogen ratio, pH, bedding age, and dry density ($P<0.01$ for all associations) were unconditionally associated with the incidence of environmental clinical mastitis (Table 4). Nonetheless, bedding moisture ($P<0.01$) remained as a sole significant predictor in the final model (Table 5 and Fig. 2). The odds of a case of environmental clinical mastitis increased 5.7% for each one-unit increase in bedding moisture (Table 5).

Bedding moisture ($P<0.01$), dry density ($P=0.04$), and carbon–nitrogen ratio ($P=0.03$) were associated with the incidence of clinical mastitis (Table 4), but moisture ($P<0.01$) was the only predictor that remained in the final multivariable model. The odds of a case of clinical mastitis increased 5.8% for each one-unit increase in bedding moisture (Table 5 and Fig. 2).

Leg cleanliness score was the only variable associated with the prevalence and incidence of subclinical mastitis in both bivariate and multivariable analysis. The odds of a new case of subclinical mastitis (cows whose SCC shifted from <200 to ≥ 200 cells/mL on 2 consecutive test days) and of a cow having SCC $>200{,}000$ cells/mL, increased 32% and 16% for each one-unit increase in leg cleanliness score, respectively (Table 5, Fig. 2).

3.3. Profile of pathogens isolated from clinical and subclinical mastitis cases

Environmental pathogens were the most frequent cause of clinical mastitis on farms B (36.7%) and C (33.8%). *E. coli* was the most prevalent environmental pathogen isolated from clinical cases of farms B (8.3%) and C (16.9%, Table 1). Contagious pathogens were the most frequent cause of clinical mastitis on farm A (52.2%), and were isolated from 6.7% and 16.9% of the cases of farms B and C, respectively. Opportunistic pathogens were isolated from 2.2%, 11.7%, and 8.5% of the clinical cases of farms A, B, and C, respectively (Table 1).

Table 3
Descriptive statistics for mastitis epidemiologic indexes, bulk milk and teat swab bacterial concentrations, and cow cleanliness scores, by farm.

Variable	Farm A					Farm B					Farm C				
	N	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max
Incidence of subclinical mastitis[a] (%)	9	20.56	14.35	0.00	44.00	12	19.08	5.21	10.00	26.00	12	23.00	6.13	16.00	37.00
Prevalence of subclinical mastitis[b] (%)	9	44.68	6.69	34.21	53.49	12	45.72	6.75	35.71	60.00	12	41.13	8.11	31.00	59.86
Incidence of clinical mastitis[c] (%)	7	15.04	8.89	2.86	25.00	11	8.84	3.93	1.85	17.24	11	4.24	3.71	0.74	14.29
Incidence of environmental clinical mastitis (%)	8	2.95	1.97	0.00	7.14	11	6.36	3.50	1.85	12.07	11	2.33	2.17	0.00	8.07
Bulk milk concentration of total bacteria (log10 cfu/mL)	9	3.19	0.53	2.63	4.36	12	3.92	1.43	1.90	6.40	12	3.52	0.52	2.67	4.63
Bulk milk concentration of coliforms (log10 cfu/mL)	9	0.00	0.00	0.00	0.00	12	1.95	1.51	0.00	4.45	12	1.45	0.99	0.00	2.51
Bulk milk concentration of streptococci (log10 cfu/mL)	9	1.95	0.99	0.00	3.08	12	2.97	1.26	1.00	4.70	12	2.92	0.50	2.23	3.78
Teat swab concentration of total bacteria[d] (log10 cfu/mL)	9	4.69	0.61	3.59	5.56	12	4.43	0.54	3.38	5.63	11	4.74	0.62	3.49	5.79
Teat swab concentration of coliforms[d] (log10 cfu/mL)	9	1.41	1.51	0.00	3.83	12	0.72	0.83	0.00	2.40	11	0.87	1.10	0.00	2.62
Teat swab concentration of sreptococci[d] (log10 cfu/mL)	9	2.46	0.78	1.30	3.90	12	1.73	0.97	0.00	2.90	11	1.44	0.88	0.00	2.54
Udder cleanliness score[e] (herd weighted mean)	9	1.26	0.18	1.09	1.61	12	1.30	0.17	1.03	1.59	12	1.29	0.27	1.05	2.04
Leg cleanliness score[e] (herd weighted mean)	9	1.60	0.41	1.18	2.53	12	1.77	0.38	1.24	2.40	12	1.66	0.45	1.19	2.63
Flank cleanliness score[e] (herd weighted mean)	9	1.36	0.21	1.10	1.67	12	1.43	0.32	1.03	2.23	12	1.40	0.30	1.10	2.11
Teat cleanliness score[e] (herd weighted mean)	9	1.86	0.26	1.63	2.44	12	2.12	0.27	1.74	2.62	11	1.72	0.42	1.13	2.58

[a] Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC $>200{,}000$ cells/mL at a given test day.
[b] Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from $<200{,}000$ to $\geq 200{,}000$ cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was $<200{,}000$ cells/mL on the previous test day (cows at risk).
[c] Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day.
[d] Teat swabs were collected from a random sample of 30 quarters at each farm visit.
[e] Cleanliness scoring was performed before milking, within the CBP area, sampling 100%, 100%, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

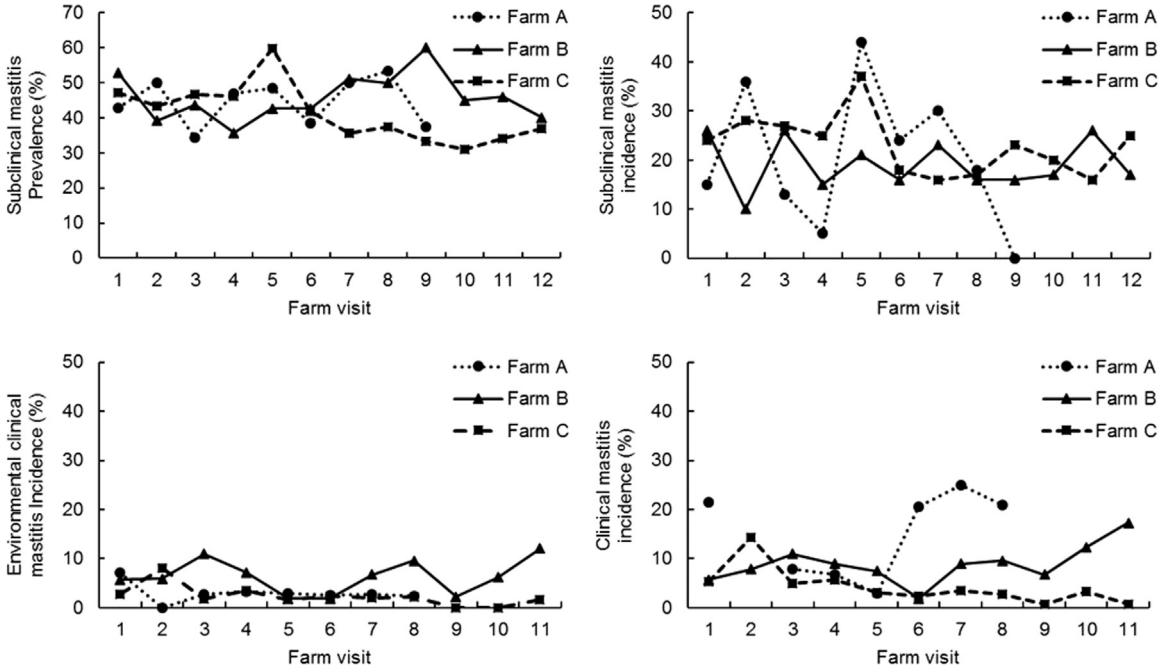

Fig. 1. Monthly mastitis epidemiologic indexes, by farm. Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day. Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC $>200{,}000$ cells/mL at a given test day. Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from <200000 to ≥ 200000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was $<200{,}000$ cells/mL on the previous test day (cows at risk).

Most subclinical mastitis cases were caused by contagious pathogens (farm A=35.2, farm B=20.7, and farm C=37.8% of all cases). *C. bovis* was the most frequent pathogen isolated from subclinical cases of farms B (17.6%) and C (26.0%). For farms, A, B, and C, environmental pathogens were isolated from 17.2%, 10.1%, and 14.8% of all cases, and opportunistic pathogens were isolated from 7.0%, 10.8%, and 7.6% of all cases, respectively. Farm A experienced an outbreak of *S. agalactiae* during the study, which was the most frequent pathogen isolated from clinical and subclinical mastitis cases (Table 1).

The prevalence of environmental pathogens that have been associated with outbreaks of untreatable mastitis, such as *Nocardia* spp, *Pseudomonas* spp, *Serratia* spp, and *Prototheca* spp was low. Only 1 case of *Prototheca* spp (subclinical) and 2 cases of *Serratia* spp (subclinical) were diagnosed during the course of the study.

Mastitis severity was recorded for 108 clinical cases with positive culture results and 46 cases with a "no growth" result. Of the 108 cases from which pathogens were isolated, the distribution of severity by pathogen group was (1) environmental ($N=35$): 5.7% severe, 22.9% moderate, and 71.4% mild; (2) contagious ($N=17$): 0% severe, 23.5% moderate, and 76.5% mild; (3) opportunistic ($N=10$ cases): 0% severe, 10% moderate, and 90% mild. Of the 46 cases with a "no growth" result, none were severe, 23.9% were moderate, and 76.1% were mild.

3.4. Cow cleanliness

For all farms, most cows remained clean (score 1) or slightly dirty (score 2) during the study period. Overall means for udder, teat, flank, and leg hygiene scores were less than 2.1 for all farms and varied little during the study (Table 3 and Fig. 3). Mean udder ($P=0.07$), teat ($P=0.32$), flank ($P=0.17$), and leg ($P=0.21$) scores were not different among seasons of the year.

Bedding wet density was unconditionally associated with leg ($P<0.04$), teat ($P<0.01$), and flank ($P=0.03$) cleanliness, and bedding dry density was positively associated with udder ($P=0.02$) and teat ($P=0.02$) cleanliness. Bedding organic matter was negatively associated with teat ($P=0.03$) and flank ($P=0.04$) cleanliness. Mean udder cleanliness increased across bedding age categories ($P<0.01$, Table 4).

Bedding wet density remained as a sole predictor of all cleanliness scores studied (Table 4). All associations were positive, but of small magnitude (Table 5 and Fig. 4). None of the bedding variables studied were associated with teat skin concentration of total bacteria, streptococci, or coliforms.

3.5. Bulk tank milk bacterial concentrations

For farms A and C, bulk milk concentration of total bacteria ranged from 2.6 to 4.6 log10 cfu/mL and remained below the Brazilian legal limit (3×10^5 cfu/mL) during the entire study period (Fig. 5). For farm B, bulk milk concentration of total bacteria increased steadly from the fourth month (1.9) and reached 6.4 log10 cfu/mL at the sixth month of the study. Subsequently, bulk milk concentration of total bacteria decreased to levels similar to those observed at the beginning of the study. A total of 2 monthly counts were greater than the official regulatory limit (Fig. 5).

Longitudinal trends of bulk milk concentration of streptococci were similar to those observed for total bacteria (Fig. 5). Trends of bulk milk concentration of coliforms were similar for farms B and C, and showed great variability during the study (Fig. 5). Farm A's bulk milk concentration of coliforms was below the detection limit of the culturing method throughout the study. Bulk milk concentrations of total bacteria ($P=0.36$), streptococci ($P=0.12$), and coliforms ($P=0.91$) were not different among seasons of the year.

Bedding dry density ($P<0.01$), wet density ($P=0.02$) and organic matter ($P=0.02$) were unconditionally associated with bulk milk concentration of total bacteria, but only wet density remained in the final model (positive association, Table 5). Bedding dry density was the only variable associated (positive association) with bulk milk concentration of streptococci ($P=0.02$) and organic

Table 4
Unconditional associations ($P < 0.05$) between explanatory variables and study outcomes.

Outcome (bold letters) and explanatory variables	Coefficient	SE	*P*-value
Incidence of clinical mastitis – environmental pathogens[a]			
Bedding moisture (%)	0.055	0.013	< 0.01
Bedding dry density (kg/m^3)	−0.006	0.002	< 0.01
Bedding carbon–nitrogen ratio	0.050	0.014	< 0.01
Bedding pH	−0.948	0.332	< 0.01
Bedding age (months)			0.01
≤ 4	0.869	0.273	
5–8	0.271	0.308	
≥ 9	Reference		
Incidence of clinical mastitis – all pathogens[a]			
Bedding moisture (%)	0.050	0.013	< 0.01
Bedding dry density (kg/m^3)	−0.004	0.002	0.04
Bedding carbon–nitrogen ratio	0.038	0.016	0.03
Prevalence of subclinical mastitis[b]			
Leg cleanliness score (herd weighted mean)	0.148	0.071	0.04
Incidence of subclinical mastitis[c]			
Leg cleanliness score (herd weighted mean)	0.279	0.129	0.04
Udder cleanliness score[d]			
Bedding dry density (kg/m^3)	0.001	0.001	0.02
Bedding age (months)			< 0.01
Intercept	1.41	0.05	
≤ 4	−0.18	0.08	
5–8	−0.26	0.08	
≥ 9	Reference		
Teat cleanliness score[d]			
Bedding wet density (kg/m^3)	0.002	0.001	< 0.01
Bedding dry density (kg/m^3)	0.002	0.001	0.03
Bedding organic matter (%)	−0.02	0.01	0.03
Bedding concentration of total bacteria (log10 cfu/g)	−0.37	0.14	0.01
Flank cleanliness score[d]			
Bedding wet density (kg/m^3)	0.001	0.001	0.03
Bedding organic matter (%)	−0.02	0.01	0.04
Leg cleanliness score[d]			
Bedding wet density (kg/m^3)	0.002	0.001	0.04
Bulk tank milk – concentration of total bacteria[e]			
Bedding wet density (kg/m^3)	0.004	0.002	0.02
Bedding dry density (kg/m^3)	0.006	0.002	< 0.01
Bedding organic matter (%)	−0.06	0.02	0.02
Bulk tank milk – concentration of streptococci[c]			
Bedding dry density (kg/m^3)	0.006	0.003	0.02
Bulk tank milk – concentration of coliforms[c]			
Bedding organic matter (%)	−0.07	0.03	0.04

[a] Associations were derived from generalized linear mixed models based on a binomial distribution. Model coefficients are log (odds ratio). Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day.

[b] Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200,000 cells/mL at a given test day.

[c] Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200,000 to ≥ 200,000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200,000 cells/mL on the previous test day (cows at risk).

[d] Associations were derived from linear mixed models based on a normal distribution. Cleanliness scoring was performed before milking, within the CBP area, sampling 100%, 100%, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

[e] Associations were derived from linear mixed models based on a normal distribution.

matter was the sole predictor (negative association) for the concentration of coliforms ($P = 0.049$, Table 5).

4. Discussion

Results of this study provide novel information on biosecurity and management of milk quality in CBP systems. By monitoring 3 herds during the course of the study, we did not observe outbreaks of environmental subclinical or clinical mastitis, nor a concerning prevalence of IMI caused by environmental pathogens that are refractory to conventional treatments (such as *Nocardia* spp and *Prototheca* spp), which have been suspected to be prevalent in this unique organic environment. The identification of compost bedding factors associated with mastitis epidemiologic indexes, cow cleanliness, and microbial quality of bulk tank milk, such as density and moisture, supports the hypothesis that changes in bedding characteristics can affect the occurrence of environmental mastitis and the quality of milk produced in CBP systems.

Because of the high bacterial concentrations found in compost bedding (Barberg et al. 2007a, 2007b; Black et al., 2014), researchers have recommended adoption of excellent pre-milking hygienic procedures, and that bedding be maintained dry and not adherent to cows (Barberg et al. 2007a, 2007b; Black et al., 2013). Nonetheless, these associations had not been scientifically demonstrated. Results of the present study indicate that bedding factors such as density and moisture are associated with mastitis epidemiologic indexes and cow cleanliness. Wet density is related to particle size and moisture. As particle size decreases and moisture increases, bedding becomes denser and more compacted.

Another consequence of denser bedding is the decrease in bedding aeration. An aerobic composting process is important to maintain an efficient microbiological decomposition of the organic material. When bedding is loose and aerated, temperature in the deep layer (20 cm deep) increases to approximately 40–55 °C as a consequence of microbial activity (Barberg et al. 2007a, 2007b; Black et al., 2014). Thus, a combination of effective aeration and high deep layer temperature facilitates moisture loss and maintenance of a dry environment to cows.

In the present study, bedding moisture was the main predictor for the incidence of environmental clinical mastitis. Bedding moisture has been reported as one of most difficult characteristics to control in CBP systems (Lobeck et al., 2011) because it can be influenced by bedding management and weather conditions. Inadequate aeration, high animal density, and lack of ventilation can lead to increased moisture levels (Janni et al., 2007; Black et al., 2014). Weather factors include air humidity and rainwater entering the CBP.

There could be different mechanisms by which bedding moisture is associated with increased risk of environmental mastitis. Moisture is essential for bacterial growth and increase in moisture levels can favor multiplication of microorganisms and therefore increase exposure to cows. Another factor that needs to be studied is the transfer of bacteria to the teat skin. It can be

Table 5
Associations between explanatory variables and study outcomes derived from multivariable analyses.

Outcome (bold letters) and explanatory variables	Coefficient	SE	*P*-value
Incidence of clinical mastitis – environmental pathogens[a]			
Intercept	−5.457	0.638	
Bedding moisture (%)	0.055	0.013	< 0.01
Incidence of clinical mastitis – all pathogens[a]			
Intercept	−4.596	0.635	
Bedding moisture (%)	0.056	0.010	< 0.01
Prevalence of subclinical mastitis[b]			
Intercept	−1.101	0.126	
Leg cleanliness score (herd weighted mean)	0.148	0.071	0.04
Incidence of subclinical mastitis[c]			
Intercept	−2.004	0.232	
Leg cleanliness score (herd weighted mean)	0.279	0.129	0.04
Udder cleanliness score[d]			
Intercept	1.40	0.67	
Bedding wet density (kg/m^3)	0.002	0.001	0.74
Wet density × Farm			< 0.01
Farm A	0.001	0.002	
Farm C	0.004	0.001	
Farm B	Reference		
Teat cleanliness score[d]			
Intercept	0.89	0.34	
Bedding wet density (kg/m^3)	0.002	0.001	< 0.01
Flank cleanliness score[d]			
Intercept	0.87	0.24	
Bedding wet density (kg/m^3)	0.001	0.001	0.03
Leg cleanliness score[d]			
Intercept	0.95	0.35	
Bedding wet density (kg/m^3)	0.002	0.001	0.04
Bulk tank milk – concentration of total bacteria[e]			
Intercept	1.53	0.82	
Bedding wet density (kg/m^3)	0.004	0.002	0.02
Bulk tank milk – concentration of streptococci[e]			
Intercept	0.96	0.73	
Bedding dry density (kg/m^3)	0.006	0.25	0.02
Bulk tank milk – concentration of coliforms[e]			
Intercept	3.55	1.22	
Bedding organic matter (%)	−0.07	0.03	0.04

[a] Associations were derived from generalized linear mixed models based on a binomial distribution. Model coefficients are log (odds ratio). Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day.

[b] Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200000 cells/mL at a given test day.

[c] Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200,000 to ≥ 200,000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200,000 cells/mL on the previous test day (cows at risk).

[d] Associations were derived from linear mixed models based on a normal distribution. Cleanliness scoring was performed before milking, within the CBP area, sampling 100%, 100%, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

[e] Associations were derived from linear mixed models based on a normal distribution.

hypothesized that smaller (denser bedding) and moisten bedding particles adhere to cows and facilitate the transfer of bacteria to the skin. Bedding wet density was positively associated with all cleanliness scores studied here. Conversely, if bedding is maintained dry and loose, transferring of bacteria to the skin may be minimized. Further research should be conducted to study physical characteristics of bedding particles (such as particle size and water retention) that can affect the transfer of bacteria to the teat skin.

It was interesting to observe that bedding moisture decreased dramatically on farm C after installation of fans over the bedding area (Favero et al., 2015). The bedding became dry and not adherent to cows, which remained in excellent hygienic conditions. Even after reaching moisture levels as low as 30%, bedding deep temperature (20 cm deep) was maintained > 40 °C, indicating that it is possible to maintain dry bedding without compromising microbiological activity.

A negative association was found between bedding pH and the incidence of environmental clinical mastitis. It has been consistently demonstrated that, during the composting process, pH increases with time. Perhaps the alkaline pH levels found in this study (overall mean > 8.8 for all farms) inhibit growth of environmental mastitis pathogens. The effect of pH on bedding bacterial populations needs to be further studied because it could be managed on the farms.

Aging of the composting process results in changes such as decrease in both density and organic matter, and increase in the water retention capacity of the material (Kiehl, 1985). As a result of the bivariate analysis, we observed that the risk of environmental mastitis decreased progressively as bedding became older. This changes could be explained by some characteristics of new bedding material such as high moisture content, organic matter, and carbon–nitrogen ratio (Favero et al., 2015). Thus, the ideal time of bedding total replacement needs to be studied because it may influence the risk of mastitis.

The distribution of environmental pathogens isolated from clinical and subclinical mastitis cases was similar to those reported from different countries and housing systems (Olde Riekerink et al., 2008; Jobim et al., 2010; Lago et al., 2011; Oliveira and Ruegg, 2014). In agreement with those studies, “no growth” was the most frequent culture result (33.3% of all cases), and *E. coli* (9.6%) and environmental streptococci (17.5% of all cases) were the most frequent pathogens isolated from clinical mastitis cases.

The distribution of pathogens isolated from subclinical mastitis was characterized by a high prevalence of contagious pathogens. *C. bovis*, *S. agalactiae*, and *S. aureus* are still highly prevalent on Brazilian dairies (Bueno et al., 2008) due to the lack of adoption of mastitis control programs. Except for contagious pathogens, coagulase-negative staphylococci and environmental streptococci were the most prevalent pathogens isolated form subclinical mastitis cases, as previously reported (Gianneechini et al., 2002; Wallace et al., 2004; Pol and Ruegg, 2007).

Cow cleanliness have been associated with milk quality outcomes at the cow (prevalence of mastitis; Schreiner and Ruegg, 2003) and herd (bulk tank milk SCC; Barkema et al., 1998; Ellis et al., 2007; Dufour et al., 2011) levels. Results of the present study corroborate with those of North American studies that have demonstrated that cows housed in the CBP are maintained in good hygienic conditions, comparable to standard systems such as well managed sand-bedded freestalls (Lobeck et al., 2011). Mean cow cleanliness score (ranging from 1 = clean to 5 = very dirty) was 2.6 (Barberg et al. 2007a, 2007b) e 3.1 (Shane et al., 2010) for a population of Minnesota CBP. Likewise, Black et al. (2013) reported mean cleanliness score of 2.2 (scale ranging from 1 = clean to 4 = very dirty) for a group of Kentucky CBP. Researchers found significant seasonal variation and reported difficulties controlling cow cleanliness during humid and rainy weather. In Brazilian conditions, where weather differences among seasons are not as extreme as in North America, cows remained in excellent hygienic conditions throughout the year studied. Housing cows in well-managed CBP could be an interesting alternative to one of the most prevalent systems worldwide, the semi-confinement (dry lot), in which cows are usually maintained in poor hygienic

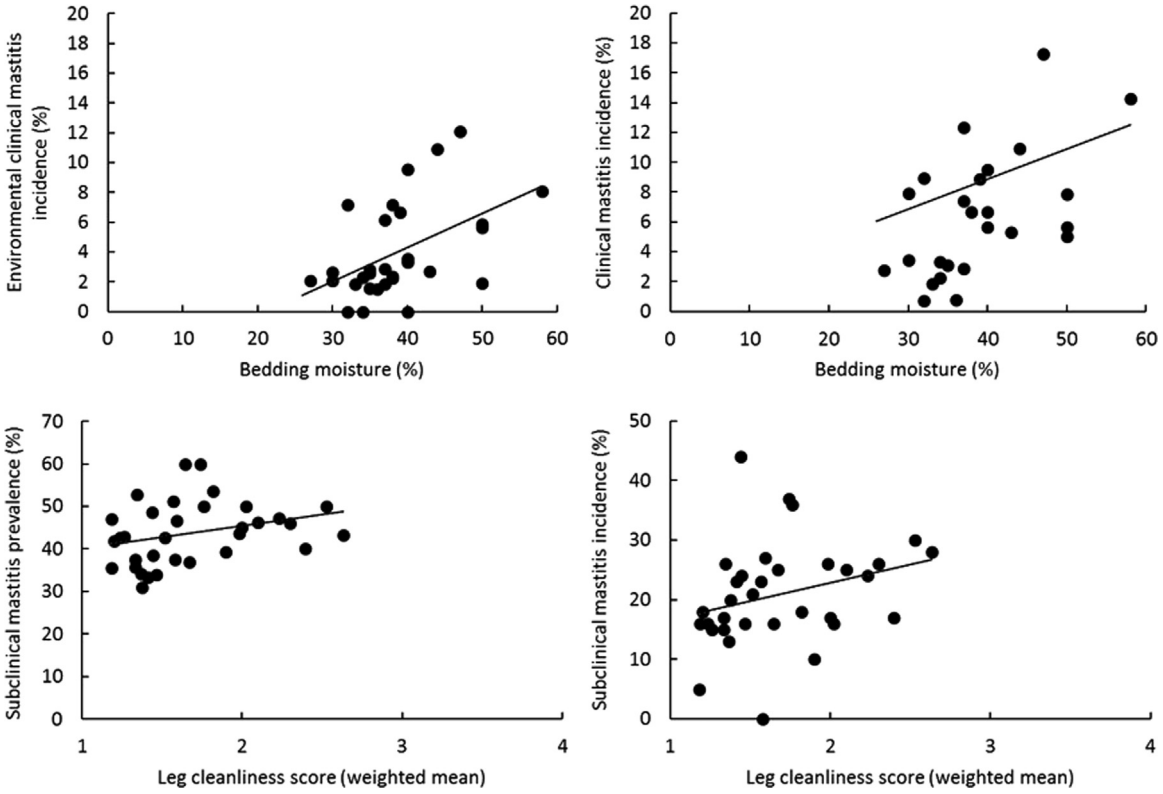

Fig. 2. Associations between mastitis epidemiologic outcomes and bedding moisture or leg cleanliness score. Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day. Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC $>200{,}000$ cells/mL at a given test day. Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from $<200{,}000$ to $\geq 200{,}000$ cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was $<200{,}000$ cells/mL on the previous test day (cows at risk). Results of cleanliness scoring were reported as weighted means, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

conditions.

4.1. Study limitations

One limitation of this study was the high prevalence of

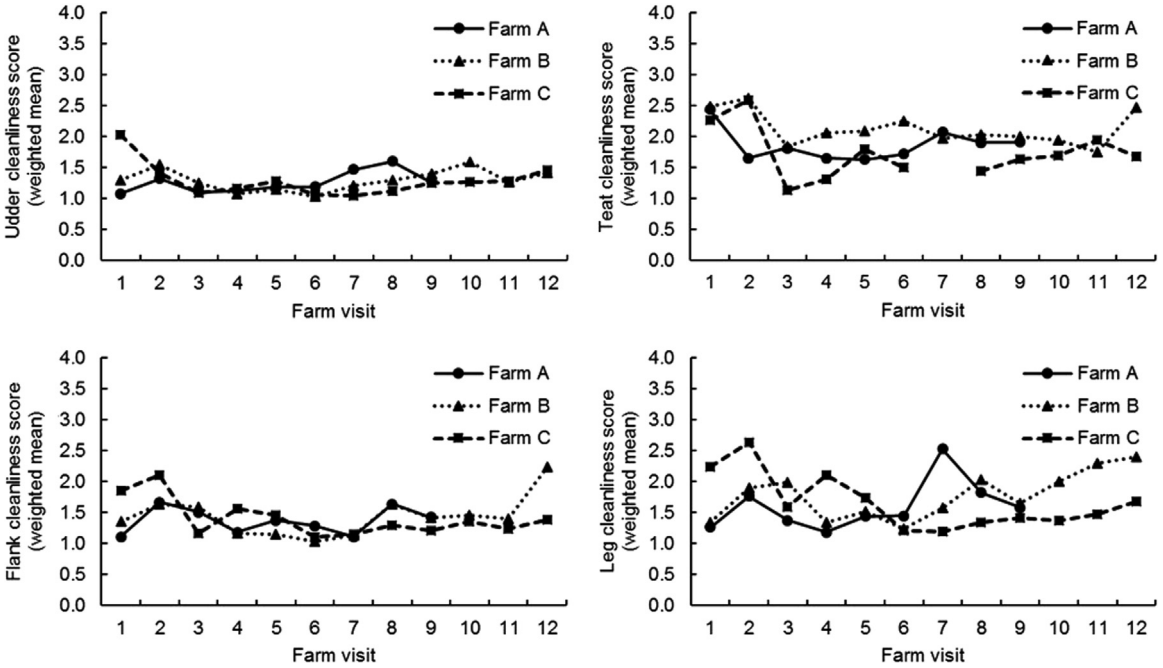

Fig. 3. Monthly udder, teat, flank and leg cleanliness scores, by farm. Cleanliness scoring was performed before milking, within the CBP area, sampling 100%, 100%, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

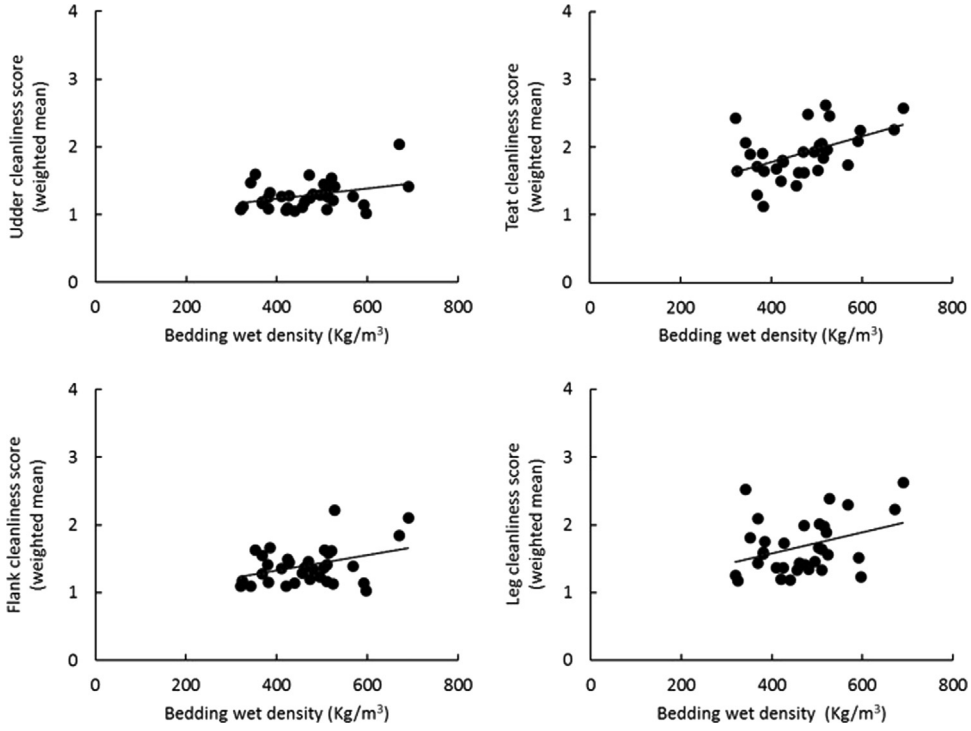

Fig. 4. Associations between bedding wet density and cow cleanliness scores. Cleanliness scoring was performed before milking, within the CBP area, sampling 100%, 100%, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty). Wet density was estimated using composite bedding samples from 12 areas of the CBP, collected from the surface and deep (20 cm) layers.

contagious pathogens observed in the herds studied, which probably resulted in difficulties to separate the effect of bedding characteristics on the occurrence of subclinical mastitis (defined based on SCC). If possible, selecting herds where contagions pathogens have been controlled will be important for future studies.

It is important to emphasize that the present study was not designed to assess whether shifting from other systems to the CBP would improve milk quality and mastitis control. Thus, longitudinal trends in mastitis epidemiologic indexes observed on the 3 farms could have been affected by several factors not related to compost bedding, such as milking machine, milking management, and profile of pathogens found on each herd. Moreover, because bedding materials were different among farms, assessment of the effect of bedding type (e.g., sawdust versus peanut shell) on mastitis epidemiologic indexes or animal hygiene should not be encouraged because one farm might not be representative of a greater population of farms that use the same bedding type. Likewise, comparison of pathogen profiles, mastitis epidemiologic indexes, and bedding characteristics among the 3 farms cannot answer relevant scientific questions because biological differences cannot be explained when derived from the study design used here.

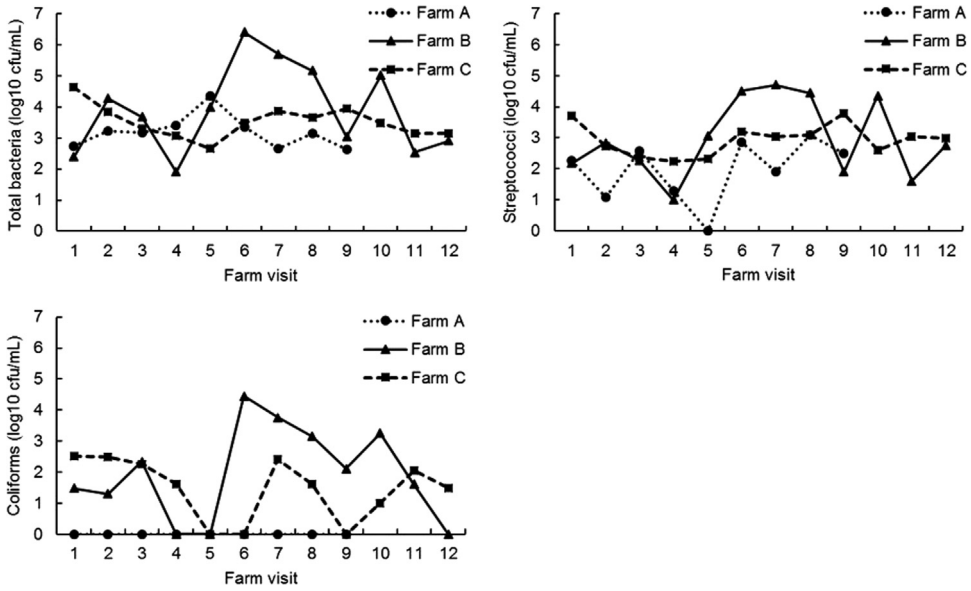

Fig. 5. Monthly bulk milk concentration of total bacteria, streptococci, and coliforms by farm.

5. Conclusions

Results of this study suggest that changes in compost bedding characteristics such as density and moisture can affect the occurrence of environmental clinical mastitis, cow cleanliness, and microbial quality of milk produced in CBP systems. Managing bedding to remain dry and loose will result in cleaner animals with decreased risk of mastitis. Cow cleanliness scoring can be useful to aid bedding management and asses the risk of subclinical mastitis.

Coliforms and environmental streptococci were the most frequent environmental pathogens isolated from clinical mastitis cases. The prevalence of IMI caused by *Nocardia* spp, yeast, *Prototheca* spp, *Serratia* spp, *Pseudomonas* spp, and other environmental pathogens that can cause outbreaks of untreatable mastitis was not concerning. No outbreaks of environmental mastitis were observed during the course of the study.

Conflict of interest statement

None.

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