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REGULAR ARTICLES

Milk yield and lactation stage are associated with positive results to ELISA for *Mycobacterium avium* subsp. *paratuberculosis* in dairy cows from Northern Antioquia, Colombia: a preliminary study

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Abstract Paratuberculosis is a slow-developing infectious disease characterized by chronic granulomatous enterocolitis. This disease has a variable incubation period from 6 months to over 15 years and is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Some studies have been conducted in cattle during the last decades in Colombia. However, those studies were designed using relatively small populations and were not aimed to establish prevalence. This study aimed to determine the MAP seroprevalence in selected dairy herds and to explore risk factors associated with the serology results. Serum samples and related data were collected from 696 randomly selected bovines in 28 dairy herds located in 12 different districts in one of the main dairy municipalities in Colombia (San Pedro de los Milagros). The samples were analyzed using a commercial ELISA kit. The information on risk factors was analyzed using a logistic regression. The apparent seroprevalence was 3.6 % (1/28) at the herd level and 2 % (14/696) at the animal level. The number of days in milk production between 100 and 200 days and over 200 days and the daily milk production between 20 and 40 L/cow and over 40 L/cow were associated with MAP seropositivity with odds ratios of 4.42, 3.45, 2.53, and 20.38, respectively. This study demonstrates the MAP seroprevalence in dairy herds from Antioquia and the possible relationship between MAP seropositivity, milk yield, and lactation stage.

Keywords Dairy cattle · Johne's disease · Milk production · *Mycobacterium avium* subsp. *paratuberculosis* · Seroprevalence · Risk factors

Introduction

Paratuberculosis (PTB), also known as Johne's disease (JD), is a severe slow-developing and incurable granulomatous enteritis (Clarke 1997). This disease affects cattle and other domestic and wild ruminants (Nielsen and Toft 2009; Sweeney et al. 2012). *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causal agent of PTB. It is a Gram-positive, facultative anaerobic, mycobactin-dependant, slow-growing, and acid-fast bacillus (AFB) that may cause a persistent infection in a host tissue's intestinal macrophages and lead to immune and inflammatory reactions (Sweeney 1996). MAP can resist environmental and chemical changes and persists in spoils, stream water, and manure slurry storages for up to a year (Sweeney 1996). MAP has been associated with the human chronic enteritis known as Crohn's disease (Sweeney et al. 2012; Atreya et al. 2014; Liverani et al. 2014).

MAP infections produce important economic losses related to cattle production in infected herds (Marce et al. 2009; Nielsen and Toft 2009). Economic losses due to reduced milk production, increased cow replacement, lower cull-cow revenue, and greater cow mortality are higher in PTB-infected herds compared to PTB-negative herds (Johnson et al. 2001; Kudahl et al. 2004; Weber 2006; Beaudeau et al. 2007; Gonda et al. 2007; Nielsen and Toft 2009; Richardson and More

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2009; McAloon et al. 2016). There are reports of infections with MAP and clinical cases of JD from all countries that have ruminant populations (Marce et al. 2009; Nielsen and Toft 2009; Juste and Pérez 2011). It is thought that this disease has a global distribution (Manning and Collins 2010). Therefore, PTB belongs to the List of Diseases of the World Organization for Animal Health (OIE) because of its international distribution and zoonotic potential, leading to not only public and animal health risks but also commercial restrictions (Anonymous 2000, 2015).

Parturition, lactation, or other stresses may provoke clinical stages of this disease (Clarke 1997; Fecteau and Whitlock 2010). The main transmission route at an individual level in natural conditions is the oral-fecal route, especially at early stages of life in animals. However, intrauterine and transmammary routes have also been considered (Lambeth et al. 2004; Whittington and Windsor 2009).

MAP infections occur in young animals, and it is generally assumed that some age resistance takes place. Animals from 0 to 6 months of age are thought to be the most susceptible to MAP infections (McGregor et al. 2012; Mortier et al. 2013). Consequently, the major sources of MAP infection are infected animals (Manning and Collins 2001) and the contamination of the udder of the calf's dam, the pasture, the feedstuff, or the implements with feces. These are described as the principal factors to avoid when the control of the disease in the herd is desired (Sweeney 1996; O'Brien et al. 2006). For an antemortem diagnosis of PTB in cattle, several tests are available and recommended. These include tests to detect antibodies against MAP, the direct detection of MAP genes, bacterial cultures of fecal samples (individual, pooled, and environmental), and tests to detect MAP in tissue samples (Collins et al. 2005). The sensitivity and specificity of tests for the antemortem diagnosis of PTB vary significantly depending on the MAP infection or clinical stage (Nielsen and Toft 2008a). Therefore, it is considered that none of the diagnostic tests are capable of detecting all subclinically infected animals (Chacon et al. 2004; Lavers et al. 2013). In any case, sampling all adult cattle in every herd, environmental sampling, serial testing, and the use of two to three diagnostic tests has been recommended for herd screening and to increase the accuracy of MAP diagnosis (Collins et al. 2005; Stevenson 2010; Serraino et al. 2014).

Different individual and herd-level factors related to within-herd contact have been shown to influence the PTB infection status in dairy cattle (Johnson-Ifearulundu and Kaneene 1998, 1999; Hacker et al. 2004; Dieguez et al. 2008). Some of those risk factors include "not cleaning maternity pens after each use" (Johnson-Ifearulundu and Kaneene 1998; Tiwari et al. 2009), "more than one cow in a maternity pen" (Wells and Wagner 2000; Tiwari et al. 2009), "presence and percentage of cows born at other dairies" (Wells and Wagner 2000; Chia et al. 2002; Tiwari et al. 2009), "contamination of udders of peri-parturient cows with manure" (Ansari-Lari et al. 2009), "winter group-housing for pre-weaned calves" (Wells and Wagner 2000; Tiwari et al. 2009; Ridge et al. 2010; Pithua et al. 2013), "animals fed colostrum from multiple cows" (Nielsen and Toft 2008b), "Bovine Viral Diarrhea Virus (BVDV)-seropositive herds" and "BVDV vaccination not done properly in calves" (Tiwari et al. 2009), "housing replacement calves with adult cattle before they were 6 months old" (Collins et al. 1994; Dieguez et al. 2008), "suckling from foster cows" (Nielsen and Toft 2008b), "feeding milk with antibiotics" (Ridge et al. 2010), "exposure of calves 0 weeks to adults feces," "young stock contact with adult feces from same equipment used for cleaning," and "feces spread on forage fed to any age group" (Goodger et al. 1996; Obasanjo et al. 1997), and "cows with more than 4 parturitions" (Jakobsen et al. 2000).

In South America and the Caribbean, few studies have reported consistent seroprevalence. Animal and herd-level prevalence of PTB from this region range from 2.7 to 72 % and from 18.7 to 100 %, respectively (Fernández-Silva et al. 2014). In Colombia, PTB was first reported in cattle in 1924, probably from imported animals (Vega-Morales 1947). After this, PTB research in cattle has been sporadic and has mainly focused on clinical, histopathological, serologic, microbiological, and/or molecular diagnosis (Vega-Morales 1947; Isaza-Triviño 1978; Góngora and Perea 1984; Mancipe et al. 2009; Ramírez-Vásquez et al. 2001; Zapata et al. 2010; Fernández-Silva et al. 2011a, b; Ramírez-Vásquez et al. 2011, 2013; Ramírez-García and Maldonado-Estrada 2013), treatment (Huber-Luna 1954), prevalence (Patiño-Murillo and Estrada-Arbeláez 1999; Fernández-Silva et al. 2011a), and molecular characterization (Fernández-Silva et al. 2011b). These studies were very useful in confirming the presence of MAP in local cattle. However, the studies were performed in a relatively small dairy cattle population.

Despite these investigative efforts, no official control or eradication program for PTB has been carried out in Colombia. Its control is considered a farmer's responsibility. The main objective of the current study was to determine the seroprevalence of MAP and explore the main risk factors associated with enzyme-linked immunoassay (ELISA)-positive results in cows of dairy herds of one municipality of the Northern Region of Antioquia, Colombia.

Materials and methods

Ethical considerations

This research was approved by the Ethics Committee for Animal Experimentation of the Universidad of Antioquia, Colombia (Act number 88, from March 27, 2014).

Study design

Twelve districts (out of 37) of a municipality located in the Northern Region of Antioquia, Colombia, that contribute 70 % of the municipality's cattle population were included in the study. Proportional allocation design of the herds to be sampled in each of the selected districts as well as an adjustment by cluster was considered.

A sample of 28 dairy herds inside the selected districts without a previous PTB diagnosis and/or without known history of PTB was selected, according to its specific weight in the dairy population of the municipality. Accounting for a loss of 28 % and an average adult population (≥2 years of age) per herd estimated to be 23, 696 animals were randomly sampled. According to the study design, 29 animals per herd were tested by ELISA. In the study region, dairy production is the main economic activity. Dairy production takes place in all places within the region, and Holstein is the predominant dairy cattle breed. In all the cases, the herds had to fulfill the following conditions to be enrolled in the study: security during sampling visits, geographical accessibility, and willingness of herd owner to participate in the study, allow sampling of all the necessary animals, and provide information regarding animal features and herd management practices. In addition, herds had to have the minimum facilities for the personnel to carry out the procedures safely on animals. All herds accomplishing these inclusion criteria were included in the random selection process.

Serum samples and information

All the herds were visited and tested once from May to July 2014. In each herd, information and whole blood samples were taken from each animal over 2 years of age. The sample collection was conducted according to standard methods to avoid unnecessary pain or stress to animals. Blood samples were taken from the coccygeal or jugular vein, collected in red-top plastic Vacutainer® tubes and transported in a refrigerated cage until their arrival at the laboratory, where they were centrifuged at 1008 RCF for 5 min to obtain the serum for the ELISA test. The obtained serum was frozen for 30 to 45 days at −20 °C. After this time, frozen samples were thawed at room temperature before being tested by ELISA. In each herd, the information on individual animal features, herd characteristics, and herd management practices were collected through questionnaires administered directly to herd owners or managers on every visit and by direct observation of the individual and herd characteristics, as well as management practices (questionnaires available upon request). The questionnaires were administered by one of the authors to ensure that recording was clear, complete, and consistent.

ELISA

A serum ELISA was performed using a preabsorbed serum ELISA Parachek®2 (Prionics AG, Switzerland) following the manufacturer's instructions. This test included a preabsorption step with *Mycobacterium phlei* to reduce cross-reactions. A herd was considered ELISA-positive if the herd had at least two serum ELISA-positive animals. This avoided the risk of confirming a herd as positive based on one single false-positive result by the test, as it is defined by the manufacturer of the diagnostic test used. An animal was considered ELISA-positive if serum sample was above or equal to the cutoff of 15 % positivity (%P), as it is defined by the manufacturer of the diagnostic test used.

Statistical analysis

All the information generated during the study was entered into Excel worksheets (Microsoft Corp., Redmond, WA, USA) and then exported to Stata 12.0 (StataCorp, 2011, Texas, USA) for statistical analysis. The data were examined for biologically implausible entries (those unlikely to be true). Any erroneous data (those incorrect, detected during the editing process of the database) were removed or corrected. Descriptive statistics were computed for all the variables of interest. Observations were stratified by district and sampling weights were computed based on the specific weight of the district on the reference population. Variables were checked for more than 30 % missing values, a case in which they should have been deleted from the analysis. None of the variables showed more than 30 % missing values. Pearson and Spearman correlation analyses were used for continuous and categorical variables, respectively. A complex design analysis was conducted according to a cluster effect and the stratified nature of the study using the Survey command. Unconditional associations between each risk factor and the outcome of interest—ELISA-positive—were computed. Associations with $p \leq 0.25$ were retained for consideration in a multivariable model. A complete multivariable logistic regression model was constructed considering a significance level of $p < 0.05$. The potential confounding effect of parturition was evaluated by refitting the final model with parturition omitted to see if the coefficients for other predictors changed substantially. The results from the final models are presented as odds ratios (OR) with 95 % CIs. The model fit was assessed using a Hosmer-Lemeshow goodness-of-fit test.

Case definition

The case definition for a MAP-infected herd was the one with at least two seropositive animals determined by serum ELISA. The case definition for a MAP-infected animal was seropositivity of an individual serum ELISA.

Pretest of the methodology

All testing procedures and questionnaires were pretested on a small scale to evaluate their effectiveness in order to accomplish the objectives of the study.

Results

Descriptive statistics

The study population was mainly composed of Holstein (77.6 %) cows (99.6 %), older than 3 years of age (74.9 %), in lactation (83.3 %), with more than 200 days in milk (57.1 %) and less than three parities (67 %) (Table 1). The individual daily milk production was predominately 20–40 L/cow (45.8 %), and the percentage of animals not born in the herd was 69.7 % (Table 1).

The herd-level characteristics of less than 50 ha (66.2 %), ≥30 and ≤60 cows in milk (45.8 %), and a daily milk production between ≥500 and ≤1400 l (46.2 %) were the most common findings regarding farm size, herd size, and herd daily milk production, respectively (Table 2). The presence of other ruminants (i.e., goats, sheep, and/or buffalo), manure spreading on pastures as a method of fertilization, and cows staying with their calf after calving was reported in 17.9, 67.9, and 85.7 % of the herds, respectively. The percentage of herds certified in good farming practices (buenas prácticas ganaderas, BPG) and percentage of tuberculosis- and brucellosis-free herds were 25 and 75 %, respectively (Table 2). The descriptive analysis of the quantitative variables is summarized in Table 3.

Table 1 Animal-level predictors in bovines from dairy herds of San Pedro de los Milagros, Antioquia, Colombia

Variable	Description	Unit/category	Observations	Distribution (%)
Breed	According to herd registers	Holstein	540	77.6
		Jersey	120	17.2
		Other[a]	36	5.2
		Total	696	
Sex	According to herd registers	Female	693	99.6
		Male	3	0.4
		Total	696	
Age	According to herd registers	2–3 years old	175	25.1
		>3 years old	521	74.9
		Total	696	
Milk production state	According to herd registers	Heifer	68	10.5
		Milking cow	538	83.3
		Dry cow	40	6.2
		Total	646	
Days in milk	Days that had passed from the first day the cow started producing milk to the moment of the testing	<100	158	22.7
		≥100–≤200	140	20.1
		>200	397	57.1
		Total	695	
Parity	Times the cow had gave birth during its life to the moment of the testing	<3	376	67
		≥3–≤8	188	32.4
		>8	132	0.6
		Total	696	
Individual daily milk production	Total milk obtained during the previous day to the moment of testing	<20	125	53.1
		≥20–≤40	312	45.8
		>40	92	1.1
		Total	529	
Born in the herd	The cow had been born in the herd or was purchased from another farm	Yes	451	30.3
		No	196	69.7
		Total	647	

[a] Other breeds included Guernsey, Ayrshire, Swedish Red, Swiss Brown, Jersey, and several crossbreeds of Holstein with Jersey, Ayrshire, Angus, Blanco Orejinegro, Brahman, and Gir

Table 2 Herd-level predictors in dairy herds of San Pedro de los Milagros, Antioquia, Colombia

Variable	Description	Unit/category	Observations	Distribution (%)
Farm size	Part of the herd dedicated to farming in hectares (Has)	<50	19	66.2
		≥50–≤99	6	23.7
		≥100	3	10.1
		Total	28	
Herd size	Number of cows in milk	<30	6	25
		≥30–≤60	11	45.8
		≥60	7	29.2
		Total	24	
Herd daily milk production	Total milk (in liters) obtained during a day in each herd considered in the screening, in average, to the moment of the testing	<500	7	26.9
		≥500–≤1400	12	46.2
		>1400	7	26.9
		Total	26	
Presence of other ruminants	Coexistence with goats, sheep, and/or buffaloes in the same installations	Yes	5	17.9
		No	23	82.1
		Total	28	
Manure spreading	Use of cow manure as a fertilizer in the pastures	Yes	19	67.9
		No	9	32.1
		Total	27	
Cow stays with the dam after calving	After parturition the cow stays with the mother in direct contact	Yes	23	85.7
		No	5	14.3
		Total	28	
BPG[a] certification	Herd certified by the Instituto Colombiano Agropecuario (ICA) as a BPG practicant	Yes	8	25
		No	20	75
		Total	28	
Tuberculosis-free certification	Herd certified by Instituto Colombiano Agropecuario (ICA) as tuberculosis-free	Yes	20	75
		No	8	25
		Total	28	
Brucellosis-free certification	Herd certified by Instituto Colombiano Agropecuario (ICA) as brucellosis-free	Yes	21	75
		No	7	25
		Total	28	

[a] Buenas Prácticas Ganaderas (Good Farming Practices)

ELISA

Fourteen of 696 of the animals had a positive ELISA test, which resulted in an animal-level apparent prevalence of 2 %. Eight of the seropositive animals were from one herd of the 28 included in the study. This herd was the only positive herd according to the case definition, resulting in a herd-level apparent prevalence of 3.6 %.

Table 3 Descriptive summary of quantitative variables in dairy herds of San Pedro de los Milagros, Antioquia, Colombia

Variable	Observations	Mean ± SD	Minimum	Maximum
Farm size (in Has)	28	50.87 ± 47.22	5	180
Herd size	24	63.66 ± 61.27	11	332
Herd daily milk production (L/day)[a]	26	1350 ± 1534	220	8132
Days in milk	532	199.67 ± 140.32	1	785
Parity	562	3.06 ± 2.00	0	12
Individual milk production (L/day)[b]	529	20.42 ± 7.39	2	51

[a] Milk produced per herd/day

[b] Milk produced per cow/day

Risk factors analysis

The two cow-level factors "days in milk" and "individual daily milk production" showed strong associations with the presence of ELISA-positive results (Table 4). Biologically plausible interactions of predictor variables were assessed and find to be nonsignificant. The Hosmer-Lemeshow goodness-of-fit test suggested that the model fits the data ($p > 0.97$). The OR for seropositivity was increased with the number of days in milk and individual daily milk production ($p < 0.01$). The number of days in milk had a similar OR pattern for the 100- to 200-day interval (OR = 4.42) as for >200 days (OR = 3.45).

Discussion

The present study was designed to identify the prevalence and explore the risk factors associated with seropositive results detected using an ELISA in one of the main dairy production areas of Colombia.

The current herd and animal-level prevalence is unknown in many countries. However, according to several authors, the prevalence of infection is increasing in some countries that do not have mandatory control programs (Salem et al. 2013; Fernández-Silva et al. 2014). Colombia lacks a mandatory program. However, no trend can be established with the currently available data. The animal- and herd-level prevalence estimated in the present study is lower than the prevalence found in cattle by other authors in European, Asian, North American, Latin American, and Caribbean countries (Clarke 1997; Nielsen and Toft 2009; Manning and Collins 2010; Fernández-Silva et al. 2014). Nonetheless, Fernández-Silva et al. (2014) reported studies in Latin American and Caribbean countries with an overall prevalence of 16.9 (13.2–20.5) and 75.8 % (50.1–101.5) in cattle, at the animal and herd levels, respectively, revealing the extreme limits that can be found in the PTB prevalence reports.

On a national scale, our results are similar to those obtained in a previous seroprevalence study in Normando cattle using an ELISA in the Colombian departments of Caldas and Tolima (animal-level 1.69 %; 3/177; Patiño-Murillo and Estrada-Arbeláez 1999). However, they contrast with MAP-detection results obtained in the department of Antioquia in which ELISA-positive results were found for 10.1 (31/307) and 70 % (10/14) at the animal and herd-level, respectively (Fernández-Silva et al. 2011a). It should be mentioned that in this previous study, serum from asymptomatic cows was analyzed by an unabsorbed ELISA test, which could affect the specificity of the findings, leading to false-positive results. On the other hand, in their study, herds were selected attempting a representation of all productive districts of the municipality (not a random sampling), and of these 14 herds, one herd had presented sporadic clinical cases compatible with paratuberculosis confirmed by PCR and histopathology (Zapata et al. 2010). These factors could have increased the prevalence reported. Our study attempts to, and finally, report a seroprevalence at the animal- and herd-level in a higher population of the department of Antioquia compared to previous studies carried out in the country and region. Those previous studies did not attempt to report prevalence in their study design and used diagnostic tests with different characteristics.

Although the results obtained (2 and 3.6 %, animal and herd-level, respectively) refer to the apparent MAP prevalence in the population being studied, no attempt to calculate the true prevalence was carried out due to a lack of information on the sensitivity and specificity of the test used, which should had been previously estimated in the same population for an accurate determination (Nielsen and Toft 2009). In any case, the low prevalence obtained could also be explained by the test's characteristics that are mainly related to its sensitivity as a response to the silent and long-lasting behavior of the disease, than to failures of the test itself (Sweeney 1996; Collins et al. 2005; Mon et al. 2012; Sorge et al. 2012). According to Lavers et al. (2015), the sensitivity of serum and milk ELISA is approximately 25.6–45.3 % and its specificity of 97.6–98.9 %, which can lead to a misclassification of the cows and reporting infected cows as negative (Nielsen et al. 2002). On the other hand, the low prevalence obtained could be related to sample handling. In the present study, the serum samples were frozen for 30 to 45 days at −20 °C, which could have led to lower scores for the MAP ELISA, as previously reported by Alinovi et al. (2009).

The risk factors identified in this study (number of days in milk and individual daily milk production) are supported by the current data that parturition, stage of lactation, and metabolic stress, induced by milk production, can act as triggers and lead to seroconversion or progression from stage II to

Table 4 Final logistic regression model assessing the effect of selected herd and cow variables on the probability for animals to be serum-ELISA-positive to MAP in San Pedro de los Milagros, Antioquia, Colombia ($n = 532$ observations)

Variable	Odds ratio	SEM	p value*	95 % CI
Days in milk				
<100	Referent			
≥100–≤200	4.42	0.86	0.00	2.89–6.76
>200	3.45	0.92	0.00	1.93–6.17
Individual daily milk production				
<20	Referent			
≥20–≤40	2.53	0.75	0.00	1.32–4.85
>40	20.38	5.54	0.00	11.26–36.88

*Significant results ($p < 0.05$)

stage III of the disease (Clarke 1997; Nielsen et al. 2002; Fecteau and Whitlock 2010). Nielsen et al. (2002) reported that in serum ELISAs, the OR of being positive is highest at the end of lactation (>203 days; OR = 5.22), possibly indicating that cows with low antibody concentrations are infected but with a cell-mediated immune response, undetectable by ELISA. This statement is hypothetical and would have to be supported by a longitudinal study with repeated samplings on the same population to understand the serological patterns.

Our study reported similar results of odds over 3.45 for cows over 200 days in milk, indicating that the probability of being ELISA-positive is different across lactation progression and is higher in the middle of the lactation. From a diagnostic point of view, it is important to recognize the differences in ELISA-positive animals in different stages of lactation and different production levels, as these findings can help establish risk assessment-based control programs and guide owners to recognize the distinctive clinical signs of PTB at an early stage.

Some variables that we hypothesized to be important risks and were previously identified by other studies for seropositivity were not significant in the logistic regression analysis, including parity ($p = 0.160$), physiological state ($p = 0.57$), cow staying with the calf after calving ($p = 0.55$), presence of other ruminants ($p = 0.62$), and manure spreading as a fertilizer in the pastures ($p = 0.57$; Goodger et al. 1996; Cetinkaya et al. 1997; Obasanjo et al. 1997; Jakobsen et al. 2000; Fredriksen et al. 2004; Dieguez et al. 2008; Nielsen and Toft 2008b; Ansari-Lari et al. 2009; Doré et al. 2012; Nielsen and Toft 2012).

Although previous studies have reported that the highest probability of a positive-ELISA is observed in older cows (parity ≥ 3; Sherman 1985; Jakobsen et al. 2000), a large herd (Braun et al. 1990; Ott et al. 1999; Jakobsen et al. 2000; Muskens et al. 2003; Hirst et al. 2004), and Jersey cows compared to larger breeds (including Holstein-Friesian; Jakobsen et al. 2000; JØrgensen 1972; McNab et al. 1991; Cetinkaya et al. 1997), no relationship between breed, parity, and herd size was found in our study. However, the role of parity as a confounder was investigated by the fitting models considering MAP ELISA-positive results, with and without parity included. No confounding effect of parity was observed.

The practice of leaving a cow with her calf after birth was also representative of the herds of the study and has been reported as a risk factor, increasing the within-herd transmission of PTB by Goodger et al. (1996), Obasanjo et al. (1997), and Ansari-Lari et al. (2009). Concerning the presence of other ruminants, Whittington et al. (2001) reported cases of bovine PTB due to S (sheep) strain that were confirmed in Australia, demonstrating the transmission opportunity between species. Manure spreading as a risk factor has been previously described (Goodger et al. 1996; Obasanjo et al. 1997), because of the potential exposure to younger and susceptible cattle.

BPG certification includes management practices which can be considered PTB-related, such as grazing strategies (i.e., rotational, rational, intelligent, stripped-rotational, altering, and extensive), fertilization strategies (i.e., organic and inorganic), other animal species in the farm (e.g., pigs, rabbits, goats, horses, buffaloes, and poultry), enteric disease cases in the last semester and their diagnosis, and tuberculosis and brucellosis sanitation status (ICA 2007).

This study had several limitations. The design chosen for this study was not optimal for the evaluation of herd-level paratuberculosis risk factors. The study would have had much more power to evaluate herd-level effects if a cross-sectional study involving many more herds had been used. However, financial resources were limited to include more herds, but authors believe that herds included in this study were good examples of the specialized dairy herds in the region in an exploratory manner.

The Survey command in Stata version 12.0 (StataCorp 2011) was used in the data analysis for several reasons. First, the variance linearization procedure used allows for the simultaneous evaluation of both cow-level and herd level risk factors, with appropriate standard error estimates. Second, it allows for the incorporation of sampling weights into all analyses to correctly account for the probability of a herd being sampled within a district.

Conclusion

In conclusion, we detected an apparent seroprevalence of 3.6 % at the herd-level and 2 % at the animal-level. The risk factors associated with MAP seropositivity were ≥100 days in milk and an individual daily milk production over 20 L/cow.

The information in this study indicates the importance of implementing protective management practices related to our results. Thus, it will be necessary to design risk-based programs in each country that are adapted to its specific conditions. Follow-up studies on herds with PTB over a long time period to investigate if the change of individual management practices leads to changes in PTB prevalence on these farms should be performed.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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