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Effects of bovine subclinical mastitis caused by *Corynebacterium* spp. on somatic cell count, milk yield and composition by comparing contralateral quarters

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ABSTRACT

Subclinical mastitis caused by *Corynebacterium* spp. (as a group and at the species level) was investigated by evaluating contralateral (healthy and infected) mammary quarters for somatic cell count (SCC), milk yield and composition. Selection of cows with subclinical mastitis caused by *Corynebacterium* spp. was performed by microbiological culture of composite samples collected from 1242 dairy cows from 21 dairy herds. For each of the selected cows, milk yield was measured and milk samples were collected at the mammary quarter level (i.e., 1140 mammary samples collected from 285 cows) for analysis of milk composition and SCC. The identification of *Corynebacterium* spp. isolates was performed by 16S rRNA gene sequencing.

One hundred and eighty *Corynebacterium* spp. isolates were identified, of which 167 (92.77%) were *C. bovis* and eight (4.44%) non-*C. bovis*; for five of the *Corynebacterium* spp. isolates (2.77%), sequencing of 16S rRNA genes did not allow identification at the species level. Mammary quarters infected with *Corynebacterium* spp. as a group had a higher geometric mean SCC (197,900 cells/mL) than healthy contralateral mammary quarters (85,800 cells/mL). Species of *Corynebacterium* non-*C. bovis* were infrequently isolated and did not change SCC, milk yield or milk solid contents when evaluated at the contralateral quarter level. Although *C. bovis* infection showed no effect on milk yield, fat, protein, casein or total solids in milk, it increased SCC and decreased lactose and milk solids non-fat content.

Introduction

Mastitis is the most costly disease affecting dairy cattle (Djabri et al., 2007; Halasa et al., 2007) and it presents most commonly in the subclinical form caused by bacteria (Djabri et al., 2002; Andersen et al., 2010). Subclinical mastitis increases the somatic cell count (SCC) and reduces the milk yield of dairy cows (Forsbäck et al., 2009). Losses occur due to damage caused by microorganisms to the secretory tissues of the mammary gland and the breakage of cell junctions, which may result in the permanent loss of milk synthesis capacity (Auldist et al., 1995).

Adoption of various strategies to control mastitis during the last few decades has resulted in a decreased frequency of clinical and subclinical mastitis caused by major pathogens. However, the frequency of subclinical mastitis caused by minor pathogens is still a challenge for dairy farmers (Haltia et al., 2006; Souto et al., 2008; Taponen and Pyorala, 2009). *Corynebacterium bovis* is a contagious microorganism frequently isolated from cases of subclinical mastitis. Despite the high frequency of isolation, *Corynebacterium* spp. are considered minor pathogens of mastitis (Bradley and Green, 2005; Schukken et al., 2009). The effects of *Corynebacterium* spp. on milk yield and composition remain largely unknown.

C. bovis has been described as a commensal of bovine mammary glands (Brooks and Barnum, 1984) and quarters infected with this bacterium may be less susceptible to intramammary infections caused by other mastitis pathogens (Rainard and Poutrel, 1982; Sordillo et al., 1989; Lam et al., 1997; Schukken et al., 1999; Blagitz et al., 2013). It has been reported that *C. bovis* only colonises the teat canal of dairy cows, and as such, it has been used as an indicator of milking hygiene (Watts et al., 2000). *C. bovis* has however

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also been reported to colonize the teat cistern, gland cistern, and mammary parenchyma (Benites et al., 2003).

Different methods have been used to estimate production losses due to subclinical mastitis in dairy cows. The most commonly used technique is based on the SCC for estimating production losses between herds, among cows, between mammary quarters, or even between identical twin cows (Petrovski et al., 2006; Pearson et al., 2013). The majority of studies have evaluated milk yield and milk composition between healthy and infected mammary quarters based on SCC (Barkema et al., 1997; Wilson et al., 1997; Forsbäck et al., 2010a, 2010b). However, these studies compared the mammary quarters of different cows and this may represent a bias due to heterogeneity between different animals and herds. To the best of our knowledge, no study has reported the effect of subclinical mastitis caused by *Corynebacterium* spp. on SCC, milk yield and composition by comparing healthy and infected contralateral mammary quarters. This approach could minimize confounding factors at both cow and herd level (such as the cow's immune status at the time of infection, management systems or environmental challenge). Such an experimental design may prove to be more reliable in evaluating the effect of *Corynebacterium* spp. on SCC, milk quality and yield.

The objectives of the present study were: (1) to determine the effect of subclinical *Corynebacterium* spp. mastitis as a group and at a species level on milk yield and SCC by evaluating the contralateral (healthy and infected) mammary quarters, and (2) to determine the effect of subclinical mastitis caused by *Corynebacterium* spp. on concentrations of milk fat, protein, lactose, casein, total solids and solids non fat.

Materials and methods

Herds and cow selection and sample collection

Twenty-one dairy herds located in the Mid-west area of São Paulo State, Brazil, were enrolled in this study over a 14-month sample collection period. To be enrolled in the study, herds needed to meet the following requirements: (1) good cow identification and recording systems and (2) proper milking management and mastitis control practices (including disposal of first streams of milk, disinfection of teats prior to and after milking using disinfectant solutions, drying teats with disposable towels, and treatment of clinical mastitis cases).

Cows were enrolled in the study based on microbiological cultures performed after the collection of two milk samples. First, composite milk samples (milk from all mammary quarters) were collected aseptically from each cow for screening for *Corynebacterium* spp. subclinical mastitis following National Mastitis Council guidelines (Oliver et al., 2004). After microbiological culture of the first milk sample, cows with *Corynebacterium* spp. were individually sampled at the quarter level within 15 days. At the second sample collection, milk yield was measured at the quarter level, and quarter milk samples were collected for microbiological culture and for analyses of composition and SCC.

To determine quarter milk yield and for analyses of composition and SCC, mammary quarters were milked individually using a bucket milking system (Intermaq Milking Systems), which was connected to the milking machine vacuum line. The equipment included a pulsator and a cluster of four liners connected to individual silicone tubing equipped with valves for vacuum release. The system allowed the milk to flow from each mammary quarter to a four-compartment stainless steel bucket. Quarter milk was stirred and weighed, and milk samples (40 mL) were collected into plastic tubes containing the antimicrobial Bronopol (2-bromo-2-nitropropane-1,3-diol) as preservative (0.05 g/100 mL milk) according to the International Dairy Federation guidelines (IDF (International Dairy Federation), 1995). Samples were kept refrigerated (4–7 °C) until analysis of composition and SCC.

This study (protocol number 2231/2011) was in conformity with the Ethical Principles in Animal Research adopted by the Ethical Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science, at the University of São Paulo.

Microbiological culture procedures

Microbiological cultures of milk samples were performed according to the National Mastitis Council guidelines (Oliver et al., 2004). Briefly, 10 μL of milk was inoculated on blood agar with 5% defibrinated bovine blood. Inverted plates were incubated aerobically at 37 °C for 72 h and observed every 24 h for colonial characteristics (shape, size, number, and colour), haemolytic ability (presence and type), and possible contamination. Smears were stained by Gram and a catalase test was performed to determine the morphology and differentiation between *Corynebacterium* spp. genera.

Milk samples with more than two morphologically different colonies were considered contaminated. Briefly, after growth on blood agar, a single small, circular colony (approximately 1 mm in diameter) with a white-grey or yellowish colour and a slightly raised, dry and/or flaky, non-haemolytic appearance and Gram-positive rods was considered as *Corynebacterium* spp. Each isolate of *Corynebacterium* spp. was inoculated in a tube containing 1 mL of trypticase soy broth (TSB, Becton Dickinson). Tubes were incubated at 37 °C for 48 h, and centrifuged at 10,000 *g* for 10 min. Pellets were washed with 1 mL of sterile Milli-Q water, centrifuged again under the same conditions, and 1 mL of TSB containing 10% glycerol was added, followed by homogenization. A loop from each microtube was streaked on a plate containing tryptic soy agar (TSA, Becton, Dickinson) supplemented with 1% Tween 80 (Sigma Chemical Company) for confirmation (Watts et al., 2000). Only microtubes containing *Corynebacterium* spp. were cryopreserved at –20 °C.

Milk composition and SCC

Concentrations of milk fat, protein, lactose, casein, total solids and solids non-fat were determined by infrared absorption system using a milk analyser (MilkoScan FT+, Foss Electric). The SCC was determined by flow cytometry using a high-capacity somatic cell counter (Fossomatic FC, Foss Electric).

Corynebacterium *spp. subclinical mastitis*

Mammary quarters were considered to have subclinical mastitis when milk samples showed isolation of >10 colonies (1000 cfu/mL) of *Corynebacterium* spp. (Andersen et al., 2010; Dohoo et al., 2011a, 2011b). Mammary quarters were considered healthy when they had no growth of bacteria within 72 h incubation of milk from either sampling and an SCC of <200,000 cells/mL (Bradley and Green, 2005).

Gene sequencing for identification of Corynebacterium *spp.*

The isolates were identified genotypically by 16S rRNA sequencing analysis as described by Watts et al. (2000). A DNA extraction protocol was performed by adding lysozyme buffer solution, lysozyme (10 mg/mL; Merck) and resin solution (10 mg/mL, Chelex100 resin, Bio-Rad Laboratories), heated in a thermocycler at 99 °C for 10 min (Vaneechoutte et al., 1995). All isolates of *Corynebacterium* spp. were submitted to amplification with a pair of primers (F – 5′GCGAACGGGTGAGTAACACG3′ and R – 5′TCTGCGATTACTAGCGACTCCG3′) as described by Huxley et al. (2004). Isolates with no amplification were submitted to a second round of PCR using pairs of primers (F – 5′AGAGTTTGATCCTGGCTCAG3′ and R – 5′AAGGAGGTGATCCAGCCGCA3′) (Watts et al., 2000).

The second protocol amplification was used to amplify non-*C. bovis* species. All PCR reactions targeted the 16S rRNA gene. After electrophoresis analysis, the purified PCR products were sequenced unidirectionally using the reverse primers. All sequences obtained from the 16S rRNA gene sequences were analysed with GenBank[1] Library Reference online data. Isolates were identified at the species level when their similarities to reference sequences were ≥98% (Watts et al., 2000).

Statistical analysis

The effects of subclinical mastitis caused by *Corynebacterium* spp. as a group and at species level on quarter milk yield, composition and SCC were evaluated. The milk yield, composition and SCC of infected quarters were compared with the same variables from healthy contralateral quarters by a strip-plot design by splitting the anterior and posterior mammary quarters in halves. Thus, the left and right contralateral mammary quarters were compared within the half and cow using the following mixed model:

$$Y = \mu + IMI + Q + (IMI \times Q) + \{[C + C(H) + (C \times Q) + [IMI \times C(Q)]\} + e$$

where Y is the dependent variable, μ is the overall mean, IMI and Q are the fixed effects of variables in that IMI is the presence or absence of subclinical mastitis caused by *Corynebacterium* spp., and Q is the contralateral quarters (right or left) within cow; IMI × Q is the interaction between the fixed effects; C is the random effect of cow, C(H) is the random effect of cows nested within herd, and *e* is the random error term.

Somatic cell count at quarter level was converted to linear scores (LS) by the formula (Schukken et al., 2003) and was presented as geometric mean:

$$LS\ SCC = \log_2 (SCC/100) + 3$$

Statistical models were analysed using the MIXED procedure of SAS version 9.2. Statistical significance was defined at $P < 0.05$.

[1] See: http://www.ncbi.nlm.nih.gov/nuccore/AF311433.1 (accessed 2 August 2015).

Results

Microbiological cultures and sequencing of 16S rRNA genes

Composite milk samples were collected from 1242 dairy cows during the first sample collection to screen cows with subclinical mastitis caused by *Corynebacterium* spp. During the second sample collection, milk yield was measured and milk samples were collected at quarter level from all cows ($n = 285$) previously diagnosed with *Corynebacterium* spp. subclinical mastitis. Thus, milk samples from 1140 mammary quarters were submitted for microbiological culture and milk composition and SCC.

After culture and sequencing of 16S rRNA genes, a total of 180 *Corynebacterium* spp. isolates were identified at species level (frequency 15.79%) and the results have been reported previously (Gonçalves et al., 2014). *C. bovis* was the most isolated *Corynebacterium* species ($n = 167$; 92.78%) from subclinical mastitis cases at the quarter level. Eight isolates (4.45%) were identified as non-*C. bovis*, namely, *C. amycolatum* ($n = 1$); *C. aquilae* ($n = 2$); *C. auriscanis* ($n = 2$); *C. casei* ($n = 1$); *C. efficiens* ($n = 1$); and *C. xerosis* ($n = 1$). For five *Corynebacterium* spp. isolates (2.77%), sequencing of 16S rRNA genes did not allow identification at the species level but the methodology suggested that the isolates could be *C. xerosis*, *C. freneyi* or *C. hansenii*; and two isolates could be *C. auriscanis* or *C. resistens.*

SCC, milk yield and composition

We hypothesized that there is no difference in milk yield and composition between contralateral healthy quarters in the same udder of a cow and so evaluated 60 pairs of contralateral mammary quarters (both healthy) as controls to observe whether milk yield and composition of samples from the same udder were similar when the quarters presented SCC <200,000 cells/mL and were culture negative (Table 1). There was no difference in milk yield, composition (fat content, protein, casein, lactose, total solids and solids non-fat) and SCC between healthy contralateral quarters.

Table 1
Milk yield, composition and somatic cell count of pairs of contralateral healthy quarters ($n = 60$).

Item	Contralateral healthy quarters[a]		SEM[b]	*P*
	Right	Left		
Milk yield[c]	1.91	1.90	102.28	0.896
FCM 3.5%[d]	1.95	1.95	170.47	0.819
SCC[e]	59,760	60,410	6.77	0.945
LS SCC[f]	2.257	2.273	0.008	0.572
Milk composition (g/kg)				
Fat	37.22	36.22	1.45	0.625
Protein	31.89	31.85	0.49	0.948
Casein	24.68	24.69	0.39	0.995
Lactose	46.19	46.11	0.24	0.822
Total solids[g]	124.92	123.75	1.76	0.639
Solids non fat[h]	87.70	87.53	0.56	0.837
Milk components yield (g/milking/quarter)				
Fat	73.72	71.32	5.51	0.757
Crude protein	61.66	60.42	3.52	0.803
Casein	19.74	19.40	1.20	0.844
Lactose	91.64	89.98	5.72	0.837
Total solids	245.88	240.24	15.16	0.792
Solids non fat	172.16	168.92	10.29	0.824

[a] Mammary contralateral healthy quarters (SCC < 200,000 cells/mL with no positive culture).
[b] Standard error of the mean.
[c] Quarter milk yield (kg) from a single milking of the day.
[d] 3.5% fat-corrected milk yield.
[e] Average geometric mean somatic cell count (cells/mL).
[f] Somatic cell count converted to linear scores.

A total of 92 (46 pairs) contralateral mammary quarters were evaluated. Milk yield, composition and SCC from 46 mammary quarters with subclinical mastitis caused by *Corynebacterium* spp. as a group were compared with contralateral healthy mammary quarters. Quarters with *Corynebacterium* spp. subclinical mastitis had higher geometric mean SCC (197,900 cells/mL) than the contralateral quarters (85,730 cells/mL). Quarters with *Corynebacterium* spp. subclinical mastitis produced less lactose (45.50 g/kg) than the contralateral quarters (45.91 g/kg). There was no effect ($P > 0.05$) of subclinical mastitis caused by *Corynebacterium* spp. on milk yield and concentrations of milk fat, protein, casein and total solids. The average milk yield from a single milking was 1.92 kg for *Corynebacterium* spp. infected quarters and 1.97 kg for healthy quarters (Table 2).

After species identification of *Corynebacterium* spp. isolates, *C. bovis* and non-*C. bovis* species were separated. Milk yield, composition and SCC from 35 quarters with subclinical mastitis caused by *C. bovis* were compared with the contralateral healthy quarters. As expected, quarters infected with *C. bovis* had higher SCC (174,280 cells/mL) than healthy contralateral quarters (87,770 cell/mL). However, no effects ($P > 0.05$) of subclinical mastitis caused by *C. bovis* were observed on milk yield or on concentrations of milk fat, protein, casein and total solids. On the other hand, quarters with *C. bovis* subclinical mastitis produced less lactose and solids non-fat ($P < 0.05$) than the contralateral quarters (Table 2).

We combined all non-*C. bovis* species isolates because a minimum number of isolates was required to allow statistical evaluation at the contralateral quarter level. Therefore, milk yield, composition and SCC from 11 quarters with subclinical mastitis caused by non-*C. bovis* species were compared with their contralateral healthy quarters. No effects ($P > 0.05$) of subclinical mastitis caused by non-*C. bovis* were observed on SCC, milk yield or composition (Table 2).

Discussion

The aim of this study was to evaluate how subclinical mastitis-causing *Corynebacterium* spp. affects the SCC, yield, and composition of milk. Subclinical mastitis caused by *C. bovis* increased SCC and decreased lactose and solids non-fat content when healthy and infected contralateral mammary quarters were compared; however, subclinical mastitis caused by non-*C. bovis* had no effect on SCC, milk yield or composition.

In general, *Corynebacterium* spp. and coagulase negative *Staphylococci* (CNS) are considered minor pathogens causing a mild increase of SCC in response to the intramammary infection. However, both groups have been frequently isolated from cases of subclinical mastitis (Gonçalves et al., 2014; Tomazi et al., 2015). Tomazi et al. (2015) described 11 CNS species causing subclinical intramammary infections. *Staphylococcus chromogenes* was the species of CNS most frequently isolated from subclinical mastitis cases (74.07%), and similar to subclinical mastitis caused by *C. bovis*, produced only a mild increase in SCC with no effect on milk yield.

Frequency of Corynebacterium *spp. isolates*

Our results indicate a high isolation frequency of *Corynebacterium* spp. (15.79%) similar to other studies reporting a high frequency of minor pathogen subclinical mastitis (ranging from 14.6% to 17.7%) (Haltia et al., 2006; Malek dos Reis et al., 2011). Schukken et al. (2009) also found a high isolation frequency of minor pathogens such as *C. bovis* (5.5%) and CNS (15%). A high frequency (14.6%) of *C. bovis* in mammary quarters was reported by Haltia et al. (2006), with an SCC of 303,000 cells/mL, which was greater than those found in the present study (174,280 cells/mL). Haltia et al. (2006) reported that the high frequency may be associated with an absence of teat dipping, since only 7/25 farms (28%) used this procedure. In our

Table 2
Milk yield, composition and somatic cell count of contralateral mammary quarters with subclinical mastitis caused by *Corynebacterium* spp. as a group and at species level.

Item	*Corynebacterium* spp.		SEM[a]	*P*	Non-*C. bovis*		SEM	*P*	*C. bovis*		SEM	*P*
	Infected	Healthy			Infected	Healthy			Infected	Healthy		
n	46	46			11	11			35	35		
Milk yield[b]	1.92	1.97	181.44	0.595	2.18	2.28	353.90	0.753	1.85	1.89	198.61	0.663
FCM 3.5%[c]	1.95	2.00	184.35	0.598	2.22	2.31	358.20	0.753	1.88	1.92	201.45	0.664
SCC[d]	197,900	85,730	32.04	0.011	239,680	78,970	97.50	0.259	174,280	87,770	27.69	0.015
LS SCC[e]	3.985	2.778	0.6035	<0.01	4.261	2.659	0.801	0.071	3.801	2.812	0.4945	<0.01
Milk composition (g/kg)												
Fat	34.79	34.91	1.69	0.836	32.31	33.95	2.44	0.289	35.44	35.12	1.97	0.611
Protein	32.49	32.25	0.66	0.334	32.94	31.68	0.96	0.226	32.44	32.55	0.78	0.262
Casein	25.18	24.97	0.53	0.288	25.42	24.45	0.81	0.234	25.20	25.27	0.61	0.399
Lactose	45.50	45.91	0.39	0.006	46.43	46.71	0.71	0.255	45.33	45.75	0.43	0.019
Total solids	122.47	122.77	1.98	0.597	121.45	122.13	2.74	0.586	122.83	123.06	2.34	0.717
Solids non fat	87.69	87.86	0.73	0.482	89.14	88.19	1.25	0.270	87.42	87.97	0.84	<0.01
Milk components yield (g/milking/quarter)												
Fat	65.51	66.71	6.12	0.730	68.77	71.62	11.20	0.768	64.81	65.77	7.06	0.794
Protein	61.55	63.46	5.43	0.541	71.80	74.17	10.81	0.808	59.12	61.05	5.98	0.514
Casein	47.75	49.24	4.23	0.537	55.60	57.51	8.42	0.800	45.95	47.44	4.66	0.515
Lactose	89.87	93.35	8.74	0.475	104.06	109.65	17.18	0.701	86.21	89.14	9.53	0.545
Total solids	235.73	242.88	21.51	0.559	266.24	277.88	41.56	0.750	228.15	234.47	24.03	0.601
Solids non fat	170.22	176.17	15.83	0.506	197.46	206.26	31.32	0.747	163.35	168.71	17.38	0.535

[a] Standard error of the mean.
[b] Quarter milk yield (kg) from a single milking of the day.
[c] 3.5% fat-corrected milk yield.
[d] Average geometric mean somatic cell count (cells/mL).
[e] Somatic cell count converted to linear scores.

study, the frequency of *Corynebacterium* spp. was similar to that of Haltia et al. (2006), although all of our dairy farms used teat dipping. The distribution of *Corynebacterium* spp. was similar to that reported by Huxley et al. (2004) and Watts et al. (2000), which showed a higher frequency of *C. bovis* (>75%), compared to non-*C. bovis* species.

Effect of Corynebacterium *spp. on SCC*

Composite milk samples from dairy cows were evaluated by Wilson et al. (1997) who described an average of 186,600 cells/mL for subclinical mastitis caused by *C. bovis* and 141,420 cells/mL for subclinical mastitis caused by non-*C. bovis.* These findings were similar to the results in our study evaluating SCC at the quarter level (Table 2).

Somatic cell count was higher in mammary quarters infected with *Corynebacterium* spp. than in healthy contralateral quarters and were higher than those reported by Bradley and Green (2005), who found a SCC of between 50,000 and 150,000 cells/mL and by Schepers et al. (1997), who reported an average SCC of 52,500 cells/mL (LnSCC 3.96) for *Corynebacterium* spp. subclinical mastitis cases at cow and quarter level, respectively. The lower average SCC described by Schepers et al. (1997) was probably due to correcting for SCC (parity, stage of lactation, production level of cows and month of sampling).

At the species level, contralateral mammary quarters had a higher SCC for quarters infected by *C. bovis* than healthy quarters. This result was similar to that described by LeVan et al. (1985) and Ngatia et al. (1991) who reported a higher SCC in mammary quarters infected with *C. bovis* compared to healthy quarters. Ngatia et al. (1991) reported that quarters infected by *C. bovis* had <200,000 cells/mL and were associated with no change in milk production and composition. On the other hand, Coulon et al. (2002) reported that SCC was similar between healthy and *C. bovis*-infected mammary quarters but they used foremilk samples.

The effect of experimental subclinical mastitis caused by *C. bovis* was also evaluated by Brooks and Barnum (1984), whose approach differed from the present study in that we evaluated naturally occurring subclinical mastitis. They reported that *C. bovis*-infected quarters had a higher SCC than healthy quarters, similar to our results but the SCC average of infected quarters was <200,000 cells/mL, and the authors suggested that *C. bovis* was an agent that colonises only the teat canal.

Effect of Corynebacterium *spp. on milk yield and composition*

Munro et al. (1984) found a decrease in lactose content during the mastitis process; this may occur due to lower lactose synthesis by damaged epithelial cells or the passage of this component of milk to the blood stream. Thus, the lower concentration of lactose in milk could be used as a criterion for mastitis detection (Auldist et al., 1995). In our study there was no decrease in fat and protein, and so the decrease in lactose content contributed to the decrease in solids non-fat content. LeVan et al. (1985) and Coulon et al. (2002) found no effect of subclinical mastitis caused by *C. bovis* on milk composition, but LeVan et al. (1985) evaluated only protein and fat content.

In the present study, milk yield, composition and SCC from 46 mammary quarters with subclinical mastitis caused by *Corynebacterium* spp. as a group were compared with the results from contralateral healthy mammary quarters. Quarters with *Corynebacterium* spp. subclinical mastitis produced less lactose than the contralateral quarters. Because less lactose was produced and there was an increase in SCC, it may be supposed that *Corynebacterium* spp. does not colonize only the teat canal, as lactose is produced by the alveolar tissue. Benites et al. (2003) isolated *Corynebacterium* spp. from the teat canal region, the teat cistern, gland cistern, and mammary parenchyma.

Our study shows that there was no effect of subclinical mastitis caused by *C. bovis* on fat content, protein, casein, total solids and milk yield when comparing healthy and infected contralateral quarters. Natzke et al. (1972) found that mammary quarters infected with *C. bovis* produced less milk than healthy quarters. Other researchers have reported that phosphorus content was lower in a *C. bovis* infected quarter than in the healthy control (Coulon et al., 2002), and this reinforces the idea that in *Corynebacterium* spp. subclinical mastitis, breakage of cell junctions may occur and change the permeability of mineral content across the blood–milk barrier.

Regarding fat and protein content, Brooks and Barnum (1984) reported that there was no difference between mammary quarters experimentally infected by *C. bovis* and healthy quarters. In the present study, we also found no effect of subclinical mastitis caused by *C. bovis* on fat and protein content.

We found no effect of subclinical mastitis caused by non-*C. bovis* on SCC, milk yield and composition at the contralateral mammary quarter level. We know of no previous studies on the effects of non-*C. bovis* on milk yield and composition.

Cows were not evaluated for parity, stage of lactation or level of production. Moreover, the milk yield of healthy mammary quarters was assessed from a single milking per day. Therefore, it is estimated that for a cow with a milk yield of 1.9 kg/quarter/single milking/day would produce around 15.2 kg of milk/cow/day, over two milkings, and 4636 kg of milk/lactation of 305 days; these figures are lower than the milk yield of 9610 kg in 328 days as reported by Schepers et al. (1997).

In general, subclinical mastitis increases the SCC and reduces milk yield of dairy cows (Forsbäck et al., 2009) but this does not seem to be the case for *C. bovis* as shown by our results. Although *C. bovis* caused a mild increase in SCC and a decrease in lactose content (g/kg), the SCC average was <200,000 cells/mL, which is considered a cut-off value for healthy mammary quarters (Malek dos Reis et al., 2011).

Conclusions

Species of *Corynebacterium* non-*C. bovis* are infrequently isolated from cases of subclinical mastitis and did not change SCC, milk yield and milk solid contents when evaluated at the contralateral quarter level. *C. bovis* was the most frequent *Corynebacterium* spp. found and had no effect on milk yield, fat, protein, casein, and total solids of milk from dairy cows evaluated at the contralateral quarter level. It did however increase SCC and decrease lactose and milk solids non-fat content.

Conflict of interest statement

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