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Original Research

Prevalence of paratuberculosis (*Mycobacterium avium* subspecies *paratuberculosis*) in specialized dairy herds in Costa Rica

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ABSTRACT:

A cross-sectional study was conducted in 194 specialized dairy herds in Costa Rica, to determine the prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in herds at a national level and by administrative regions. Bulk milk samples were taken twice, three months apart; the sera was analyzed using an immunoassay. Overall MAP prevalence of herds was 79.4%; a total of 70.1% of the herds showed low prevalence and 9.3% showed high prevalence. Changes were observed in 43.8% of the herds analyzed between the first and the second sampling: 16 of 47 changed from negative to positive, while 45 of 122 changed from positive to negative. Spatial analysis of the distribution of MAP seropositive and negative herds indicated no significant difference in the average central location of both groups. Significant differences were found in MAP prevalence based on differences in life zones and precipitation. A greater proportion of MAP negative farms was found in the lower montane rain forest (Z= -2.0289; P= 0.0424), and in precipitation ranges of 4000 -8000 mm (Z= -2.9920; P= 0.0028), while a greater proportion of seropositive farms were found in the precipitation ranges of 1000-2000 mm (Z= 2.5137; P= 0.0121). Considering the epidemiology of MAP and the low sensitivity of the Paratuberculosis ELISA, it may be concluded that a considerable amount of potentially infected herds were not detected, resulting in an estimated high prevalence, nationwide. This study may be detecting only a part of the problem that paratuberculosis poses for the dairy industry in Costa Rica.

Keywords:
Paratuberculosis, epidemiology, dairy cattle, tropics, Costa Rica

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INTRODUCTION

Paratuberculosis, or Johne's disease, is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and produces a granulomatous enteritis which manifests in persistent or recurring diarrheas and does not respond to the therapeutic treatment with antibiotics (Chiodini *et al*., 1984). It is distributed worldwide and affects cattle, sheep and goats (Nielsen and Toft, 2009; Fernández-Silva *et al*., 2014). MAP is a facultative intracellular aerobic bacillus, alcohol-acid resistant, mycobactin-dependent, with slow growth (8 to 12 weeks) and difficult to culture. Its cellular wall is a physical barrier that provides the bacillus with great resistance to physical factors such as heat, cold, light and desiccation as well as to disinfectants (Manning, 2001; Bannantine *et al*., 2003).

Cattle shed minimum amounts of MAP intermittently in their feces during the subclinical phase of infection, which may last years and high amounts in the clinical phase of the disease, which may last approximately three years (Wu *et al*., 2007). Through time, this causes considerable environmental contamination and an insidious dissemination of infection in the herd (Whittington *et al*., 2004). According to Jörgensen (1977), 85% of the animals that acquire the disease will be infected in the first postpartum weeks through ingestion of MAP contaminated colostrum and milk. Calves are most susceptible to MAP infection, since they can be infected by low dose (10^3-10^4/g) of feces. In general, this happens because milk comes from an infected animal, and it was not sufficiently heated or was contaminated with feces (Wu *et al*., 2007). It is believed that another 5% of animals will be infected during their first years of life through food contaminated with bovine feces or infected wildlife reservoirs (Greig *et al*., 1997; Robino *et al*., 2002; Judge *et al*., 2006). Finally, around 10% of animals will be infected in the uterus, if mothers are in the clinical phase of the disease (Wu *et al*., 2007).

In 95% of the cases, introduction of MAP into uninfected herds take place through introduction of infected bovines into the herd, since diagnostic techniques show low sensitivity, making it difficult in detecting MAP when animals are purchased (Khol *et al*., 2013; Biet and Boschiroli, 2014); in the remaining 5% of cases, the agent may be transmitted through contaminated food or water and due to high resistance of MAP in the environment (Greig *et al*., 1997; Robino *et al*., 2002; Whittington *et al*., 2004; Judge *et al*., 2006). If an infected individual is introduced to a herd of 100 animals, 22% of them will become infected during a 15-year period; of these, 10% will be disseminators of the disease and 6% will be eliminated because of MAP infection (Valentin-Weigand and Goethe, 1999).

Traditional bacteriological diagnosis – through cultures – can detect animals between 5 to 10 years after they have been infected; this is the gold standard for MAP diagnosis, with a detection limit of 50 to 100 MAP/g feces, and a sensitivity of 10% to 50%. Complementing the culture with molecular tests such as Polymerase Chain Reaction (PCR) increases the sensitivity to 100%. Generally the DE insertion IS900 segment is amplified by PCR (Gierke, 2010). PCR may also be used to identify subclinical excretors, showing a detection limit of 10^4 MAP/g in feces. In contrast, detection of MAP in individual or bulk milk samples through conventional PCR seems to be difficult, while using Real-Time PCR allowed to detect 300 copies/ml of milk with a 100% sensitivity (Gierke, 2010). The great disadvantage of all direct diagnostic assays is that only a positive result is certain, since excretion of MAP in feces and milk occurs intermittently, and that individuals tested in the subclinical phase does eventually not excrete the agent on the day when the sample was taken, producing a false negative result (Stratmann, 2002). Other disadvantages with these techniques are its cost (Real-Time PCR) and the long time needed for culturing which makes it relatively inaccessible for producers.

Serological techniques are low-cost and allow quick analysis of a large number of samples. They can also detect MAP positive animals between 2 to 5 years after they have been infected (Collins *et al*., 2005). Immuno-enzymatic assays (ELISA) may be carried out on individual sera as well as in individual milk samples for an early diagnosis of infected animals (up to 2 years before clinical symptoms appear) as well as in bulk milk samples, to determine the sanitary condition of the herd. The assays that are available in the market report variable sensitivities and specificities (Jark *et al*., 1997; Winterhoff *et al*., 2002; Geisbauer *et al*., 2007; Geue *et al*., 2007; Woodbine *et al*., 2009; Fernández-Silva *et al*., 2011), and may produce false negative results due to low sensitivity or in final phases of the disease, when the immune response is weak or non-existent leading the owner of a herd to believe that the agent is absent in the animals (Wilson *et al*., 2010). On the other hand, false positive results have been reported due to the cross-reactions with other mycobacteria. However, use of immunoassays is recommended because they are simple, practical and cost efficient for MAP detection in dairy herds (Collins *et al*., 2006).

In Europe, MAP prevalence in herds is reported to be as high as 86% in Denmark, 71% in the Netherlands, and 42.5% to 89.0% in the United Kingdom (Caldow *et al*., 2009; Geraghty *et al*., 2014). In the USA, MAP prevalence has not been precisely determined, but it is estimated that between 40% and 68% of dairy herds are infected (Geraghty *et al*., 2014). In Canada, different prevalences are reported in different provinces, ranging between 60% and 80% (Wolf *et al*., 2014). Several studies in Latin America and the Caribbean showed individual and herd prevalences ranging between 16.9% and 75.8% (Fernández-Silva *et al*., 2014).

In Costa Rica, a study carried out in 1996 with a serum bank of bovines collected by the Ministry of Agriculture to detect brucellosis in bovines established 18.7% of herds positive to MAP and 11.9% of bovines seropositive (Dolz *et al*., 1999); however, only five animals per herd were analyzed, suggesting a significant underestimation of prevalences.

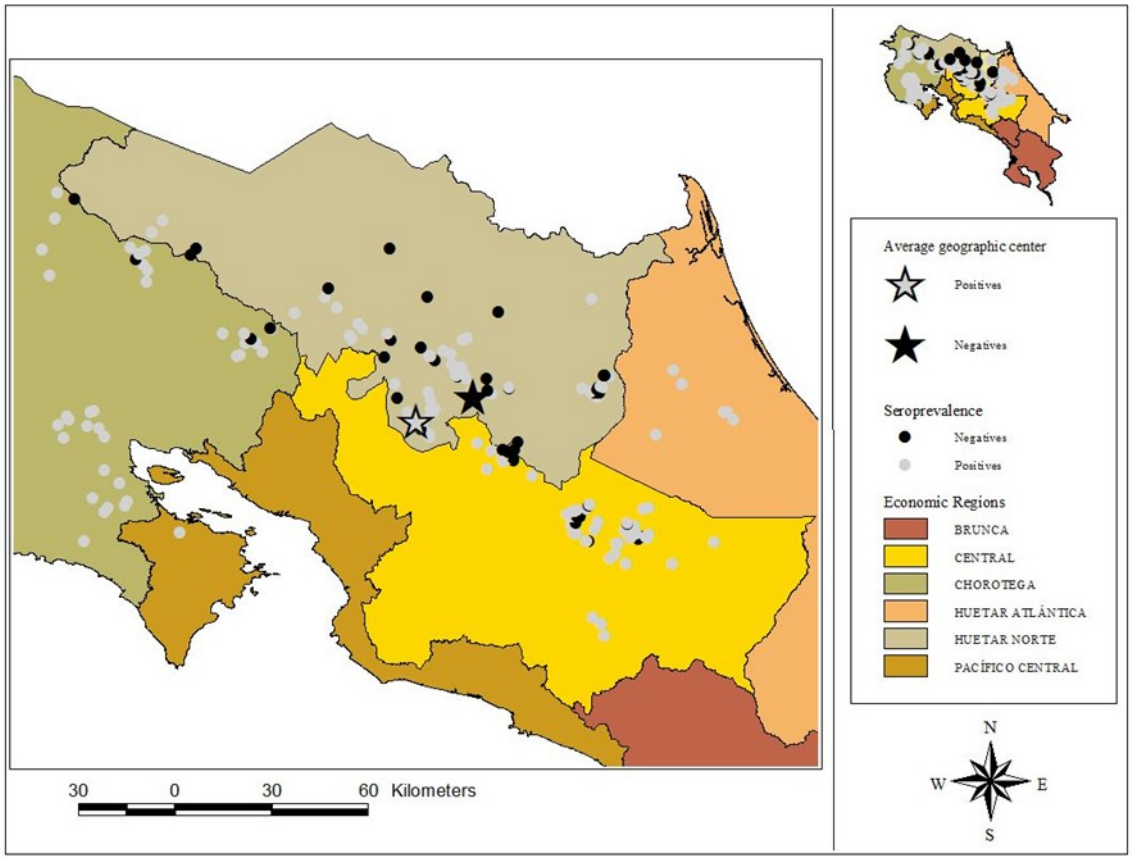
On the other hand, the potential association of MAP with Crohn's disease in humans has increased the importance of studying MAP in different parts of the world, leading to a recommendation to reduce exposure to this agent (Vanleeuwen, 2008). Given the importance of MAP prevalence on bovine production as well as the possible risk for human health, this work intended to determine MAP prevalence in specialized dairy herds nationwide and by administrative regions analyzing bulk milk samples.

MATERIALS AND METHODS

Type of study and sample size

A descriptive transversal study was carried out to determine seroprevalence of MAP in specialized dairy herds in Costa Rica. The population studied consisted of 1.550 specialized dairy farms, associated with a nationwide cooperative, distributed in all dairy zones throughout the country, with the farm as the sample unit. The farms were classified by administrative regions in which they were located (Central, Huetar Norte, Huetar Atlántica and Chorotega). The percentage of farms to be included in the sample was calculated based on an expected prevalence of 18.7% (Dolz *et al*., 1999), with an acceptable error of 5% and a 95% confidence level. Samples were taken from a total of 201 farms. For logistical reasons, by the end of the study it was possible to analyze only 194 farms. The number of farms to be analyzed per administrative region was determined through proportional allocation: 57 in the Central region, 57 in the Huetar Norte region, 51 in the Chorotega region and 29 in the Huetar Atlántica region (Figure 1). The farms in each region were selected through a simple random sampling procedure from a previously obtained list. To obtain a better prevalence estimate, farms were sampled two times, once during the dry season (February

Figure 1: Average center of MAP positive and negative farms in the specialized dairy herds of Costa Rica, 2007



- March) and again during the rainy season (May - June) of 2007. One hundred and sixty nine farms were analyzed in the first sampling period while 194 farms were analyzed in the second sampling.

Milk sampling

Detection of MAP antibodies was carried out in bulk milk samples. The sample was taken following the recommendations of the manufacturer, as follows: in herds with 50 cows or more being milked, a bulk milk sample was taken from the first 50 animals that were milked; in herds with less than 50 cows being milked, a bulk milk sample was taken from all the animals. Milk samples were transported to the laboratory under refrigeration (4ºC to 7ºC), where they were centrifuged at 10.000 x g for 10 minutes and the supernatant (milk sera) was stored at –20ºC until it was analyzed.

Serological analysis of bulk milk samples

Milk sera samples were analyzed with the indirect paratuberculosis immune-enzymatic assay (ParaTB-ELISA) from Svanova, Uppsala, Sweden, which reported a sensitivity of 90% and a specificity of 94% . Lipoarabinomannan (LAM) antigen of the cellular wall of MAP was absorbed to the microtiter plates, the assay was carried out following the instructions of the manufacturer. Milk sera samples were diluted in the ratio of 1:10, while control sera was diluted in the ratio of 1:100; all samples were tested in duplicates on the plate. To validate the assay, Optical Densities (OD) of the duplicates of the positive control, negative control and milk samples were verified which do not differ by more than 25% between them; it was also verified that positive and negative controls were within the limits specified by the manufacturer. With the optical densities obtained

Table 1. Global and regional MAP prevalence in specialized dairy herds in Costa Rica, 2007

Region	Stratum	Sampling Period 1		Sampling Period 2		General Results		CI 95%	
		n	%	n	%	n	%	LL	UL
Global	Negative	47	27.8^{a}	85	43.8^{b}	40	20.6	14.9	26.3
	Positive	122	72.2^{a}	109	56.2^{b}	154	79.4	73.7	85.1
	Low	*115*	*68.0^{a}*	*97*	*50.0^{b}*	*136*	*70.1*	*63.7*	*76.6*
	High	*7*	*4.1^{a}*	*12*	*6.2^{a}*	*18*	*9.3*	*5.2*	*13.3*
	Total	169	*100.0*	194	100.0	194	100.0		
Central	Negative	9	$18.0^{\alpha a}$	25	$43.9^{\alpha b}$	10	17.5^{α}	7.7	27.4
	Positive	41	$82.0^{\alpha a}$	32	$56.1^{\alpha b}$	47	82.5^{α}	72.6	92.3
	Low	*36*	*$72.0^{\alpha \pi a}$*	*28*	*$49.1^{\alpha b}$*	*39*	*68.4^{α}*	*56.4*	*80.5*
	High	*5*	*$10.0^{\alpha a}$*	*4*	*$7.0^{\alpha a}$*	*8*	*14.0^{α}*	*5.0*	*23.1*
	Total	50	100.0	57	100.0	57	100.0		
Chorotega	Negative	3	$8.1^{\alpha a}$	18	$35.3^{\alpha b}$	5	9.8^{α}	1.6	18.0
	Positive	34	$91.9^{\alpha a}$	33	$64.7^{\alpha b}$	46	90.2^{α}	82.0	98.4
	Low	*34*	*$91.9^{\beta a}$*	*31*	*$60.8^{\alpha b}$*	*44*	*86.3^{β}*	*76.8*	*95.7*
	High	*0*	*$0.0^{\alpha a}$*	*2*	*$3.9^{\alpha a}$*	*2*	*3.9^{α}*	*-1.4*	*9.3*
	Total	37	100.0	51	100.0	51	100.0		
Huetar Atlántica	Negative	11	$40.7^{\beta a}$	17	$58.6^{\alpha a}$	10	$34.5^{\alpha \beta}$	17.2	51.8
	Positive	16	$59.3^{\beta a}$	12	$41.4^{\alpha a}$	19	$65.5^{\alpha \beta}$	48.2	82.8
	Low	*16*	*$59.3^{\alpha a}$*	*11*	*$37.9^{\alpha a}$*	*18*	*$62.1^{\alpha \beta}$*	*44.4*	*79.7*
	High	*0*	*$0.0^{\alpha a}$*	*1*	*$3.4^{\alpha a}$*	*1*	*3.4^{α}*	*-3.2*	*10.1*
	Total	27	100.0	29	100.0	29	100.0		
Huetar Norte	Negative	24	$43.6^{\beta a}$	25	$43.9^{\alpha a}$	15	$26.3^{\alpha \beta}$	14.9	37.8
	Positive	31	$56.4^{\beta a}$	32	$56.1^{\alpha a}$	42	$73.7^{\alpha \beta}$	62.3	85.1
	Low	*29*	*$52.7^{\beta \alpha a}$*	*27*	*$47.4^{\alpha a}$*	*35*	*61.4^{α}*	*48.8*	*74.0*
	High	*2*	*$3.6^{\alpha a}$*	*5*	*$8.8^{\alpha a}$*	*7*	*12.3^{α}*	*3.8*	*20.8*
	Total	55	100.0	57	100.0	57	100.0		

CI: Confidence Interval; LL: Lower Limit; UL: Upper Limit. Different superscripts indicate statistical differences with a value α=0.05 between the percentages of the regions from one sampling period to another, while different Greek letters indicate differences in percentages between regions and between each sampling period.

from the different milk samples, Positive Percentage (PP) was calculated, with respect to the average of the positive control sera, using the following formula: PP = (average OD of sample x 100): average OD of positive control. Herds with samples that yielded PP lower than 5% were considered as negative, whereas herds with samples showing PP 5%-16%, and higher than 16%, were considered with low prevalence and with high prevalence, respectively.

Statistical analysis

The number and percentage of MAP negative and positive (low and high) dairy herds by administrative zone and nationwide were determined based on the results obtained with ELISA in each sampling period. To estimate the maximum observed prevalence, the highest PP was chosen from each farm from the two sampling

Table2: MAP category variation (negative, low prevalence, or high prevalence) in specialized dairy herds in Costa Rica, between the first and second sampling periods, 2007

		Sampling Period 2			Total
		Negative	Low Prevalence	High Prevalence	
Sampling period 1	Negative	31	14	2	47
	Low Prevalence	44	63	8	115
	High Prevalence	1	5	1	7
	Total	76	82	11	169

periods. For prevalence levels, the confidence interval was calculated at 95%. Likewise, a descriptive analysis was carried out on variations between the results of the first and second sampling period, which produced a change in the prevalence category in farms. A comparison of prevalence levels was carried out, taking the maximum observed prevalence by administrative zone. When Pearson's chi-square test was used, prevalence was classified in two categories (negative and positive), and with Fisher's exact test three different strata (negative, low prevalence and high prevalence) were analyzed.

Spatial analysis

A database provided by the cooperative of milk producers, which included geographical locations (latitude, longitude) of the studied farms was used, along with serological results (the highest measurement of the two sampling periods) per farm. To determine spatial distribution, the average center of the location of MAP seropositive and negative farms was established with the Crime Stat III program (Levine, 2004). A *t* test was used for unpaired groups to compare the averages of sero-positive and negative farms. To analyze macro-environmental factors, the points that represented the farms were overlaid on environmental strata obtained from the Atlas Digital de Costa Rica 2014 (Ortiz-Malavasi, 2014) using the Spatial Join command of Arc GIS 10.2. The environmental strata used were life zones, precipitation, altitude, and type of soil (scale 1: 200 000). A two proportion test with 0.05 significance level was used to determine if there was a difference between MAP seropositive and negative farms.

RESULTS

In the first sampling period, 169 farms were analyzed, of which 47 (27.8%) were found MAP negative and 122 (72.2%) positive: 115 were determined with low prevalence of MAP, and seven with high prevalence (Table 1). Differences in the number of MAP negative and positive farms (P=0.03) were found at a regional level, where the Chorotega region presented the highest percentage of MAP positive farms (91.9%, 34/37), while the lowest percentage of MAP positive farms (56.4% and 59.3%) was detected in the Huetar Norte and Huetar Atlántica regions, respectively (Table 1). Of the farms that were found MAP positive, a substantial majority were classified as with low prevalence, from 52.7% (29/55) in the Huetar Norte region, to 91.9% (34/37) in the Chorotega region (Table 1). The region where most of the farms were detected with high MAP prevalence was the Central region (10.0%; 5/50), while in the rest of the regions, farms with high MAP prevalence ranged between 0 and 3.6% (Table 1).

In the second sampling period, more MAP negative farms were detected at regional and national level than in the first sampling period; additionally a lower number of farms with low prevalence were detected; however, the number of farms with high MAP

prevalence increased, especially in the Huetar Norte region. There was also an increase of herds with high prevalence in the Huetar Atlántica and Chorotega regions; in contrast to the Central region, which showed a reduction in herds with high MAP prevalence. Details on the results of the second sampling period are presented in Table 1.

Taking the highest PP value detected with ELISA for each farm (General Results, Table 1), global MAP prevalence was 79.4%. Of the total of 194 farms analyzed, 136 (70.1%) showed low prevalence and 18 (9.3%) showed high prevalence of MAP (Table 1). At a regional level, the Central region presented the largest number of MAP positive farms (47/57, 82.5%) and the greatest number of farms with high prevalence (8/57, 14.0%) at national level. The Central and Huetar Norte regions showed prevalences similar to the global prevalence, while in the Huetar Atlántica region 65.5% of the farms were MAP positive. Details of the results, and their respective confidence intervals are presented in Table 1.

Of a total of 169 farms whose samples were taken in two different periods, 95 (56.2%) showed agreement in their classification in both moments: 31 were MAP negative, 63 had low prevalence and one had high prevalence. Of the 47 MAP negative farms in the first sampling period, 16 (34.0%) were MAP positive (14 with low prevalence, and two with high prevalence) in the second sampling period; while of 115 farms with low prevalence in the first sampling period, 44 (38.3%) farms were MAP negative, and eight (6.7%) changed to high prevalence in the second sampling period. Finally, of the seven farms with a high MAP prevalence detected in the first sampling period, one changed to negative and five changed to low prevalence. As may be observed, the greatest proportion of changes was determined towards an immediate lower or higher category, with very few cases changing from the negative category to that of high prevalence, or vice versa (Table 2).

When the average geographic center of MAP positive and negative farms was calculated, a spatial difference was observed in the location of both groups; however, no significant difference was determined when comparing latitude and longitude between groups (Figure 1).

Macro-environmental characterization showed no differences in the average altitude and type of soil when comparing MAP seropositive and negative farms. However, significant differences were determined for life zones and precipitation. A greater proportion of MAP negative farms were located in the lower montane rain forest ($Z= -2.0289$; $P= 0.0424$), and with precipitation ranging 4000 to 8000 mm ($Z= -2.9920$; $P= 0.0028$), while a greater proportion of MAP seropositive farms was found in areas with precipitation ranging from 1000 to 2000 mm ($Z= 2.5137$; $P= 0.0121$).

DISCUSSION

Global prevalence of farms with MAP antibodies in milk for the country (72%: 68% with low prevalence and 4% with high prevalence), is consistent with reports from Latin America, North America and Europe, which indicate prevalences of 42.5% to 89% among dairy herds (Caldow *et al*., 2009; Geraghty *et al*., 2014, Fernández-Silva *et al*., 2014). However, it is not consistent with what was reported by Dolz *et al*. (1999) who stated that in Costa Rica, the percentage of MAP positive farms was established lower than 20%. This could be due to the fact that the 1999 study was carried out in a population that included beef, dairy and dual-purpose cattle, while the present study only analyzed specialized dairy farms. As reported in the literature, beef and dual-purpose cattle seem to be more resistant to MAP infections than cows of specialized dairy farms (Chiodini *et al*., 1984). Furthermore, the number of animals analyzed per herd in the study of Dolz *et al*., (1999) was very small, which does not provide certainty about the absence of infection in the herd. Finally, it is probable that during the time

elapsed between the two studies, the disease spread among Costa Rican herds, since according to Valentin-Weigand and Goethe (2006), with the introduction of an infected individual into a herd, around 22% of the animals will be infected after 15 years. It is important to take this information into consideration, because if adequate prevention and control measures are not applied, there is a high probability that an important proportion of the herds with low MAP prevalence detected in the present study will become high prevalence herds in the medium or long term. It is therefore necessary to maintain surveillance on these herds and, if possible, analyze sera of the animals individually, to confirm the results obtained in the present work, to detect MAP positive animals, and implement and execute herd sanitation measures. As is well-described in the literature, the agent may be present in a herd even though clinical symptoms have not been reported in any animal, because of the very long incubation period of the disease, ranging from two to five or more years (Collins *et al*., 2005).

Prevalences determined at the regional level are similar to those reported in 1999 in the Chorotega and Huetar Norte regions, while the Central and Huetar Atlántica regions showed a reduction of herds with high prevalence (Dolz *et al*., 1999). It is interesting to note the significant amount of MAP-positive herds in the Chorotega region (90.2%). Probably, the increase of dairy herds in this region, the use of specialized breeds for dairy production and the mobilization of animals from the Central and Huetar Norte regions to the Chorotega region without any control of MAP infection have caused this situation.

The reason for sampling bulk milk in two different periods in each farm, three months apart, was to determine MAP presence or absence with greater certainty and to establish approximate prevalence levels in the herds, since normal movements that take place within a specialized dairy herd (exit of dry cows and entry of mother cows) and the infection phase of a MAP-infected animal, may produce underestimations of prevalence, or even false negative results (Hendrick *et al*., 2005). In the present study, 74 (43.8%) of the farms showed different results in different sampling periods, which may have been due to the entry or exit of infected cows to and from milking stations during the study. This situation is particularly important if the result is a possible false negative result. In this study, 59% of the MAP negative farms in the first sampling period were diagnosed as MAP positive in the second sampling. In addition, recently infected bovines with MAP present a non-reactive phase in which a poor antibody response is reported, which is not detected by ELISA, producing false negative results (Coussens, 2004). After three months to two years, animals enter into a reactive phase in which antibodies can be detected (Waters *et al*., 1999).

Another possible explanation is that concentrations of MAP antibodies in bulk milk and therefore the ELISA results, were influenced by the amount of milk produced by each cow, by the MAP prevalence of the herd, and the parity of participating cows. During a longitudinal study, Nielsen and Toft (2009) found that cows that increased their milk production (>5Kg) changed their MAP ELISA results in milk from seropositive to seronegative. They also determined that cows with more than one delivery were easier to detect as seropositive primiparous cows. In conclusion, they recommend the use of MAP ELISA milk tests during early or late lactation of the cows, when milk production is low, and to include only primiparous cows (Nielsen and Toft, 2009).

Finally, the differences between results from the two sampling periods may have also been due to the MAP ELISA test used in the present work. In the literature, different sensitivities (27.8 to 90%), and similar specificities (90 to 94%) are reported (Jark *et al*., 1997; McKenna *et al*., 2005, Fernandez-Silva *et al*., 2011). Thus it can be concluded that, false negative

results may have occurred, but unlikely false positive results.

Macro-environmental characterization determined higher proportion of seronegative farms in areas with high precipitation and water saturated soils (lower montane rain forest), indicating that too much water may have a negative consequence for the survival of the bacteria. We hypothesize that this may occur because the bacteria is washed away from the topsoil and thus making it unavailable to the grazing cattle, what has to be investigated in further studies.

The MAP diagnosis in bulk milk samples showed its ability to detect paratuberculosis antibodies; however, due to the range of responses (negative, low and high prevalence), it is important to keep in mind that, any result other than negative, may be a warning signal. In this aspect, bulk milk sampling seems to be useful to obtain information about the situation of a region or country with respect to MAP prevalence and to control MAP negative herds. The present study indicates that two bulk milk samplings may produce different results in the same farm, showing prevalence changes. It is therefore important to analyze the MAP status of herds at multiple times, since the results obtained from the multiple samplings will be closer to the sanitary reality of the herd studied (Holstad *et al*., 2005, Lavers *et al*., 2014). In MAP positive herds (with low or high prevalence), it is also recommended to analyze animals individually at intervals of at least three months to identify positive bovines, which must be eliminated to reduce the presence of shedders of MAP within the herd, in order to control and eradicate the disease. It is concluded that immune-enzymatic assays are useful to obtain an estimate of a herd's sanitary status (Shawn *et al*., 2006).

CONCLUSION

Considering the epidemiology of MAP, where most individuals are infected during their first weeks but are not detected until several years later and given the high prevalence estimated throughout the country, this study may be detecting only a part of the problem that paratuberculosis represents for the milk industry in Costa Rica. Milk producers should implement MAP epidemiological surveillance in their herds through bulk milk analysis at least twice a year and remember that a single analysis of bulk milk with MAP negative results does not guarantee that the herd is free from the agent.

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