Journal MVZ Cordoba
2020; 25(2):e1782.
https://doi.org/10.21897/rmvz.1782

Original

Detection of *anti-Mycobacterium avium* subsp. *paratuberculosis* antibodies in cows of low-tropic dairy herds in the Provinces of Sucre and Córdoba, Colombia

Nathalia Correa-Valencia[1] M.Sc; Ferney Arango L[2] MV; Jorge A. Fernández-Silva[1*] Dr.Med.Vet.

[1]Universidad de Antioquia, Facultad de Ciencias Agrarias, Escuela de Medicina Veterinaria, Grupo Centauro, Colombia.
[2]Universidad de Antioquia, Facultad de Ciencias Agrarias, Escuela de Medicina Veterinaria, Colombia.
*Correspondencia: jorge.fernandez@udea.edu.co

Received: September 2019; Accepted: February 2020; Published: July 2020.

ABSTRACT

Objective. To report the frequency of seropositivity against *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and to explore the factors associated with the positive outcome, both at herd and animal level. **Materials and methods.** A cross-sectional study was carried out on 204 dairy cows, result of the crossing of different breeds, from five low-tropic dairy herds, located in three municipalities of the Provinces of Sucre and Córdoba (Colombia) in 2018. The animals were randomly selected, and blood samples were collected from each one. A commercial ELISA kit was used to analyze the sera. Information regarding factors related to herds and animals was collected through questionnaires. Descriptive statistics were calculated for all variables (i.e. herd size, presence of other ruminants co-grazing with cattle in the last 2 years, age of the animals, parity, and days since the last birth), and the association between these variables and the result to ELISA ($p<0.05$) was explored. **Results.** The 17.2% (35/204; 95% CI: 12.0-22.3%) of the cows were positive for MAP by the ELISA test, and the five herds had seropositive animals. The herd-level variable presence of other ruminants in co-grazing with cattle in the last 2 years and the animal-level variables age and parity were associated with the positive ELISA results. **Conclusions.** The present study found that 17.2% of the cows and 100% of the herds were MAP positive by the ELISA test. Additionally, associated variables were identified and may be of interest to both producers and veterinarians of production animals, guiding their approach to disease management.

Keywords: Epidemiology; cattle; serology (*Source: National Agricultural Library Thesaurus*).

RESUMEN

Objetivo. Reportar la frecuencia de la seropositividad a *Mycobacterium avium* subsp. *paratuberculosis* (MAP) y explorar los factores asociados al resultado positivo, tanto a nivel de hato como de animal. **Materiales y métodos.** Se llevó a cabo un estudio transversal en 204 vacas lecheras resultado

How to cite (Vancouver).
Correa-Valencia N, Arango LF, Fernández-Silva J. Mycobacterium avium subsp. paratuberculosis antibodies in cows of low-tropic dairy herds in the Provinces of Sucre and Cordoba, Colombia. Rev MVZ Cordoba. 2020; 25(2):e1782. https://doi.org/10.21897/rmvz.1782

ISSNe: 1909-0544

del cruce de diferentes razas, en cinco hatos de zonas de trópico bajo ubicadas en tres municipios de los departamentos de Sucre y Córdoba (Colombia) en 2018. Los animales fueron seleccionados aleatoriamente y se colectaron muestras de sangre de cada uno. Se utilizó un kit comercial de ELISA para analizar los sueros. La información con respecto a los factores relacionados con los hatos y los animales se recopiló mediante cuestionarios. Se calcularon las estadísticas descriptivas para todas las variables (i.e. tamaño del hato, presencia de otros rumiantes en copastoreo con el ganado bovino en los últimos 2 años, edad de los animales, paridad y días desde el último parto), y se exploró la asociación entre dichas variables y el resultado a ELISA ($p<0.05$). **Resultados.** El 17.2% (35/204; IC 95%: 12.0-22.3%) de las vacas resultaron positivas a MAP mediante la prueba de ELISA, y los cinco hatos tenían animales seropositivos. La variable a nivel de hato presencia de otros rumiantes en copastoreo con el ganado bovino en los últimos 2 años y las variables a nivel de animal *edad* y *paridad* se encontraron asociados con los resultados positivos de ELISA. **Conclusiones.** El presente estudio encontró que el 17.2% de las vacas y el 100% de los hatos fueron positivos a MAP mediante la prueba de ELISA. Adicionalmente se identificaron variables asociadas que pueden ser de interés tanto para los productores como para los veterinarios de animales de producción, y orientar su enfoque para el manejo de la enfermedad.

Palabras clave: Epidemiología; ganado; serología (*Fuente: National Agricultural Library Thesaurus*).

INTRODUCTION

Paratuberculosis (PTB) or Johne's disease, is a chronic contagious disease, affecting both domestic and wild ruminants, being characterized by a weight loss and chronic diarrhea. The PTB causes emaciation and finally the death of the animal. The disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), triggering a granulomatous enteropathy in affected animals, that results in poor absorption of essential nutrients, loss of proteins and consequently the clinical picture described. The bacterium is eliminated through the feces and milk of the affected animals, especially in advanced stages of the disease. The main mechanism of transmission is the fecal-oral route but intrauterine transmission has been also reported (2). MAP has also been associated with Crohn's disease, a human inflammatory bowel disease (3,4).

The disease has an unapparent subclinical phase that can last for years. Animals generally become infected at an early age, with clinical signs manifesting in the productive stage (5,6). The negative impact of the infection is important because of its effect on the economy of the farms, expressed in the decrease of milk production, reduction of the productive life of the cow and predisposition to other pathologies (6).

Distributed worldwide (1), the PTB is currently considered as an emerging disease despite its recognition for more than a century (1895)(7). Previous studies have identified some factors that can influence MAP status. Most of these studies have been conducted using the herd as the unit of analysis and used serological results to establish the MAP diagnosis in animals, as well as the subsequent identification of factors associated (8). In Colombia, the PTB was first reported in cattle in 1924 (9). Since then, the disease has been explored mainly in cattle and sheep, with some studies focused primarily on the agent detection and local prevalence estimates —mainly in specialized dairy systems (9,10), but no epidemiological information on MAP in low-tropic dairies is available so far in the country.

In tropical livestock, multiple production systems coexist in different thermal floors, different degrees of intensification, and are located in socio-economic environments of very diverse nature, in which the sustainable and competitive production of protein of animal origin is possible, thanks in large part to the use of cattle breeds adapted to the climatic, economic, and management conditions characteristic of each region (11).

One of such is the low-tropic dairy production system, which is defined as a management system in which milk from crossed-European biotypes cattle are produced, considering that only the cow —and no its breeding, constitutes the unit of production during the lactation. Therefore, the profits defined from the fattening of the calves is not representative of the herd, different from what is seen in dual-purpose systems (12). In Colombia, this system is characterized by the presence of small and medium producers with very limited physical, technical, and financial resources, and often located in marginal production areas (13).

The production of meat and milk in the country is basically based on this type and on dual-purpose systems, which comprise about 80.5% of the total national livestock inventory, and contributes with 50 and 70% of Colombian milk and meat to the internal markets, respectively (14).

Due to the representativeness of this productive system in the country, this study aimed to report the frequency of MAP seropositivity and to assess the identification of herd and animal-level factors that could be associated with the seropositive status of 204 cows from five low-tropic dairy herds located in the Province of Sucre (Colombia).

MATERIALS AND METHODS

Ethical considerations. The ethics committee for animal experimentation (CEEA) of the Universidad de Antioquia, Colombia approved this study (Act #118, June 5th, 2018).

Design and study population. A cross-sectional study, using a convenience sampling was carried out. Cattle from five low-tropic dairy herds, located in three different districts of three different municipalities of the Provinces of Sucre and Córdoba (Colombia) were randomly selected to be sampled for the detection of MAP antibodies. The altitude of the study area ranges between 44 and 117 m.a.s.l., corresponding to a tropical lower montane region, according to the Holdridge classification of life zones (15).

The geographical position and its relief determine the weather of the Province of Sucre. The predominant type of weather in the North and Center of the Province is warm-semi-arid, while to the South is of the semi-humid and humid warm type. The various reliefs of the Province are of warm thermal floor with temperatures that oscillate between 25.5 and 28.7°C in annual average (16). The geographical position and the relief determine the weather of the Province of Córdoba, as well. The Province territory is made up of 60% by the Great Plain of the Caribbean and the rest by the last foothills of the Andes mountain range giving rise to different climates. The 96% of the territory corresponds to the warm thermal floor, 3% corresponds to tempered thermal floor, and 1% to cold thermal floor. Most of the Province records temperatures between 26 and 28°C (17).

The animals tested in each herd were randomly selected and proportionally allocated, according to the specific weight of each herd in the population of cows with an age equal to or greater than 2 years (Table 1).

Blood samples were collected form the coccygeal vein. After allowing the retraction of the blood clot at environment temperature, the serum was immediately separated by centrifugation at 2000 rpm for 5 min. Serum samples were refrigerated at 4°C until their arrival at the laboratory (Universidad de Antioquia, Medellín, Colombia) where they were frozen at -20°C until analysis, done 8 days later.

Table 1. *Mycobacterium avium* subsp. *paratuberculosis* ELISA results in sera of cows of five low-tropic dairy herds, Provinces of Sucre and Córdoba (Colombia).

Herd	Province	Municipality	District	Total population of cows ≥ 2 years of age*	Sample weight (%)	Animals tested	Positive animals
1	Sucre	Sincelejo	Laguna flor	307	51.4	105	12
2	Sucre	Sampués	San Luis	55	9.2	19	9
3	Sucre	Sampués	San Luis	89	15	31	4
4	Sucre	Sampués	San Luis	81	13.6	28	8
5	Córdoba	Chinú	Nuevo oriente	65	10.8	21	2
		Total		597	100	204	35

*Information obtained from the herd administrator.

Enzyme-linked immunosorbent assay (ELISA) test. Serum samples were analyzed using the commercial test Cattletype® MAP (Qiagen Leipzig GmbH, Leipzig, Germany) an indirect pre-absorbed ELISA kit, according to instructions of the manufacturer. The results were considered as valid if the mean value (MV) of the measured OD value for the positive control was ≥ 0.7, and if the MV of the measured OD value for the negative control was ≤ 0.2. An ELISA-positive animal was defined at a sample-to-positive ratio (S/P%) of ≥ 0.4, as suggested by the manufacturer. The sensitivity (Se) and specificity (Sp) reported for the commercial kit used are 60-85 and 99%, respectively.

Information collection and data analysis. A one-page questionnaire was administered to the herd manager at the time of sample collection in a face-to-face interview. The same researcher (NC) administered the questionnaire.

All questionnaires included an introductory paragraph, clarifying the justification and importance of the questions, how data were going to be used as well as a confidentiality and anonymity agreement.

Herd-level variables considered included *herd size* (number of cows in production at the time of sampling) and *presence of other ruminants co-grazing with cattle in the last 2 years* (i.e. goats, sheep, buffaloes). The variables at the animal-level considered were, *age* (in months), *parity*, and *days since the last birth*.

All the information generated during the study was entered into Excel worksheets (Microsoft Corp., Redmond, WA, USA) and then exported to Stata 14.0 (StataCorp, 2017, Texas, USA) for statistical analysis. Descriptive statistics were calculated for all variables, both at the herd and animal-level, and the association between these variables and the result from the ELISA test for MAP was explored using Pearson's *chi*-square test and Fisher's test (p<0.05).

RESULTS

The study herds belonged to the same owner and were administered by the same manager, sharing several management practices and animal genetics (crossed-European biotypes), with no predominant breeds. The breed-crossing strategy for all the herds was to maintain the proportion F1 (*Bos indicus* × *Bos taurus*) and, based on a routine phenotypic evaluation, were females were reproductively programmed with biotypes oriented to *B. taurus* or *B. indicus.*

The five herds were geographically close to each other, and there was an active flow of animals between them, so all are considered as open herds. As a management practice, the calves were weaned at 9 months old; however, they were in direct contact with their dams since birth, and during all the following mornings, considering that, at night, they were housed in separated spaces. There was no daily milk record since cows suckled their calves in the morning. The five herds were certified as brucellosis-free, but neither certified as tuberculosis-free nor enrolled in good farming practices (GFP) programs at the moment of sampling.

An explanation by the researchers to the administrative personal and people in charge of the animals about compatible PTB symptoms was needed (i.e. diarrhea and progressive weight loss, refractory to treatment) because they had not heard of the disease before, not even recognized the name. Then, and according to the manager, none of the herds has presented clinical cases compatible with PTB until sampling.

Blood samples from 204 cows older than 2 years of age. It was found that the 17.2% (35/204; 95% CI: 12.0-22.3%), and 82.8% (169/204; 95% CI: 77.7-88%) of the samples produced MAP-positive and negative results by ELISA, respectively. In the five herds, ELISA detected at least one seropositive animal. The serological results for the study population are shown in table 1.

The average *herd size* for the study population was 92.6 animals (55-179 cows in production). The statistical analysis found that this variable was not associated with the serological status for MAP (p=0.36). In turn, the variable *presence of other ruminants in co-grazing with cattle in the last 2 years* was found to be associated with MAP seropositivity by ELISA (p=0.020). It is important to emphasize that herd 1 was the only one with meat sheep that shared spaces with the calves during the night, from the birth of them until the calves were 3 months old. A descriptive summary of the animal-level quantitative variables evaluated is shown in table 2. Of the three variables analyzed, two were found to be significantly associated with MAP seropositivity (i.e. *age*, *parity*; p≤0.05).

Table 2. Descriptive summary of the animal-level variables evaluated in five low-tropic dairy herds, Provinces of Sucre and Córdoba (Colombia), and their association with anti-*Mycobacterium avium* subsp. *paratuberculosis* ELISA results.

Variable	MVV	SD	Smallest value	Largest value	p-value
Age	7.8 years	± 2.29	2.2	13.83	0.03*
Parity	3.4 births	± 1.74	0	9	0.05*
DSL	154 days	± 88.73	0	685	0.11

MVV=Mean valued of variable in MAP-ELISA positive animals; SD=Standard deviation; DSL= Days since the last delivery. *Statistically Significant results ($p \leq 0.05$).

DISCUSSION

According to the available information, and to the literature consulted, this is the first study evaluating MAP-serostatus and exploring factors associated in Colombian low-tropic dairy herds. In Colombia, the disease has been mainly explored in dairy cattle, beef cattle, and sheep, and have been primarily focused on the detection of the agent and the estimation of local prevalence (9, 10). The agent and the disease are present in Colombia; nevertheless, a partial epidemiological information is available. Therefore, it is still necessary to gather information on epidemiological links, considering the different livestock production systems.

Given the particularities in management strategies and genetics used in low-tropic dairy production systems, it is difficult to compare frequencies of detection of MAP-antibodies and factors associated with positive results. Nevertheless, the proportion of ELISA positive results obtained from animals (17.2%) is consistent with animal-level reports in cattle from other Latin-American countries using ELISA (10). However, a higher frequency was expected by the researchers, since several reported risk factors were observed during sampling (e.g. movement of animals between herds, purchasing of replacements from external sources, cattle co-grazing with sheep, low level of information about PTB by the manager, permanence of the dam with the calve after birth, colostrum and milk feeding from multiple cows).

No attempt to report prevalence as frequency indicator of the study population was carried out since the number of herds sampled is not considered representative of the production system in the study area nor the country, and then, a convenience sample was used. Nevertheless, we used a study design that considered proportionality and randomization of the animals sampled into each herd.

On the other hand, some particularities of tests used in the study could have affected MAP-infection frequency of MAP positive animals reported herein, due to false-negative results. The most regularly tests used in the diagnosis of PTB are fecal culture (FC), ELISA, and PCR (17). The ELISA test has shown advantages as a screening test, over molecular and microbiological approaches, mainly because of the low cost of obtaining the samples and because it provides results quickly and has shown to be highly specific (≥ 99%) (19). The Se-relative disadvantages are more related to the stage of the disease than to the test *per se* (the more the disease has advanced, the more Se is the test to detect anti-MAP antibodies (8). Furthermore, ruminants can develop the disease with no antibodies response or an inconsistent response against MAP (20), which could explain the relatively low frequency found in the present study.

The herd-level variable identified in the present study as associated with anti-MAP seropositivity (*presence of other ruminants co-grazing with cattle in the last 2 years*) was first reported in 2011 (21) as a risk factor for MAP infection. In addition, the literature has reported bovine-PTB cases related to S (sheep) strain that was confirmed in Australia and Iceland under pasture-based systems, indicating the transmission opportunity between species (22). Then, a serological response can be detected, because MAP is an intracellular pathogen that does not replicate in the environment, so possible sources for contamination would be other domestic ruminants such as goats or sheep.

The animal-level variables *age* and *parity* can have a collinear behavior, because one variable can be an exact linear combination of the other. This could be explored from a multiple regression model approach, which was far from our scope. Nevertheless, at this level these two variables can be observed as giving the same information, because of the older the cow, the greater the number of births and vice versa. Previous reports have established the highest probability of a positive test among older cows (e.g. parity >4) and the lowest among first parity (23,24).

The association observed with *parity* in our study is in agreement with the chronic nature of the infection which implies that the cows are relatively old (usually 2-5 years old), before even showing symptoms of the disease (8). In our study, we tested cows close to 14 years of age, which implies a higher detection probability using ELISA. It is important to emphasize that the variables previously mentioned and their associations with MAP-serostatus come from reports on dairy cattle in specialized systems for milk production, which differs in several aspects —mainly those related to genetics and management, from low-tropic dairy systems observed in Colombia, which were the study population of the present study. However, to the authors, it is considered viable to keep them as comparable associated factors, given the inherent characteristics of the disease and its causal agent.

The present study found that 17.2% of the cows and 100% of the study herds were positive for MAP by ELISA. Additionally, associated variables were identified and may be of interest to both producers and veterinarians of production animals, guiding their approach to disease management.

Our findings support the need for further studies to explore possible links in the dynamics of infection in different cattle production systems in Colombia, using bacteriological and molecular techniques to analyze specific features and population-level specific risk factors.

Conflict of interest

The authors are not aware of any financial or personal relationships with other people or organizations that could inappropriately influence the work reported in this paper.

Acknowledgments

The authors thank the manager and owner of the study herds for their willing assistance.

REFERENCES

1. Fecteau ME. Paratuberculosis in cattle. Vet Clin North Am Food Anim Pract. 2018; 34(1):209-222. https://doi.org/10.1016/j.cvfa.2017.10.011

2. Sweeney RW, Whitlock RH, Rosenberger AE. *Mycobacterium paratuberculosis* isolated from foetuses of infected cows not manifesting signs of the disease. Am J Vet Res. 1992; 53:477-480. https://doi.org/10.1016/j.cvfa.2011.07.012

3. Atreya R, Bülte M, Gerlach GF, Goethe R, Hornef MW, Köhler H, et al. Facts, myths and hypotheses on the zoonotic nature of *Mycobacterium avium* subspecies *paratuberculosis*. Int J Med Microbiol. 2014; 304(7):858–867. https://doi.org/10.1016/j.ijmm.2014.07.006

4. Kuenstner JT, Naser S, Chamberlin W, Borody T, Graham DY, McNees A, et al. The Consensus from the *Mycobacterium avium* ssp. *paratuberculosis* (MAP) Conference 2017. Front Public Heal. 2017; 5:1–5. https://doi.org/10.3389/fpubh.2017.00208

5. McAloon CG, Roche S, Ritter C, Barkema HW, Whyte P, More SJ, et al. A review of paratuberculosis in dairy herds — Part 1: Epidemiology. Vet. J 2019; 246:59–65. https://doi.org/10.1016/j.tvjl.2019.01.010

6. McAloon CG, Whyte P, More SJ, Green MJ, O'Grady L, Garcia A, et al. The effect of paratuberculosis on milk yield—A systematic review and meta-analysis. J Dairy Sci. 2016; 99(2):1449–1460. https://doi.org/10.3168/jds.2015-10156

7. Nielsen SS, Toft N. A review of prevalences of paratuberculosis in farmed animals in Europe. Prev Vet Med. 2009; 88(1):1–14. https://doi.org/10.1016/j.prevetmed.2008.07.003

8. Barkema HW, Orsel K, Nielsen SS, Koets AP, Rutten VPMG, Bannantine JP, et al. Knowledge gaps that hamper prevention and control of *Mycobacterium avium* subspecies *paratuberculosis* infection. Transbound Emerg Dis. 2017; 65(1):125-148. https://doi.org/10.1111/tbed.12723

9. Correa-Valencia N, García-Tamayo YM, Fernández-Silva JA. *Mycobacterium avium* subsp. *paratuberculosis* in Colombia (1924-2016): A review. Rev Colomb Cienc Pecu 2018; 31(3):165-179. https://doi.org/10.17533/udea.rccp.v31n3a01

10. Fernández-Silva JA, Correa-Valencia NM, Ramírez NF. Systematic review of the prevalence of paratuberculosis in cattle, sheep, and goats in Latin America and the Caribbean. Trop Anim Health Prod. 2014; 46(8):1321–1340. https://doi.org/10.1007/s11250-014-0656-8

11. Galeano AP, Manrique C. Estimación de parámetros genéticos para caracteristicas productivas y reproductivas en los sistemas doble propósito del trópico bajo colombiano. Rev la Fac Med Vet y Zootec. 2010; 57(2):119–131. https://revistas.unal.edu.co/index.php/remevez/article/view/17342/18179

12. Holmann F, Rivas L, Carulla J, Rivera B, Giraldo LA, Guzmán S, et al. Producción de leche y su relación con los mercados; caso colombiano. CIAT. 2004; 1–80. http://ciat-library.ciat.cgiar.org/Articulos_Ciat/tropileche/books/Produccion_leche_relacion_mercados_caso_Colombia.pdf

13. DANE. Departamento Administrativo Nacional de Estadística. Colombia. Censo Nacional Agropecuario, 2014. https://www.dane.gov.co/files/images/foros/foro-de-entrega-de-resultados-y-cierre-3-censo-nacional-agropecuario/CNATomo2-Resultados.pdf

14. Espinal LS. Zonas de vida o formaciones vegetales de Colombia: Mapa geológico. Memoria explicativa sobre el mapa ecológico, Bogotá (Colombia). Instituto Geográfico Agustín Codazzi (IGAC) 1977. Vol. 11-13.

15. IDEAM. Instituto de hidrología, meteorología y estudios ambientales, Colombia. Mapas y gráficos del tiempo y el clima, 2018. http://institucional.ideam.gov.co/jsp/mapas-y-graficos-del-tiempo-y-el-clima_882

16. Toda Colombia, 2019. https://www.todacolombia.com/departamentos-de-colombia/sucre/clima.html

17. Toda Colombia. Clima Departamento de Córdoba. 2019. https://www.todacolombia.com/departamentos-de-colombia/cordoba/clima.html

18. Stevenson K. Diagnosis of Johne's disease: Current limitations and prospects. Cattle Pract. 2010; 18:104–109.

19. Alinovi CA, Ward MP, Lin TL, Wu CC. Sample handling substantially affects Johne's ELISA. Prev Vet Med. 2009; 90(3–4):278–283. https://doi.org/10.1016/j.prevetmed.2009.04.004

20. Münster P, Völkel I, Wemheuer W, Schwarz D, Döring S, Czerny CP. A longitudinal study to characterize the distribution patterns of *Mycobacterium avium* ssp. *paratuberculosis* in semen, blood and faeces of a naturally infected bull by IS*900* semi-nested and quantitative real-time PCR. Transbound Emerg Dis. 2013; 60(2):175–187. https://doi.org/10.1111/j.1865-1682.2012.01336.x

21. Barrett DJ, Mee JF, Mullowney P, Good M, McGrath G, Clegg T, et al. Risk factors associated with Johne's disease test status in dairy herds in Ireland. Vet Rec. 2011; 168(15):0–2. https://doi.org/10.1136/vr.c6866

22. Whittington RJ, Taragel CA, Ottaway S, Marsh I, Seaman J, Fridriksdottir V. Molecular epidemiological confirmation and circumstances of occurrence of sheep (S) strains of *Mycobacterium avium* subsp. *paratuberculosis* in cases of paratuberculosis in cattle in Australia and sheep and cattle in Iceland. Vet Microbiol. 2001; 79(4):311–322. https://doi.org/10.1016/S0378-1135(00)00364-3

23. Eisenberg SWF, Veldman E, Rutten VPMG, Koets AP. A longitudinal study of factors influencing the result of a *Mycobacterium avium* ssp. *paratuberculosis* antibody ELISA in milk of dairy cows. J Dairy Sci. 2015; 98(4):2345–2355. https://doi.org/10.3168/jds.2014-8380

24. Laurin EL, Sanchez J, Chaffer M, McKenna SLB, Keefe GP. Assessment of the relative sensitivity of milk ELISA for detection of *Mycobacterium avium* ssp. *paratuberculosis* infectious dairy cows. J Dairy Sci. 2017; 100(1):598–607. https://doi.org/ 10.3168/jds.2016-11194