Journal of Dairy Research

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Influence of *Mycoplasma bovis* infection on milk production and quality of Holstein dairy cows

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Research Article

*Deceased.

Cite this article: Torres FD, Madureira KM, Nascimento da Silva K, Azevedo C, de Moura TM, Skorei MR and Gomes V (2022). Influence of *Mycoplasma bovis* infection on milk production and quality of Holstein dairy cows. *Journal of Dairy Research* **89**, 416–418. https://doi.org/10.1017/S0022029922000814

Received: 13 May 2022
Revised: 16 August 2022
Accepted: 19 November 2022

Keywords:
Milk production; milk quality; mycoplasmosis; somatic cells count

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Abstract

We wished to determine if *Mycoplasma bovis* infection can negatively impact milk quality and production in Holstein dairy cows. For this Research Communication, milk samples (271) from Holstein cows from 3 herds were screened for *M. bovis* by real-time PCR. Positive ($n = 21$) and negative animals ($n = 21$) were matched by herd, age, lactations and days in milk (DIM). Pairs were evaluated in 7 stages of lactation: D1–50, D51–100, D101–150, D151–200, D201–250, D251–300, and D ≥ 301. A mixed model was used to assess the effect of groups (*M. bovis*$^+$ × *M.bovis*$^-$), time (lactation) and groups × time interaction. Cows positive for *M. bovis* had lower average milk production per day and high somatic cells count (SCC).

Known as an emerging disease, *Mycoplasma bovis* (*M. bovis*) is the most important species within the genus *Mycoplasma*. This infectious agent is closely related to mastitis, reducing production and milk quality, in addition to the manifestation of bronchopneumonia, polyarthritis and otitis media (Royster and Wagner, 2015; Al-Farha *et al*., 2017). In Brazil, *M. bovis* investigations are rare and poorly diagnosed by the technicians and producers. *M. bovis* has no growth on traditional culture systems, nevertheless, only a few dairy facilities have begun to investigate if *M. bovis* is present in dairy cows presenting clinical signals of mastitis, but with negative microbiological results. Therefore, the present study aimed to investigate the presence of *M. bovis* in milk samples of Holstein dairy cows, and evaluate its potential impact on milk parameters, quality and production throughout lactation.

Material and methods

This study was approved by the Animal Care and Use Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo (no 7265271118). This research was conducted in 3 commercial dairy farms, located in Carambeí, Paraná state, Brazil. These farms had previous history of clinical mastitis and positive results for *Mycoplasma* spp. detected by qPCR. Herds A, B and C were composed of 1660, 530 and 460 lactation Holstein cows producing around 64,000, 21,200 and 18,400l per day, respectively, housed in free stall systems.

Milk samples were initially collected from a total of 271 cows, from first to sixth lactations, from farm *A* ($n = 92$), *B* ($n = 95$) and *C* ($n = 84$). For milk sampling, a pre-dipping was performed and milk aliquots were collected from each teat in individual plastic sterile tubes and pooled in the laboratory, forming a single sample for each cow. Samples were then screened by real time PCR to determine presence or absence of *Mycoplasma bovis*. Ultimately, the animals used for further analyses in this study included cows that were positive in PCR ($n = 21$) and cows that were negative in PCR ($n = 21$).

After the initial screening, the 21 *M. bovis*$^+$ and 21 *M. bovis*$^-$ cows were matched in pairs according to herd (farm $A = 12$, $B = 4$ and $C = 5$), age, number of lactations and days in milk (DIM), to avoid herd and covariate effects. Milk measurements were recorded monthly from February 2018 until October 2019 so as to include data from an entire lactation from each cow. The following milk quality variables were determined: total solids (%), fat (%), protein (%), lactose (%), urea (mg/dl) and somatic cell count (SCC, expressed by cells/ml). The milk production was analyzed considering the following parameter: average of milk production per day (l) and milk yield production adjusted for 305 d of lactation (projected milk production –

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Table 1. Parameter estimates and standard errors from linear mixed models to evaluate the effect of *Mycoplasma bovis* on production parameters during a lactation period of Holstein cows in 3 dairy facilities in Carambeí, Paraná state, Brazil, 2018–2019

Parameter	*M. bovis*$^+$	*M. bovis*$^-$	Time	Group	Time × Group
Milk/day (l)	35.36 ± 11.98	42.38 ± 11.17	0.001	0.019	0.027
Fat (%)	3.89 ± 0.85	3.83 ± 1.06	0.001	0.604	0.401
Protein (%)	3.32 ± 0.4	3.22 ± 0.4	0.001	0.106	0.727
Lactose (%)	4.6 ± 0.25	4.65 ± 0.21	0.360	0.520	0.465
Total solids (%)	12.74 ± 1.18	12.63 ± 1.35	0.001	0.456	0.582
SCC (cells/ml)	608.49 ± 1630.78	197.85 ± 649.84	0.007	0.009	0.001
Urea (mg/dl)	12.99 ± 3.35	14.18 ± 3.86	0.762	0.218	0.011
PMP 305 (days)	9541.99 ± 2878.08	11 348.27 ± 2587.77	0.001	0.090	0.017

SCC, somatic cell count; PMP, projected milk production on 305 d of lactation.

PMP). This parameter was studied in the following stages of DIM: D1–50; D51–100; D101–150; D151–200; D201–250; D251–300; and D ≥ 301.

Conventional microbiological culture was performed for all milk samples according to the National Mastitis Council (2017). All animals included in both experimental groups of this research were negative in culture at time of grouping. *Mycoplasma bovis* was diagnosed using qPCR. The DNA extraction from milk samples was carried out using the commercial kit MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems) in accordance with the manufacturer's standard protocol, with an additional step: centrifuge of 500 µl of milk sample (1000 × ***g*** for 5 min), to remove the fat pre-extraction. The nucleic acid was purified from individual samples and DNA was quantified in the Qubit® equipment. The quantitative PCR (qPCR) technique was used to detect the presence/absence of the pathogens of interest and the reactions were performed in the QuantStudio 5 Real-Time PCR System (Applied Biosystems™). The detection of DNA from *Mycoplasma* (non-*M. bovis*) and *Mycoplasma bovis* was done using the commercial Bactotype® Mastitis HP2+ PCR Kit (INDICAL Bioscience). The first round of PCR was developed in a pool of 2 cows, in case of a positive result for any pathogen; this pool was dismembered and the second round of qPCR was done individually.

Statistical analyses were performed using SPSS program. Means and deviation analyses, variables distributions by histograms, q-plot and normality tests were conducted on production parameters. A mixed model was used to assess the effect of groups (*M. bovis*$^+$ × *M. bovis*$^-$), time (lactation) and the interaction of groups and time. The correlation matrices unstructured, autoregressive and compound symmetric were tested. Models were chosen according to Akaike's Information Criterion (AIC). The Generalized Estimation Equation (GEE) model allowed analyses of the effect of the time, group and the interaction between time and groups in variables with non-normal distributions. The most appropriate model was determined according to the QIC: Quasi Information Criterion. The time and groups were defined as fixed effects and the milk parameters as dependent variables. Differences with $P \leq 0.05$ were considered significant.

Results and discussion

We evaluated the intramammary infection caused by *Mycoplasma bovis* on milk quality and quantity from Holstein cows from three farms located in Carambeí, Paraná state, Brazil. Similar primary studies were conducted in South Australia (AL-FARHA *et al.*, 2017) and Estonian dairy farms (Timenon *et al.*, 2017).

Screening process of cows to establish the experimental groups defined a *M. bovis* prevalence of 7.75% (21/271). Farm *A* presented the highest prevalence (13.04%, 12/92), while farm *B* (4.21%, 4/95) and farm *C* (5.95%, 5/84) had similar profile. Junqueira *et al.* (2020) reported a prevalence of 35.3% (6/17) for *M. bovis* in milk in the same geographical region, however these authors worked with milk samples from cows presenting clinical mastitis, which increases the probability of positive results.

The effect of group, effect of time and interaction between group and time are shown in Table 1, and the comparison between experimental groups and time-analysis are shown in the online Supplementary Table S1. Effect of group was detected for the average of milk production per day ($P = 0.019$) and SCC ($P = 0.009$). The interaction between time and group was observed for the average of milk production per day ($P = 0.027$), SCC ($P = 0.001$), urea ($P = 0.011$) and projected milk production ($P = 0.017$). Time effects were significant for all variables, except lactose and urea.

The comparison between groups by the Student's t-test in each stage of lactation showed lower milk production per day on D101–D105 and then in each period from D151 onwards until D300. In addition, the projected milk production on 305 d of lactation was also decreased in the *M. bovis* positive group on D201–250, D251–300 and D > 300. Somatic cell count was higher for the *M. bovis* dairy cows around the peak of lactation on D101–150 and D101–D150.

M. bovis causes immunosuppression and persistent infection of the mammary gland, which results in a constant inflammatory state. Also, neutrophil extracellular trap (NET) induced by *M. bovis* infection contributes to increase in cellularity. This immune disruption of the mammary gland by *M. bovis* infection is closely related to increased SCC, as seen in the positive cows in this research (Gondaira *et al.*, 2020). The negative impact of *M.bovis* intramammary infection on milk production and quality were also reported by Timonen *et al.* (2017) and Al-Farha *et al.* (2017).

Concentrations of fat, protein and total solids were similar between experimental groups. Despite the statistical differences detected for urea, the variation between means in each experimental group throughout lactation does not allow any conclusion regarding the *M. bovis* intramammary status.

https://doi.org/10.1017/S0022029922000814 Published online by Cambridge University Press

This study evaluated milk parameters thought lactation, however, the screening of the dairy cows for *M. bovis* and other mastitis pathogens was done in only a specific time by using qPCR. The establishment of the infectious status of the mammary gland requires serial microbiological analysis of milk (Royster and Wagner, 2015). In addition, future studies should investigate the association between traditional culture and PCR due to the absence of a reference test to detect *M. bovis* intramammary infection. Sachse *et al.* (1993) compared various methods for the detection on *M.bovis*: conventional isolation and identification from culture, direct and indirect enzyme-linked immunosorbent assay (ELISA) technique, and polymerase chain reaction (PCR). They concluded that PCR is potentially superior to all the other techniques, due to its high sensitivity, specificity and speed. On the other hand, Justice-Allen *et al.* (2011) reported that SYBR PCR protocol was only slightly more sensitive in the detection of *M. bovis* in bulk milk tank, compared with traditional microbiological culture.

In conclusion, infection caused by *M. bovis* reduced the average milk per day and increased the SCC in Holstein cows throughout the lactation. Further studies increasing sample size and replicates milk samples from the same cows should be done to take into account the intermittent release of *M. bovis* in milk through time.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029922000814

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https://doi.org/10.1017/S0022029922000814 Published online by Cambridge University Press