PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 43:e07187, 2023
DOI: 10.1590/1678-5150-PVB-7187

Original Article
Livestock Diseases

Detection of paratuberculosis in dairy cows from southern Brazil[1]

Diorges H. Setim[2], Carlos Bondan[3], Caroline C. Cortese[4], Jéssica C. Peretti[4], Fernanda L. Facioli[5], Ricardo Zanella[2,5], Márcio M. Costa[2,6] and Adriana C. da Motta[2,4]*

ABSTRACT.- Setim D.H., Bondan C., Cortese C.C., Peretti J.C., Facioli F.L., Zanella R., Costa M.M. & da Motta A.C. 2023. **Detection of paratuberculosis in dairy cows from southern Brazil**. *Pesquisa Veterinária Brasileira 43:e07187, 2023*. Departamento de Patologia Animal, Hospital Veterinário, Universidade de Passo Fundo, BR-285 Km 292,7, Bairro São José, Passo Fundo, RS 99052-900, Brazil. E-mail: acmotta@upf.br

Bovine paratuberculosis causes chronic, incurable diarrhea and weight loss, resulting in decreased cattle production. The disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), an obligate intracellular mycobactin-dependent mycobacterium that replicates slowly in the host and has heightened environmental resistance. In countries where the disease is found and the damage has been quantified, direct and indirect economic losses are extremely high. Local epidemiological data is of paramount importance for the implementation of control programs. Our objective was to verify whether paratuberculosis is present in commercial dairy herds in different mesoregions of RS. Therefore, a prospective, cross-sectional and observational study was performed on dairy cattle from five mesoregions of the RS state, Brazil. Milk samples taken from individual cows on commercial farms were tested using indirect ELISA tests and classified as negative, suspicious, or positive. In herds containing at least one positive cow, we conducted convenience sampling of feces directly from the rectal ampulla to identify MAP through PCR. Of the 362 cows tested, 20 were seroreactive for paratuberculosis from two mesoregions. The PCR tests were all positive; cows with a negative ELISA and positive PCR results probably indicate that the MAP was ingested and eliminated without causing infection. We found that paratuberculosis is likely endemic in the northwest and northeast mesoregions.

INDEX TERMS: Milk, diagnosis, paratuberculosis, MAP, dairy cows, *Mycobacterium* spp., PCR, ELISA.

RESUMO.- [**Detecção da paratuberculose em vacas leiteiras no sul do Brasil.**] A paratuberculose bovina causa diarreia crônica e incurável, perda de peso e resulta em diminuição da produção. A doença é causada pelo *Mycobacterium avium* subsp. *paratuberculosis* (MAP), micobactéria intracelular obrigatória, dependente de micobactina, que se replica lentamente no hospedeiro e possui elevada resistência ambiental. Nos países onde a doença é encontrada e os danos foram quantificados, as perdas econômicas diretas e indiretas são extremamente altas. Os dados epidemiológicos locais são de suma importância para a implementação de programas de controle. Nosso objetivo foi verificar se a paratuberculose está presente em rebanhos leiteiros comerciais em diferentes mesorregiões do RS. Para tanto, foi realizado um estudo prospectivo, transversal e observacional em bovinos leiteiros de cinco mesorregiões do estado do RS, Brasil. Amostras de leite individuais, provenientes de vacas leiteiras de fazendas comerciais foram testadas com ELISA indireto e classificadas

[1] Received on November 21, 2022.
Accepted for publication on December 12, 2022.

[2] Programa de Pós-Graduação em Bioexperimentação, Universidade de Passo Fundo (UPF), BR-285 Km 292,7, Bairro São José, Passo Fundo, RS 99052-900, Brazil. *Corresponding author: acmotta@upf.br

[3] Departamento de Clínica de Grandes Animais, Hospital Veterinário (HV), Universidade de Passo Fundo (UPF), BR-285 Km 292,7, Bairro São José, Passo Fundo, RS 99052-900, Brazil.

[4] Departamento de Patologia Animal, Hospital Veterinário (HV), BR-285 Km 292,7, Universidade de Passo Fundo (UPF), Campus I, São José, Passo Fundo, RS 99052-900, Brazil.

[5] Departamento de Reprodução Animal, Hospital Veterinário (HV), Universidade de Passo Fundo (UPF), Passo Fundo, RS 99052-900, Brazil.

[6] Departamento de Patologia Clínica Veterinária, Hospital Veterinário (HV), Universidade de Passo Fundo (UPF), Passo Fundo, RS 99052-900, Brazil.

como negativas, suspeitas ou positivas. Em rebanhos contendo pelo menos uma vaca positiva, realizamos amostragem por conveniência, em que foram coletadas fezes diretamente da ampola retal, para identificar MAP por meio da PCR. Das 362 vacas testadas, 20 foram sororreativas para paratuberculose, oriundas de duas mesorregiões. Os testes de PCR foram todos positivos. Vacas com resultado negativo no teste ELISA e PCR positivo provavelmente indicam que o MAP foi ingerido e eliminado sem causar infecção. Sugere-se que a paratuberculose é provavelmente endêmica nas mesorregiões noroeste e nordeste.

TERMOS DE INDEXAÇÃO: Leite, diagnóstico, paratuberculose, MAP, vacas leiteiras, *Mycobacterium* spp., PCR, ELISA.

INTRODUCTION

Bovine paratuberculosis is also known as Johne's disease (JD). It is a chronic progressive condition caused by a bacterial infection in the gastrointestinal tract by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), resulting in progressive granulomatous enteritis (Ayele et al. 2005). Paratuberculosis is a highly contagious, wasting disease associated with a sharp decrease in the body condition score, progressing to cachexia and death (Buergelt et al. 1978, Manning & Collins 2001). Cattle are the most affected animals by paratuberculosis, though other species can develop it (Dalto et al. 2012, Balseiro et al. 2019).

The disease has gained attention worldwide (Kuenstner et al. 2017) due to the direct and indirect economic losses it causes (Raizman et al. 2009, Bhattarai et al. 2013, Garcia & Shalloo 2015). Studies on paratuberculosis show that this complex disease results in changes to several physiological parameters, including lower production (Raizman et al. 2009, Bates et al. 2018), decreased reproductive rates (Garcia-Ispierto & López-Gatius 2016), and increased levels of mastitis (Wilson 1995, Rossi et al. 2017). Recent research has highlighted the role of genetic susceptibility to MAP infections (Kiser et al. 2021).

Paratuberculosis is found worldwide, including in Brazil (OIE 2018), though studies of the disease are scarce. In Brazil, the first reported case of paratuberculosis occurred in the state of Rio de Janeiro from an imported bovine. In subsequent years, more disease cases were identified in the states of Mato Grosso, São Paulo, Santa Catarina, Minas Gerais, Goias, Paraíba, Maranhão and Rio Grande do Sul (RS) (Yamasaki et al. 2013). Most reported cases refer to clinical disease followed by serological evaluation of the herds. To the best of our knowledge, only one robust and current study has informed the detection of antibodies anti MAP in cattle from the northeast region, more precisely in the state of Pernambuco (Yamasaki et al. 2013). In addition, paratuberculosis has already been detected in the southern (Fiss et al. 2015) and metropolitan (Driemeier et al. 1999) regions of RS. However, these reports are from herds where the disease was only initially detected by observing clinical cases. Therefore, there is no information on other regions of this state. It is globally known that Brazil is a country of continental size, in which the RS state has an area equivalent to or larger than the size of many nations. In this context, research involving paratuberculosis is paramount to better understand how to detect the disease in different Brazilian regions. Research on this disease in Brazil provides reliable data for implementing control and prevention measures to minimize economic and sanitary losses and improve the export market. Therefore, there is a clear need for epidemiological surveys to complement data obtained in the country so far.

The RS state is home to over 1.2 million lactating dairy cows in commercial herds that sell milk to the state's dairy industry (IBGE 2020). The magnitude of the impacts of this disease underscores the need for further research to inform control measures in commercial dairy farms. The present study was carried out in five mesoregions in RS to verify whether paratuberculosis was present in commercial dairy herds throughout the state.

MATERIALS AND METHODS

Study population, sample calculation, and sampling. Milk samples were collected from farms that perform regular milk control at the "Serviço de Análise em Rebanhos Leiteiros" (Service of Analysis of Dairy Herds − SARLE) of the "Universidade de Passo Fundo" (UPF), northern RS. The following formula was used to identify the necessary sample size (Pfeiffer 2010):

$$n = Z^2 \frac{P(1-P)}{D^2}$$

Where Z is the standard normal distribution value, corresponding to the desired confidence level (Z = 2.56 for 95% confidence intervals); P is the expected prevalence, which has been calculated at 36.8% nationwide, according to Yamasaki et al. (2013); and D is a maximum acceptable error in the estimate, which we assigned as 0.05.

The target sample size was 357 animals. Collections were carried out in the mesoregions with the largest population of lactating dairy cows, combined with the sample collection of SARLE (Table 1).

The 362 samples collected from lactating cows came from 68 dairy farms from five mesoregions of RS (northwest, northeast, central-east, southeast, and southwest).

Table 1 shows that the number of samples obtained from the central-east and southwest regions falls short of the target. Additional samples were obtained from the two regions with the largest herd of lactating cows (northwest and northeast regions) to compensate, ensuring the total sample size was greater than the target.

Sample collection and storage. Milk samples from commercial dairy herds were sent from farms to SARLE in bottles containing bronopol at 18 to 20°C. Once they arrived at SARLE, a random design was carried out to select the farms from which samples would be used. A second random design was performed on the farms chosen to select 10% to 15% of samples per herd. This process was carried out until the necessary sample quantity was obtained. No disease history information was provided.

Table 1. Mesoregion-level stratification of the sample population based on available data on the size of the dairy cattle herd in each mesoregion*

Mesoregion	Number of cattle	% of total	Projection	Collected
Northwest	923,459	67.2	239	260
Northeast	163,279	11.89	43	51
Central-east	136,485	9.94	36	20
Southwest	78,271	5.7	21	11
Southeast	71,044	5.3	19	20
TOTAL	1,372,934	100	357	362

* EMATER (2019).

From each of the samples chosen, two aliquots of 1mL each were collected and stored in sterile 2mL plastic tubes. Immediately, centrifugation was carried out for 5 min at 2000 rpm. The obtained supernatant (fat) was discarded to facilitate the ELISA washing steps. The samples were then pipetted and stored in 1mL sterile plastic tubes, followed by freezing at -20°C prior to testing, which was carried out within no more than 15 days. The aliquots were thawed at 4°C and subsequently maintained at 18°C to perform the ELISA tests.

Sample processing for ELISA. Commercial indirect enzyme-linked immunosorbent assays (ELISA; IDEXX Laboratories, Inc., Westbrook, USA) were performed according to the manufacturer's guidelines, including a pre-incubation step with *Mycobacterium phei* to neutralize cross-reactions (Selim et al. 2019).

Every ELISA test used both double positive and negative controls. At the end of processing, the optical density values were measured at 450 nm and recorded. The results were interpreted as negative (A/P%≤20%), suspected (20%<A/P%<30%), and positive (A/P%≥30%). We considered suspected or positive cases (samples that immunologically reacted to MAP) as seroreactive cases.

Statistical analysis. The variables obtained from the ELISA test were organized in cross tables to generate descriptive statistics and verify positive cases' frequency (absolute and relative). Thus, the association between mesoregion and positive cases and differences in proportions were analyzed by Yates's chi-squared test. Pearson's correlation was used to verify the relationship between the herd size and the number of positive or suspected cases. Statistical analyses were carried out in R, with *p*-values ≤0.05 considered significant.

Sampling and processing for polymerase chain reaction (PCR). Feces samples were collected from 16 cows and taken directly from the rectal ampulla for subsequent MAP identification through PCR. We conducted convenience sampling using the following criteria: at least one seroreactive sample in the herd tested, samples collected from an equal number of negative and seroreactive cases, and farmers' permission to collect samples.

DNA extraction was carried out with an Invitrogen extraction kit following the manufacturer's instructions. Following DNA extraction, the amplification reactions were performed on a final volume of 25µL, containing 5µL of genomic DNA, 1µL of specific primers for IS900 at 10µM (forward primer: 5'-GACGACTCGACCGCTAATTG-3' and reverse primer: 5'-CCGTAACCGTCATTGTCCAG-3'), 5.5µL of Mili-Q ultrapure water, and 12.5µL of PCR Master Mix (Promega®) according to the manufacturer's specifications. The temperature profile of the reactions was carried out in an XP Thermal Cycler (Bioxer Technology Co. Ltd.) with initial denaturation at 96°C for 5 min, followed by 35 cycles at 95°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 3 min, and a final cycle at 72°C for 10 min (Taddei et al. 2008). The amplified 99 bp product corresponding to the MAP DNA was detected by gel electrophoresis in 2% agarose gel with a blue/green stain. The electrophoresis product was viewed using UV light and photo-documented.

The present study has been approved by the UPF Ethics Committee on the Use of Animals (CEUA), number 049/2019.

RESULTS

ELISA results

Of the 362 cows tested, 14 had their samples identified as positive, six as suspected, and the other 342 as negative cases using the ELISA test (Table 2). Therefore, the positivity rate for paratuberculosis among the cows sampled was 5.6%. The 14 positive and six suspected cases were from 13 municipalities in the northwest and northeast mesoregions of RS (Table 3). The 362 cows were from 36 municipalities across RS (Fig.1). No association between the investigated mesoregion and the number of positive samples for ELISA was observed (Table 2).

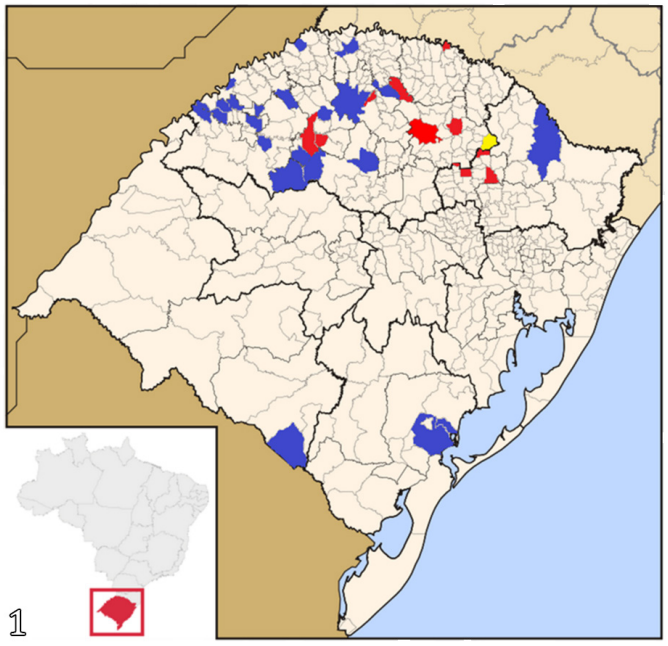

Fig.1. Map of the state of Rio Grande do Sul (RS) showing its municipalities. The inset in the lower-left corner highlights the location of RS in Brazil. The municipalities from which samples were obtained are highlighted based on ELISA test results. All negative samples (blue), suspected samples (yellow), positive cases present (red). Note that suspected cases may also be present in the municipalities colored red.

Table 2. Detection of paratuberculosis in the different mesoregions of the Rio Grande do Sul

Mesoregions	Tested cows	Positive	Suspected	Seroreactive %*	*P*-value
Northwest	260	10 (3.84%)	4 (1.53%)	14 (5.38%)	
Northeast	51	4 (7.8%)	2 (3.92%)	6 (11.76%)	
Southeast	20	0	0	0	0.868
Central-East	15	0	0	0	
Southwest	11	0	0	0	
TOTAL**	362	14 (3.92%)	6 (1.68%)	20 (5.6%)	

* Seroreactive animals are defined as the total number of animals with antibodies classified as suspect or positive, ** percentages are calculated relative to the total number of tested samples.

In the northwest mesoregion, 10 cows out of 260 tested positive (3.84%), along with four cows classified as suspected (1.53%) from nine herds. This region's total prevalence of seroreactive cases (positive + suspected cases) was 5.37%. Within the herds containing positive cases, antibodies were detected in between 1.7% and 10% of the animals tested.

In the northeast mesoregion, four positive (7.8%) and two suspected cases (3.92%) were detected on farms. For this region, positive + suspected cases accounted for 11.72% of total cases. Regarding the farms with positive cases, between 2% to 10% of tested cows were found to have antibodies.

All tested cows from the other municipalities were ELISA-negative. The municipalities for which no positive results were recorded are Aceguá (southwest mesoregion); Turuçu and Pelotas (southeast mesoregion); Vacaria (northeast mesoregion); Rio Pardo, Sinimbu, Novo Cabrais, Santa Cruz do Sul, and Estrela (central-east mesoregion); Ibirubá, Taquaruçu do Sul, Palmeira das Missões, Frederico Westphalen, Guarani das Missões, Esperança do Sul, Campina das Missões, Independência, Roque Gonzales, Pirapó, Porto Vera Cruz, Senador Salgado Filho, São Paulo das Missões, Nova Ramada, Ijuí, São Miguel das Missões, Vitória das Missões, Augusto Pestana, Jóia, Boa Vista do Cadeado, Novo Xingu, and Rondinha (northwest mesoregion).

We found no correlation between the herd size and the number of positive cases (r=-0.34, p=0.51) in the northeast mesoregion. However, there was a positive correlation between the herd size and the number of positive cases (r=0.74, p<0.001) in the northwest mesoregion, as well as for the grouped northwest and northeast mesoregions (r=0.46, p<0.001). This correlation is stronger when suspected cases are counted as positive (r=0.55, p<0.001).

PCR results

Fecal samples were collected from equal numbers of cows that tested negative for ELISA or suspected and positive cases for ELISA. PCR results showed that all samples had the presence of MAP DNA (Table 4).

DISCUSSION

A serological study applying the ELISA test on dairy herds in Brazil showed seropositivity ranging from 4.7% to 65.5% (Yamasaki et al. 2013). Our study detected a 5.26% seropositivity in all tested samples (20 seroreactive cases). In herds with only one cow with a suspected seropositive result, there are likely more suspected and/or positive cases in the herd. A previous study has detected high numbers of suspected cases in herds with positive cases and the other way around (Ozsvari et al. 2020).

We obtained data on the number of lactating cows per herd, which differed between mesoregions and the prevalence of seroreactive cases. Although the sample size was statistically sufficient, the correlation between total positive cases and herd size was not statistically significant in the northeast mesoregion. This finding may reflect the greater heterogeneity in herd size in this region and the smaller number of herds sampled. On the other hand, herd size was significantly and positively correlated with seropositivity in the northwestern mesoregion, meaning that larger herds are more likely to have animals with paratuberculosis antibodies. This finding is similar to a previous study on a herd from the USA (Corbett et al. 2018), although RS herds are mostly family-owned and have fewer animals than herds in that country.

The correlation between herd size and the number of seropositive individuals was also positive and significant when the northwest and northeast mesoregions were grouped. Positive cases were found in herds smaller than 30 lactating cows and herds with more than 100 lactating cows. Even so, the greater positivity rates in larger herds are robust and expected, especially in the presence of this disease.

These facts show that herd size is not a primary factor for detecting paratuberculosis in dairy cows, although the disease is more likely to be found in larger herds. This may also be since the number of samples taken was proportional to the herd population. These results are consistent with findings from other countries (Lombard 2011, Selim et al. 2019).

PCR was used complementarily to ELISA in the present study. These DNA tests showed that all cows (with positive, suspected, and negative cases) were eliminating the pathogen

Table 3. Municipalities in which cows with paratuberculosis were detected by Elisa test, with the positive, suspect, negative, and seroreactive cases and the size of the sampled herd

Municipality	Region	Posit.	Susp.	Negat.	% Seroreactive*	Lactating cows/herd
Água Santa	Northwest	1	0	17	5.5	120
Água Santa	Northwest	1	0	3	25	29
Bozano	Northwest	1	0	5	16.6	40
Ibiraiaras	Northwest	0	1	2	33.3	30
Ijui	Northwest	1	0	3	25	30
Novo Barreiro	Northwest	1	0	2	33.3	25
Mariano Moro	Northwest	0	1	19	5	135
Mariano Moro	Northwest	1	0	0	100	11
Passo Fundo	Northwest	3	2	20	25	196
Ronda Alta	Northwest	1	0	6	16.6	47
Nova Prata	Northeast	1	1	2	100	32
São Jorge	Northeast	1	1	14	14.28	110
Serafina Correa	Northeast	2	0	2	100	33
TOTAL (11)	2	14	6	95	5% - 100%	838

* Seroreactive animals are defined as the total number of animals with antibodies classified as suspect or positive; Posit. = positive, Susp. = suspect, Negat. = negative.

in their feces. This is expected to occur with greater intensity in positive cases than in suspected ones and is not expected in animals that test negative (Beaver et al. 2017). However, our study did not quantify MAP but focused solely on detecting it. Cows with a negative result in the ELISA test but with a positive PCR result indicate that the MAP pathogen had been ingested orally and eliminated without an infection (Plain et al. 2015, Kawaji et al. 2020). For such an effect to happen, the cows need to come into contact with the pathogen through ingestion (Begg et al. 2018). As such, the food or water sources that the animals use are likely contaminated with MAP.

The mesoregions with only negative samples for both ELISA and PCR also had a smaller sample size since the number of dairy cows in those regions was smaller, highlighting the need for further studies in these regions. Indeed, cases of clinical paratuberculosis have been reported in the southeastern mesoregion (Fiss et al. 2015) and the metropolitan region (Driemeier et al. 1999, Gomes et al. 2002), while our study found no positive results in the southeastern region and did not include any samples from the metropolitan region.

In herds where paratuberculosis was detected by ELISA, between 5% and 100% of the cows tested positive. This suggests that disease monitoring and screening using samples from bulk tanks would be a reliable way to track the disease (Lavers et al. 2014). Samples from bulk tanks are very useful for checking the initial status of the disease on a large scale and subsequently testing all herds with positive or suspected tank samples. Moreover, they allow the entire commercial herd to be screened at once and for the results to be evaluated in a control program developed specifically for the reality of the farms (McSpadden et al. 2013).

Seroreactivity in the northwest and northeast mesoregions was 5.37% and 11.72%, respectively. The combined result of both regions was 17% of seroreactive cases, given that the dairy herds in these regions total 552,078 cows over 36 months. Thus, in absolute numbers, these regions may have around 1 million dairy cows that are seroreactive for paratuberculosis. These numbers should be a warning signal for local livestock operations since the present study is restricted to lactating dairy cows. This value is likely to rise when all categories of dairy cattle in the state are considered.

A dairy herd of 1,135,498 cows produced 4.27 billion liters of milk annually (EMATER 2019). The 180,000 seroreactive cows for paratuberculosis estimated in our study represent 15.85% of the total lactating cows in the state. Infected cows may produce up to 12% less of their productive potential (Bates et al. 2018). This decrease in production results in an estimated loss of approximately 675 million liters per year. Furthermore, this milk, which amounts to 15.85% of total production, may contain viable MAP (Carvalho et al. 2012, Botsaris et al. 2016) and could end up getting mixed with milk that does not contain the pathogen since there are no tests for it in the industry (MAPA 2018).

Table 4. Municipalities in which milk and fecal samples were collected for the ELISA and PCR tests, respectively, along with the results of both tests

Municipalities	ELISA result	PCR result
Nova Prata(4 samples)	ELISA susp.	Positive
	ELISA+	Positive
	ELISA-	Positive
	ELISA-	Positive
Passo Fundo(8 samples)	ELISA susp.	Positive
	ELISA susp.	Positive
	ELISA+	Positive
	ELISA+	Positive
	ELISA-	Positive
	ELISA-	Positive
	ELISA-	Positive
	ELISA-	Positive
São Jorge(4 samples)	ELISA+	Positive
	ELISA susp.	Positive
	ELISA-	Positive
	ELISA-	Positive
TOTAL (3)	16	16

susp. = Suspect.

CONCLUSIONS

Paratuberculosis is likely to be endemic in the northwest and northeast mesoregions of the RS state, and implementing a disease control program is an important step for the industry.

The sample size per herd was not essential for detecting positive cases of paratuberculosis, though positive results are more likely in larger herds.

The detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in the feces of cows that tested negative for ELISA indicates the constant contact of these cows with the pathogen, likely via ingestion, and their elimination without causing infection.

Acknowledgments.- This study was supported by the Brazilian agency "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), Brazil.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

REFERENCES

Ayele W.Y., Svastova P., Roubal P., Bartos M. & Pavlik I. 2005. *Mycobacterium avium* subspecies *paratuberculosis* cultured from locally and commercially pasteurized cow's milk in the Czech Republic. Appl. Environ. Microbiol. 71(3):1210-1214. <https://dx.doi.org/10.1128/AEM.71.3.1210-1214.2005> <PMid:15746320>

Balseiro A., Perez V. & Juste R.A. 2019. Chronic regional intestinal inflammatory disease: A trans-species slow infection? Comp. Immunol. Microbiol. Infect. Dis. 62:88-100. <https://dx.doi.org/10.1016/j.cimid.2018.12.001> <PMid:30711052>

Bates A., O'Brien R., Liggett S. & Griffin F. 2018. The effect of sub-clinical infection with *Mycobacterium avium* subsp. *paratuberculosis* on milk production in a New Zealand dairy herd. BMC Vet. Res. 14(1):93. <https://dx.doi.org/10.1186/s12917-018-1421-4> <PMid:29540214>

Beaver A., Sweeney R.W., Hovingh E., Wolfgang D.R., Gröhn Y.T. & Schukken Y.H. 2017. Longitudinal relationship between fecal culture, fecal quantitative PCR, and milk ELISA in *Mycobacterium avium* ssp. *paratuberculosis*-infected cows from low-prevalence dairy herds. J. Dairy. Sci. 100(9):7507-7521. <https://dx.doi.org/10.3168/jds.2017-12928> <PMid:28647332>

Begg D.J., Plain K.M., Silva K., Gurung R., Gunn A., Purdie A.C. & Whittington R.J. 2018. Immunopathological changes and apparent recovery from infection revealed in cattle in an experimental model of Johne's disease using a lyophilised culture of *Mycobacterium avium* subspecies *paratuberculosis*. Vet.

Microbiol. 219:53-62. <https://dx.doi.org/10.1016/j.vetmic.2018.03.029> <PMid:29778205>

Bhattarai B., Fosgate G.T., Osterstock J.B., Fossler C.P., Park S.C. & Roussel A.J. 2013. Comparison of calf weaning weight and associated economic variables between beef cows with and without serum antibodies against or isolation from feces of *Mycobacterium avium* subsp. *paratuberculosis*. J. Am. Vet. Med. Assoc. 243(11):1609-1615. <https://dx.doi.org/10.2460/javma.243.11.1609> <PMid:24261812>

Botsaris G., Swift B.M.C., Slana I., Liapi M., Christodoulou M., Hatzitofi M., Christodoulou V. & Rees C.E.D. 2016. Detection of viable *Mycobacterium avium* subspecies *paratuberculosis* in powdered infant formula by phage-PCR and confirmed by culture. Int. J. Food Microbiol. 216:91-94. <https://dx.doi.org/10.1016/j.ijfoodmicro.2015.09.011> <PMid:26421832>

Buergelt C.D., Hall C., McEntee K. & Duncan J.R. 1978. Pathological evaluation of paratuberculosis in naturally infected cattle. Vet. Pathol. 15(2):196-207. <https://dx.doi.org/10.1177/030098587801500206> <PMid:664186>

Carvalho I.A., Pietralonga P.A.G., Schwarz D.G.G., Faria A.C.S. & Moreira M.A.S. 2012. Recovery of viable *Mycobacterium avium* subspecies *paratuberculosis* from retail pasteurized whole milk in Brazil. J. Dairy Sci. 95(12):6946-6948. <https://dx.doi.org/10.3168/jds.2012-5657> <PMid:23021748>

Corbett C.S., Naqvi S.A., Bauman C.A., De Buck J., Orsel K., Uehlinger F., Kelton D.F. & Barkema H.W. 2018. Prevalence of *Mycobacterium avium* ssp. *paratuberculosis* infections in Canadian dairy herds. J. Dairy Sci. 101(12):11218-11228. <https://dx.doi.org/10.3168/jds.2018-14854> <PMid:30316607>

Dalto A.C., Bandarra P.M., Pavarini S.P., Boabaid F.M., De Bitencourt A.P.G., Gomes M.P., Chies J., Driemeier D. & Da Cruz C.E.F. 2012. Clinical and pathological insights into Johne's disease in buffaloes. Trop. Anim. Health Prod. 44(8):1899-1904. <https://dx.doi.org/10.1007/s11250-012-0154-9> <PMid:22528538>

Driemeier D., Cruz C.E.F., Gomes M.J.P., Corbellini L.G., Loretti A.P. & Colodel E.M. 1999. Aspectos clínicos e patológicos da paratuberculose em bovinos no Rio Grande do Sul. Pesq. Vet. Bras. 19(3/4):109-115. <https://dx.doi.org/10.1590/s0100-736x1999000300004>

EMATER 2019. Relatório Socioeconômico da Cadeia Produtiva do Leite no Rio Grande do Sul. Empresa de Assistência Técnica e Extensão Rural, Porto Alegre. Avaliable at <https://www.ufsm.br/app/uploads/sites/370/2019/12/RELATORIO-LEITE-2019_2.pdf> Accessed on Aug. 20, 2022.

Fiss L., Santos B.L., Albuquerque P.P.F., Mota R.A., Marcolongo-Pereira C., Adrien M.L., Soares M.P. & Schild A.L. 2015. Paratuberculose em bovinos de corte na região sul do Rio Grande do Sul. Pesq. Vet. Bras. 35(5):437-442. <https://dx.doi.org/10.1590/S0100-736X2015000500008>

Garcia A.B. & Shalloo L. 2015. The economic impact and control of paratuberculosis in cattle. J. Dairy Sci. 98(8):5019-5039. <https://dx.doi.org/10.3168/jds.2014-9241> <PMid:26074241>

Garcia-Ispierto I. & López-Gatius F. 2016. Early foetal loss correlates positively with seroconversion against *Mycobacterium avium paratuberculosis* in high-producing dairy cows. Reprod. Domest. Anim. 51(2):227-231. <https://dx.doi.org/10.1111/rda.12670> <PMid:26841136>

Gomes M.J.P., Driemeier D., Ribeiro V.R., Wunder Jr. E.A., Asanome W., Lanzon L.F. & Wald V.B. 2002. Doença de Johne: Isolamento do *Mycobacterium avium* subsp. *paratuberculosis* (Map) em um rebanho leiteiro infectado na região Sul do Brasil. Acta. Scient. Vet. 30(2):113-118. Avaliable at <http://hdl.handle.net/10183/19744> Accessed on Aug. 25, 2022.

IBGE 2020. Rebanhos da pecuária do Rio Grande do Sul. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro. Avaliable at <https://cidades.ibge.gov.br/brasil/rs/pesquisa/18/16459?ano=2020> Accessed on Aug. 28, 2022.

Kawaji S., Nagata R., Minegishi Y., Saruyama Y., Mita A., Kishizuka S., Saito M. & Mori Y. 2020. A novel real-time PCR-based screening test with pooled fecal samples for bovine Johne's disease. J. Clin. Microbiol. 58(12):e01761-20. <https://dx.doi.org/10.1128/JCM.01761-20> <PMid:32938740>

Kiser J.N., Wang Z., Zanella R., Scraggs E., Neupane M., Cantrell B., Van Tassell C.P., White S.N., Taylor J.F. & Neibergs H.L. 2021. Functional variants surrounding endothelin 2 are associated with *Mycobacterium avium* subspecies *paratuberculosis* infection. Front. Vet. Sci. 8:625323. <https://dx.doi.org/10.3389/fvets.2021.625323> <PMid:34026885>

Kuenstner J.T., Naser S., Chamberlin W., Borody T., Graham D.Y., McNees A., Hermon-Taylor J., Hermon-Taylor A., Dow C.T., Thayer W., Biesecker J., Collins M.T., Sechi L.A., Singh S.V., Zhang P., Shafran I., Weg S., Telega G., Rothstein R., Oken H., Schimpff S., Bach H., Bull T., Grant I., Ellingson J., Dahmen H., Lipton J., Gupta S., Chaubey K., Singh M., Agarwal P., Kumar A., Misri J., Sohal J., Dhama K., Hemati Z., Davis W., Hier M., Aitken J., Pierce E., Parrish N., Goldberg N., Kali M., Bendre S., Agrawal G., Baldassano R., Linn P., Sweeney R.W., Fecteau M., Hofstaedter C., Potula R., Timofeeva O., Geier S., John K., Zayanni N., Malaty H.M., Kahlenborn C., Kravitz A., Bulfon A., Daskalopoulos G., Mitchell H., Neilan B., Timms V., Cossu D., Mameli G., Angermeier P., Jelic T., Goethe R., Juste R.A. & Kuenstner L. 2017. The consensus from the *Mycobacterium avium* ssp. *paratuberculosis* (MAP) conference 2017. Front. Publ. Health 5:208. <https://dx.doi.org/10.3389/fpubh.2017.00208> <PMid:29021977>

Lavers C.J., Barkema H.W., Dohoo I.R., McKenna S.L.B. & Keefe G.P. 2014. Evaluation of milk ELISA for detection of *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds and association with within-herd prevalence. J. Dairy Sci. 97(1):299-309. <https://dx.doi.org/10.3168/jds.2013-7101> <PMid:24239088>

Lombard J.E. 2011. Epidemiology and economics of paratuberculosis. Vet. Clin. N. Am., Food Anim. Pract. 27(3):525-535. <https://dx.doi.org/10.1016/j.cvfa.2011.07.012> <PMid:22023831>

Manning E.J.B & Collins M.T. 2001. *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. Rev. Sci. Tech. OIE 20(1):133-150. <https://dx.doi.org/10.20506/rst.20.1.1275> <PMid:11288509>

MAPA 2018. Instrução Normativa Nº 77, de 26 de novembro de 2018. Diário Oficial da União, Brasília. Avaliable at <https://www.in.gov.br/web/dou/-/instrucao-normativa-n-77-de-26-de-novembro-de-2018-52749887> Accessed on Aug. 21, 2022.

McSpadden K., Caires K. & Zanella R. 2013. The effect of mycobacterium avium subspecies paratuberculosis exposure on animal health. Acta Scient. Vet. 41(1):1095. Avaliable at <https://www.redalyc.org/articulo.oa?id=289031817001> Accessed on Oct. 21, 2022.

OIE 2018. Paratuberculosis (Johne's disease), p.544-559. In: OIE (Eds), Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 8th ed. World Organisation for Animal Health (OIE).

Ozsvari L., Lang Z., Monostori A., Kostoulas P. & Fodor I. 2020. Bayesian estimation of the true prevalence of paratuberculosis in Hungarian dairy cattle herds. Prev. Vet. Med. 183:105124. <https://dx.doi.org/10.1016/j.prevetmed.2020.105124> <PMid:32889487>

Pfeiffer D.U. 2010. Veterinary Epidemiology: an introduction. Wiley-Blackwell, Chichester, p.xiv, 135.

Plain K.M., Waldron A.M., Begg D.J., Silva K., Purdie A.C. & Whittington R.J. 2015. Efficient, validated method for detection of mycobacterial growth in liquid culture media by use of bead beating, magnetic-particle-based nucleic acid isolation, and quantitative PCR. J. Clin. Microbiol. 53(4):1121-1128. <https://dx.doi.org/10.1128/JCM.03521-14> <PMid:25609725>

Raizman E.A., Fetrow J.P. & Wells S.J. 2009. Loss of income from cows shedding *Mycobacterium avium* subspecies *paratuberculosis* prior to calving compared with cows not shedding the organism on two Minnesota dairy farms. J. Dairy. Sci. 92(10):4929-4936. <https://dx.doi.org/10.3168/jds.2009-2133> <PMid:19762809>

Rossi G., Grohn Y.T., Schukken Y.H. & Smith R.L. 2017. The effect of *Mycobacterium avium* ssp. *paratuberculosis* infection on clinical mastitis occurrence in dairy cows. J. Dairy Sci. 100(9):7446-7454. <https://dx.doi.org/10.3168/jds.2017-12721> <PMid:28711261>

Selim A., Ali A-F. & Ramadan E. 2019. Prevalence and molecular epidemiology of Johne's disease in Egyptian cattle. Acta Trop. 195:1-5. <https://dx.doi.org/10.1016/j.actatropica.2019.04.019> <PMid:31002806>

Taddei R., Barbieri I., Pacciarini M.L., Fallacara F., Belletti G.L. & Arrigoni N. 2008. *Mycobacterium porcinum* strains isolated from bovine bulk milk: Implications for *Mycobacterium avium* subsp. *paratuberculosis* detection by PCR and culture. Vet. Microbiol. 130(3/4):338-347. <https://dx.doi.org/10.1016/j.vetmic.2008.02.007> <PMid:18378411>

Wilson D. 1995. Financial effects of *Mycobacterium paratuberculosis* on mastitis, milk production, and cull rate in clinically normal cows. Agri-Practice 16(3):12-18. Avaliable at <https://agris.fao.org/agris-search/search.do?recordID=US9605801> Accessed on Oct. 22, 2022.

Yamasaki E.M., Brito M.F., Mota R.A., McIntosh D. & Tokarnia C.H. 2013. Paratuberculose em ruminantes no Brasil. Pesq. Vet. Bras. 33(2):127-140. <https://dx.doi.org/10.1590/S0100-736X2013000200001>