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Factors associated with *Mycobacterium avium* subsp. *paratuberculosis* in dairy cows from Northern Antioquia, Colombia¤

Factores asociados con el estatus serológico de Mycobacterium avium subsp. paratuberculosis en vacas lecheras del norte de Antioquia, Colombia

Fatores associados ao estado sorológico de Mycobacterium avium subsp. paratuberculosis em soro de gado leiteira no norte de Antioquia, Colombia

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Abstract

Background: Johne´s disease (JD) is a severe enteritis that affects ruminants and has been diagnosed in cattle and sheep in Colombia. However, epidemiological information on the disease in this country is scarce. **Objective:** to identify factors associated with the JD serum enzyme-linked immunosorbent assay (ELISA) status of dairy cows. **Methods:** a cross-sectional study was carried out in 307 asymptomatic adult Holstein dairy cows from 14 herds in nine districts of Belmira and San Pedro de los Milagros municipalities during November, 2007. From 19 to 25 cows, ≥ 2 years of age were randomly selected and blood sampled from every herd. A commercial ELISA kit was used to analyze sera. Information regarding cow related factors (age, farm-born, parity, and daily milk yield) and herd management practices (i.e. herd size, herd average milk production, current presence of symptomatic animals, cattle purchase, own animals grazing in foreign pastures, feeding of calves before weaning, manure spread on pastures, and sighting of birds in feed storing areas) was collected using questionnaires. Descriptive statistics were computed for all variables and a multivariable logistic regression model was constructed ($p<0.05$). **Results:** ten percent (31/307; 95% CI: 7.0-14.0%) of the animals were positive by ELISA. In 70% (10/14) of the herds, ELISA detected at least one positive animal. Cow and herd factors “parity” and “feeding of calves before weaning” showed

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weak and strong associations with ELISA positive results, respectively. The odds ratio (OR) for JD seropositivity increased 20% (OR = 1.20; 95% CI: 0.98-1.47; p = 0.067) in cows with > 1 parity. The OR was 0.74 times lower (OR = 0.26; 95% CI: 0.096-0.70; p<0.01) in herds feeding calves with pooled colostrum from several cows, compared to herds feeding calves with colostrum from their own dams. **Conclusion:** JD seroprevalence was 10 and 70% at animal and herd-level, respectively. Cow and herd factors "parity" and "feeding of calves before weaning" showed weak and strong association with positive results, respectively.

Keywords: *cattle, epidemiology, paratuberculosis, serology.*

Resumen

Antecedentes: la enfermedad de Johne (EJ) es una enteritis severa que afecta rumiantes, y ha sido diagnosticada en bovinos y ovejas en Colombia. Sin embargo, la información epidemiológica de la enfermedad en el país es escaza. **Objetivo:** identificar los factores asociados con el estatus serológico de la EJ mediante ensayo de inmunoabsorción ligado a enzimas (ELISA) en vacas lecheras. **Métodos:** se realizó un estudio transversal en 307 vacas lecheras adultas asintomáticas de 14 hatos en 9 veredas en los municipios de Belmira y San Pedro de los Milagros, en noviembre de 2007. De cada hato se seleccionaron aleatoriamente 19-25 vacas, mayores de 2 años de edad y se tomaron muestras de sangre. Se utilizó un kit comercial de ELISA para analizar el suero. Se recolectó información mediante cuestionarios sobre características individuales de las vacas y prácticas de manejo de los hatos. Se realizó un estudio estadístico descriptivo para todas las variables de interés y se construyó un modelo de regresión logística multivariada, considerando un nivel de significancia de p<0,05. **Resultados:** el 10% de los animales fue positivo a ELISA (31/307; 95% CI: 7,0-14%). En el 70% (10/14) de los hatos al menos un animal fue detectado como positivo por ELISA. Los factores a nivel individual y a nivel hato "número de partos" y "tipo de alimentación de terneras antes del destete" mostraron una asociación débil y fuerte con los resultados positivos de ELISA, respectivamente. El odds ratio (OR) para la seropositividad a EJ aumentó en un 20% (OR = 1,20; 95% CI: 0,98-1,47; p = 0,067) en vacas con más de un parto, mientras que fue 0,74 veces menor (OR = 0,26; 95% CI: 0,096-0,70; p<0,01) en hatos que alimentan sus terneras con mezcla de calostro proveniente de varias vacas, en comparación con aquellos hatos que solo utilizan el calostro de la propia madre. **Conclusión:** la seroprevalencia de EJ fue del 10 y del 70% a nivel individual y a nivel hato, respectivamente. Los factores a nivel individual y a nivel hato "número de partos" y "tipo de alimentación de terneras antes del destete" mostraron una asociación débil y fuerte con los resultados positivos de ELISA, respectivamente.

Palabras clave: *epidemiología, ganado, paratuberculosis, serología.*

Resumo

Antecedentes: a doença de Johne (DJ) é uma enterite grave que afeta o ruminantes, e tem sido diagnosticada em bovinos e ovinos na Colômbia. No entanto, a informação epidemiológica da doença no país é escassa. **Objetivo:** identificar os fatores associados ao estado sorológico de DJ em vacas leiteiras por ensaio imunossorvente ligado a enzima (ELISA). **Métodos:** estudo transversal foi realizado em 307 vacas leiteiras adultos assintomáticos em 9 de 14 aldeias nos municípios de Belmira y San Pedro de los Milagros, em novembro de 2007. Em cada rebanho foram selecionados aleatoriamente 19-25 vacas com mais de 2 anos de idade e amostras de sangue foram tomadas. Um kit de ELISA comercial é utilizado para testar soros. As informações foram coletadas por meio de questionários sobre as características individuais de animais e práticas de gestão do rebanho. A análise estatística descritiva foi realizada para todas as variáveis de interesse e modelo de regressão logística multivariada foi construído, considerando um nível de significância de p<0,05. **Resultados:** dez por cento dos animais foram positivos no ELISA (31/307; 95% CI: 7,0-14%). No 70% (10/14) os rebanhos pelo menos, um animal foi detectado como positivo por ELISA. Os fatores a nível individual como a nível do rebanho "paridade" e "tipo de alimentação bezerros antes do desmame" mostraram uma associação fraca e forte com os resultados positivos de ELISA, respectivamente. O odds ratio (OR) para DJ soropositividade aumentou em um 20% (OR = 1,20; 95% CI: 0,98-1,47; p = 0,067) em vacas com mais de um nascimento, enquanto era 0,74 vezes menor (OR = 0,26; 95% CI: 0,096-0,70; p<0,01) em rebanhos que alimentam seus filhotes com a mistura de colostro de várias vacas, em comparação com apenas dados de uso que o colostro da mãe. **Conclusão:** DJ soroprevalência foi de 10 e 70% a nível individual como a nível do rebanho, respectivamente. Os fatores a nível individual e do rebanho "paridade" e "tipo de alimentação bezerros antes do desmame" mostrou fraca associação e forte com os resultados positivos de ELISA, respectivamente.

Palavras chave: *epidemiologia, gado, paratuberculosis, sorologia.*

Introduction

Johne´s disease (JD) or paratuberculosis is a severe enteritis that affects cattle and other domestic and wild ruminants (Harris and Barletta, 2001). The causal agent of JD is *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a Gram–positive, facultative, mycobactin-dependant, slow growing, and acid-fast bacillus (Chiodini *et al.,* 1984; Sweeney, 1996). MAP has also been associated with the human inflammatory bowel disease known as Crohn's disease (Chacon *et al.,* 2004; Atreya *et al.,* 2014; Sechi and Dow, 2015).

Johne´s disease causes substantial economic losses to the cattle industry due to increased premature culling, replacement costs, decreased milk yield, reduced feed conversion efficiency, fertility problems, reduced slaughter values, and increased susceptibility to other diseases or conditions (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, 2009; McAloon *et al.,* 2016). It is known that this disease has a global distribution (Barkema *et al.,* 2010; Manning and Collins, 2010).

Prevention and control of JD demands knowledge on the disease presence, frequency, and distribution as well as those factors influencing its entrance and perpetuation in herds. Herd management practices can increase or decrease the probability of MAP entering or circulating in a dairy cattle population. These practices vary, not only between countries or agro ecological zones, but also between regions and herds, thus requiring determining specific factors associated with JD status of animals or herds in each region.

Previous studies have identified a number of cow and herd management factors that can influence MAP status. Most of these studies have been conducted using the herd as the unit of analysis, and have used serological results to establish the MAP diagnosis of animals, as well as the subsequent identification of risk factors (Collins *et al.,* 1994; Goodger *et al.,* 1996; Johnson-Ifearulundu and Kaneene, 1999; Jakobsen *et al.,* 2000; Hacker *et al.,* 2004; Dieguez *et al.,* 2008; Nielsen *et al,* 2008; Tiwari *et al*., 2009; Sorge *et al.,* 2012; Pieper *et al*., 2015; Vilar *et al*., 2015). However, other diagnostic methods, such as PCR (Ansari-Lari *et al.,* 2009), fecal culture (Obasanjo *et al.,* 1997), and mixed methods (Kobayashi *et al.,* 2007) have been used to establish JD prevalence and risk factors.

A previous study reported MAP seroprevalence of 3.6% (14/696) and 2% (1/28) at the herd and animal-level, respectively. In addition, days in milk between 100-200 days (OR = 4.42) and >200 days (OR = 3.45), and the daily milk yield between 20-40 L/cow (OR = 2.53) and >40 L/cow (OR = 20.38) were identified as risk factors for MAP seropositivity (Correa-Valencia *et al.,* 2016).

The objective of this study was to identify cow and herd management factors associated with the serological individual status to MAP (based on ELISA -enzyme-linked immunosorbent assay- results) in 307 asymptomatic lactating Holstein cows from 14 herds in San Pedro de los Milagros, Antioquia (Colombia).

Materials and methods

Ethical considerations

The study was conducted following the Statute of Good Scientific Practices of the Justus-Liebig-University Giessen (Statute of Justus Liebig University Giessen for Ensuring Good Academic Practice, 29th May 2002).

Herds and animals

In November 2007, 14 herds located in nine districts of two municipalities (Belmira and San Pedro de los Milagros) in the Northern dairy region of Antioquia province, Colombia, were sampled for detecting anti-MAP antibodies. Blood samples were collected from 307 asymptomatic Holstein cows over two years of age. One of the herds presented sporadic clinical cases compatible with JD, which was confirmed by PCR and histopathology (Zapata *et al.,* 2010; Ramírez *et al.,* 2011). The animals tested in every herd were randomly selected (Table 1).

Enzyme–linked immunosorbent assay (ELISA)

The indirect unabsorbed ELISA, based on the detection of MAP−lipoarabinomannan (LAM,

Table 1. Results of *Mycobacterium avium* subsp. *paratuberculosis* ELISA of dairy cows in 14 dairy herds located in 9 district of two municipalities in Northern Antioquia, Colombia.

Municipality	District	Herd ID	Total number of cows in population	Number of samples collected and tested	Number of MAP−ELISA positive samples
San Pedro de los Milagros	Monterredondo	1[a]	102	20	3
Belmira	Playas	2	75	19	0
	Zona Urbana	3	128	21	3
	Labores	4	300	29	1
		5	100	19	0
		6	176	25	3
		7	102	23	1
	El Yuyal	8	140	20	4
		9	74	22	6
	Santo Domingo	10	181	23	5
	Amoladora	11	83	20	1
		12	75	20	0
	Zafra	13	67	21	4
	Zancudito	14	96	25	0
Total		14	1699	307	31

[a]Herd with history of JD (Zapata *et al.,* 2010).

[b]Corresponds to a representative sample of animals ≥2 years of age.

Svanovir Para−TB Ab ELISA Kit®, Svanova Biotech AB, Uppsala, Sweden) was used to detect anti-MAP antibodies. Percent positivity (PP) by ELISA considered the optical density values (OD) obtained at 450 nm, using the following formula:

PP = mean OD value (sample or negative control) / mean OD value (positive control) x 100.

An ELISA-positive case was defined as an animal with a PP ≥3 and a negative case as an animal with a PP ≤31. Eight out of 307 (2%) doubtful results (PP >31 - <53) were considered negative. Regarding the factors associated with MAP status, a positive or negative ELISA result was considered the outcome or dependent variable.

Factors associated with MAP serologic status

Information regarding cow and herd management practices was collected to determine factors that could be associated with the cow serological status. Cow factors and herd management practices have been associated with JD status in previous studies using the same diagnostic test (ELISA). Two questionnaires were administered to herd managers and/or owners during sample collection. The first questionnaire asked for information regarding cow factors such as identification (number and name), age, farm-born, parity, and daily milk yield. The second questionnaire asked for information regarding herd management practices related to maintenance or transmission of MAP within and between herds. Questions related to herd management practices were distributed into four groups; Group 1: general information of the herd (farm size, access to veterinary services, daily milk production, and cattle population); Group 2: information regarding presence of the disease in the herd (current presentation of symptomatic animals, and cases of Johne's disease observed during the last two years); Group 3: management factors affecting MAP transmission between herds (cattle purchase, own animals grazing on foreign pastures, foreign animals grazing on own pastures); and Group 4: factors regarding housing and hygiene (existence of a calving area, type of calf housing before weaning, feeding of calves before weaning, spreading of manure on pastures, and bird sighting in feed storing areas, Table 2).

Table 2. Cow and herd predictors associated with *Mycobacterium avium* subsp. *paratuberculosis* ELISA status in 307 dairy cows of Northern Antioquia, Colombia

Variable group	Variable	Unit / Category	Observations	Distribution
Cow factors	Age (years)	>2-5	139	45,3
		>5-10	151	49,2
		>10	17	5,5
			307	100,0
	Parity	1	50	16,3
		2	77	25,1
		3	50	16,3
		4	45	14,7
		5	34	11,1
		6	27	8,8
		7	16	5,2
		8	5	1,6
		9	2	0,7
		10	1	0,3
			307	100,0
	Cow born in farm	Yes	257	83,7
		No	50	16,3
			307	100,0
	Cow daily average milk yield (lt)	Low (<20 lt/day/cow)	151	49,2
		High (>20 lt/day/cow)	156	50,8
			307	100,0
Herd traits	Access to veterinary services	Yes	241	78,5
		No	66	21,5
			307	100,0
	Farm size (hectares)	<50 Ha	210	68.4
		>50 Ha	97	31.6
			307	100,0
	Herd daily average milk production (liters)	<801 lt	165	53,7
		>800 lt	142	46,3
			307	100
	Herd population (heads)	≤78	85	27,7
		≥77-<144	127	41,4
		≥143	95	30,9
			307	100
Information about presence of disease in the herd	Current presence of symptomatic animals in herd (the day of the visit)	Yes	45	14,7
		No	262	85,3
			307	100,0
	Cases of the disease in the last two years	Yes	102	33,2
		No	205	66,8
			307	100,0

Factors affecting disease transmission between herds	Cattle purchase	Yes	181	59,0
		No	126	41,0
			307	100,0
	Own animals grazing in foreign pastures	Yes	114	37,1
		No	193	62,9
			307	100,0
	Foreign animals grazing in own pastures	Yes	73	23,8
		No	234	76,2
			307	100,0
Factors related to housing and hygiene	Existence of calving areas	Yes	82	26,7
		No	225	73,3
			307	100,0
	Type of calf housing before weaning	Stall	19	6,2
		Pasture	259	84,4
		Other housing type	29	9,4
			307	100,0
	Feeding of calves before weaning	Colostrum from the own dam	173	56,4
		Colostrum mix from several cows	134	43,6
			307	100,0
	Spreading of manure on pastures	Yes	126	41,0
		No	181	59,0
			307	100,0
	Birds sighting in feed storing areas	Yes	89	29,0
		No	218	71,0
			307	100,0

Case definition

The case definition for a MAP-infected animal was a cow with an ELISA seropositive result (PP≥53).

Statistical analysis

The data collected during the study (Table 2) was saved in Excel (Microsoft Corp., Redmond, WA, USA) and then exported to Stata 12.0 (StataCorp, 2011, Texas, USA) for statistical analysis. The data was examined for biologically implausible entries. Erroneous data were removed or corrected. Descriptive statistics were computed for all variables (Table 3 and 4). An initial exploratory analysis of the data was conducted using Chi−square and Fisher's exact tests. Results of the initial exploratory analysis revealed that "parity", "herd population (heads)", "own animals grazing in foreign pastures", "feeding of calves before weaning", and "spread on pastures" were significantly associated with cow serological status (data not shown). Subsequently, Pearson and Spearman correlation analysis was executed for continuous and categorical variables, respectively. An unconditional mixed−effects logistic regression analysis, grouped by herd to account for clustering, was performed. The criteria of Hosmer-Lemeshow ($p<0.25$) was used to retain variables for the multivariable model. A multivariable mixed−effects logistic regression model, grouped by herd, was performed using a significance level of $p<0.05$. The results from the final model are presented as odds ratios (OR) with 95% confidence intervals (CIs). The response variable was the serum status ELISA for MAP (positive or negative). The cluster effect was random and all risk factors (parity, own animals grazing in foreign pastures, feeding of calves before weaning, spreading of manure on pastures, birds sighting in feed storing areas) were fixed effects. The logistic model took the general form: (Y) ~ binary outcome (probability π), Logit (π) = $ß_0$ intercept + $ß_1$ Parity$_{ij}$ + $ß_2$ average daily milk production of the herd (lt) + $ß_3$ herd population (heads) + $ß_4$ own animals grazing in foreign pastures$_j$ + $ß_5$ feeding of calves before weaning$_j$ + $ß_6$ spreading of manure on pastures$_j$ + $ß_7$ birds sighting in feed storing areas + e_{ij} Where Y_{ij}= outcome variable at i-th cow and the jth farm π = fitted probability of the outcome, $ß_1$ to $ß_5$ = coefficients associated with each covariate, e_{ij} = random residual effect.

Results

ELISA

Ten percent (10.1%, 31/307; 95% CI: 6.7-13.5%), and 89.9% (276/307; 95% CI: 86.5-93.3%) of the samples produced positive and negative results by ELISA, respectively. In 70% (10/14) of the herds, ELISA detected at least one seropositive animal (Table 1).

Factors associated with MAP serologic status

Analysis of correlation revealed collinearity (>0.80) between the variable “age” (years) and “parity” according to previous concepts (Dohoo *et al.,* 2010); therefore, the former was excluded from the analysis. The results of the univariable analysis revealed that the factors “parity”, “herd daily average milk production (liters)”, “whole cattle population”, “own animals grazing in foreign pastures”, “feeding of calves before weaning”, “manure spread on pastures”, and “birds sighted in feedstuff store” were significantly associated with cow serological status ($p<0.25$; Table 4). These variables were selected for the multivariable analysis. In the final logistic regression model, the factors identified were as follows: “parity” (OR = 1.20), and “feeding of calves before weaning” (OR = 0.26; Table 5).

Discussion

The proportion of ELISA positive results obtained from animals (10.1%) and herds (70%) is consistent with the apparent prevalence of JD in other Latin-American countries, using both unabsorbed and absorbed ELISAs (Fernandez-Silva *et al.,* 2014).

The factors “feeding of calves before weaning” and “parity” were significantly associated with the ELISA status of the cow in the initial exploratory analysis, in both the bivariable and multivariable analysis.

Table 3. Descriptive summary of quantitative variables in dairy cows of the Northern dairy region of Antioquia, Colombia, according to *Mycobacterium avium* subsp. *paratuberculosis* ELISA results.

Group	Variable	Mean value of variable in MAP−ELISA positive animals	Standard deviation	Smallest value	Largest value
Cow-level factors	Age[a]	6.3 years	2.1	3	10.6
	Parity	4.0 births	1.6	2	7
	Individual daily average milk yield	19.9 liters	4.4	10	28
General information of herd	Farm size	51.1 hectares	25.1	14	106
	Herd daily average milk production	1089.7 liters	464.3	600	2000
	Whole herd population	133.0 heads	56.9	77	274

[a]Variable excluded from the unconditional analysis due to collinearity (>0.80).

Table 4. Unconditional analysis of factors associated with the *Mycobacterium avium* subsp. *paratuberculosis* ELISA status in 307 dairy cows of Northern Antioquia, Colombia.

Variable group	Variable	Category	No of animals sampled	No of ELISA positive animals		P
				n	%	
Cow-level factors	Parity	1	50	0	0.0	0.163[a]
		2	77	9	11.7	
		3	50	1	2.0	
		4	45	9	20.0	
		5	34	6	17.6	
		6	27	4	14.8	
		7	16	2	12.5	
		8	5	0	0.0	
		9	2	0	0.0	
		10	1	0	0.0	
	Cow born in farm	Yes	257	28	10.9	0.270
		No	50	3	6 .0	
	Cow daily average milk production (lt)	Low (<20 lt/day/cow)	151	14	9.3	0.636
		High (>20 lt/day/cow)	156	17	10 .9	
General information of herd	Access to veterinary services	Yes	240	25	10.4	0.875
		No	66	6	9 .1	
	Farm size (hectares)	<50 Ha	210	18	8.6	0.291
		>50 Ha	97	13	13.4	
			307			
	Herd daily average milk production (lt)	< 801 lt	165	12	7.3	0.178[a]
		> 800 lt	142	19	13.4	
			307			
	Whole herd cattle population (heads)	≤78	85	4	4.7	0.058[a]
		≥77 - <144	127	12	9.4	
		≥143	95	15	15.8	
			307			
Information about the presentation of disease in the herd	Current presentation of symptomatic animals in herd	Yes	45	3	6 .7	0.587
		No	262	28	10 .7	
	Presentation of disease in the last 2 years	Yes	102	13	12.7	0.549
		No	205	18	8.8	

Factors affecting transmission between herds	Cattle purchase	Yes	181	19	10.5	0.803
		No	126	12	9 .5	
	Own animals graze in foreign pastures	Yes	114	7	6 .1	0.196[a]
		No	193	24	12.4	
	Foreign animals graze in own pastures	Yes	73	4	5.5	0.277
		No	234	27	11.5	
Factors related to housing and hygiene	Existence of specific calving place	Yes	82	8	9.8	0.983
		No	225	23	10.2	
	Type of calf housing before weaning	Stall	19	0	0.0	0.808
		Pasture	259	30	11.6	
		Other type of housing	29	1	3.4	
	Feeding of calves before weaning	Colostrum from the own dam	173	25	14.5	0.020[a]
		Colostrum mix from several cows	134	6	4.5	
	Spreading of manure on pastures	Yes	126	21	16.7	0.008[a]
		No	181	10	5.5	
	Bird sighting in feedstuff store	Yes	89	5	5.6	0.202[a]
		No	218	26	11.9	

[a]Variables used for the multivariable analysis (p<0.25).

Table 5. Factors associated to *Mycobacterium avium* subsp. *paratuberculosis* ELISA status (multivariable logistic regression).

Factor	**Odds Ratio**	**Standard error**	**z**	**95% CI**	**P**
Parity	1.20	0.12	1.83	0.98-1.47	0.067
Feeding of calves before weaning	0.26	0.13	−2.666	0.09-0.70	0.008
Cons	0.31	0.23	−1.56	0.07-.34	0.120

Wald chi^2 = 9.76; p = 0.0076.

Mycobacterium avium subspecies *paratuberculosis* (MAP) has been isolated from colostrum of subclinically infected cows (Streeter *et al.,* 1995; Pithua *et al.,* 2011; Stabel *et al.,* 2014; Laurin *et al.,* 2015; Jenvey *et al.,* 2016). Additionally, the practice of feeding calves with colostrum collected from known MAP infected cows (Dieguez *et al.,* 2008), as well as the practice of feeding calves pooled colostrum from multiple cows (Nielsen *et al.,* 2008) have been identified as risk factors for MAP in dairy herds. Therefore, our results indicating a protective factor (OR = 0.26) disagree with this previous evidence and should be interpreted carefully. It is also possible that

MAP contaminated teats can increase the likelihood of infection via suckling directly from an infected dam, compared to feeding from a colostrum mix of MAP free cows (Pithua *et al.,* 2011). In our study, information regarding the method of colostrum feeding was not collected; therefore, this hypothesis cannot be confirmed. "Parity" was associated with JD serological status in previous studies, in which high parity (≥5) was associated with the probability of a positive ELISA result (Jakobsen *et al.,* 2000). Additionally, the probability of a positive ELISA result was two to three times lower for first parity cows relative to cows in other parities (Nielsen *et al.,* 2002).

Factors "born in a foreign herd" (Wells and Wagner, 2000; Tiwari *et al.,* 2009; Sorge *et al.,* 2012), "comingle with foreign cattle" (Fredriksen *et al.,* 2004) and "manure spread on pastures" (Obasanjo *et al.,* 1997), have previously been associated with JD status of herds. However, the present study found no significant associations in the multivariable analysis in relation to the herd ELISA status. This could be explained by the fact that our study was smaller concerning sample size in terms of number of herds. With our small sample size and our cow-based analysis, it is possible that significant associations, although present, could not be detected. In order to achieve a higher power, bigger sample sizes should be used. Unfortunately, a bigger sample size was not affordable at the time of the present exploratory study.

In Colombian dairy production systems, the purchase and exchange of animals between herds is relatively common. Animals regularly purchased for replacement or for fattening, are kept in the herd of origin until weaning and then transported to their final destination. This practice increases the risk of JD transmission to free herds due to introduction of young, subclinically infected, animals (Manning and Collins, 2010). Similar to what was found in the present study, spreading manure on pastures has been reported as a factor associated with the MAP culture results in the univariate, but not in the multiple logistic regression analysis in a previous study in the United States (Obasanjo *et al.,* 1997). In Colombia, it is common practice to spread slurry (cattle feces alone or in combination with pig feces) as manure for pasture. This type of mix has already been identified as a potential source of MAP for cattle, considering its survival capacity for long periods (up to 252 days at 5 °C) in cattle, pig, and cattle-pig slurry (Jorgensen *et al.,* 1977). MAP can withstand simulated composting, manure packing and liquid storage of manure from dairy farms (Grewal *et al.,* 2006).

The low number of cattle and herds sampled is the main weakness of this study, but it can significantly contribute to the knowledge of JD in the country. Another disadvantage is the use of ELISA results as the outcome or dependent variable. ELISA is a very useful and economic tool to determine JD infection status of animals but it has low sensitivity to detect antibodies in asymptomatic adult cattle (Nielsen and Toft, 2011). However, ELISA has been widely used for prevalence and risk factor determinations, it is inexpensive, easy to perform, and results can be compared with previous national and international studies.

Conclusion

"Parity" was a risk factor for MAP status while "feeding of calves before weaning" appeared to be protective for the serological status to MAP in the herds tested. Additional studies are necessary to increase epidemiological knowledge of JD in Colombia.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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