

Communication

[Comunicação]

***Mycobacterium avium* subspecies *paratuberculosis* and bovine leukemia virus seroprevalence and associated risk factors in dairy herds in Minas Gerais state, Brazil**

[Soroprevalência do *Mycobacterium avium* subespécie *paratuberculosis* e vírus da leucemia bovina e fatores de risco associados em rebanhos leiteiros de Minas Gerais, Brasil]

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In 2016, Brazil produced 33.62 billion liters of milk. The state of Minas Gerais, the country's largest dairy producer, accounted for 26.7% of national production (Ranking..., 2017). This significant commercial presence in the agricultural market points to the need for more effective sanitary measures and investigations of diseases in dairy herds to ensure product competitiveness and quality.

Paratuberculosis (PTB) or Johne's disease is an infection characterized by chronic granulomatous enteritis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which affects cattle, sheep, goats and wild ruminants (Fecteau, 2017). Enzootic bovine leukosis EBL is caused by an RNA virus of the Retroviridae family, the bovine leukemia virus (BLV). Considering the economic impact of PTB and EBL and the importance of an epidemiological analysis to support prophylactic measures, our objective here was to conduct a seroepidemiological study of PTB and EBL in cattle herds in the largest dairy basin in the state of Minas Gerais, Brazil. This research was approved by the Ethics Committee on Animal Use of the University Center of Patos de Minas (UNIPAM), under Protocol no. 54/17. The study was carried out in the municipality of Lagoa

Formosa, located in the region of Alto Paranaíba, state of Minas Gerais, Brazil. To determine the sample size, the prevalence estimation method was used on a simple random sample. Estimated prevalence was 75.8% for paratuberculosis (Fernández-Silva *et al.*, 2014) and 80.89% for leukosis (Ambrósio, 2015). Thus, for the sample size an expected prevalence of 81% was adopted with a 95% confidence level and a statistical error of 5%, indicating a minimum total sample size of 236 samples.

Forty farms were selected randomly in the north, south, east and west regions according to the zoning map provided by the municipal government. The samples were collected from a total of 10 farms per region. The herds consisted of animals of various breeds and ages, in different stages of lactation, raised in intensive or semi-intensive systems and subjected to mechanical and/or manual milking. Blood samples were drawn exclusively from lactating cows. A total of 236 blood samples were collected by coccygeal venipuncture, using sterile 10mL syringes and 25mm × 0.7mm needles. The blood was stored in tubes without anticoagulant and kept on ice in styrofoam boxes. The samples were sent to the Laboratory of Infectious Diseases of UNIPAM,

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where they were centrifuged at 3000g for 5min to extract the serum. They were then frozen in Eppendorf microtubes at -20°C until the tests were performed.

Serological diagnosis of PTB was performed using a commercial kit for indirect ELISA antibody testing for *Mycobacterium avium* subsp. *paratuberculosis* (IDEXX Laboratories Inc. Westbrook, ME, USA). The presence of antibodies against BLV was evaluated using a commercial agar-gel immunodiffusion (AGID) test (Tecpar Laboratories, Paraná, Brazil), following the manufacturer's instructions. Reading and interpretation of the test results was carried out under strong light after 24, 48 and 72h of incubation in a dark environment to ascertain the appearance of precipitation lines.

A structured questionnaire with closed-ended questions was created and applied to farm administrators to obtain information on the major risk factors associated with the animal-level and herd-level target prevalence. The variables investigated for MAP were age; presence of chronic diarrhea; raw milk consumption; veterinary service; newborn calf management; cleansing the teats before allowing the calf to suckle; contact by calves with adult animal feces; presence of dung heaps; and trade of animals. The variables investigated for BLV were age; use of disposable needles; reuse of veterinary obstetric

gloves; veterinary service; presence of bloodsucking flies; trade of animals; application of oxytocin during milking; blood transfusion; and type of reproductive management (natural mating and/or artificial insemination).

To calculate frequency, the number of cows with antibodies against MAP and BLV in relation to the number of sampled animals was established, using descriptive statistical analysis, by means of absolute and relative frequency. The statistical analysis of association between the investigated variables and the serological status was evaluated considering the Odds Ratio nonparametric test with a 95% confidence interval as the dependent variable. A p-value of <0.05 was considered statistically significant. The analyses were performed using GraphPad version 5.0 software (San Diego, California, USA).

In eight of the 40 herds evaluated (20%, 95% CI: 7.60–32.39%), at least one animal had antibodies against MAP. Among the 236 cows examined, 14 (6%, 95% CI: 2.98–9.02%) were reactive. The animals presented no clinical symptoms suggestive of infection by MAP. As for the risk factors investigated in the epidemiological survey, a significant association was identified between MAP and newborn calf management (P<0.0001) and contact by calves with adult animal feces (p=0.0258; Table 1).

Table 1. Distribution of cows from dairy herds with antibodies against MAP, according to the evaluated risk factors, in the municipality of Lagoa Formosa, MG, Brazil, 2017.

Variable	Cows			OR*	CI 95%	P
	Total	Reagents	Frequency (%)			
Animal trade						
Yes	104	6	5.76	0.9490	0.3186-2,827	1,0000
No	132	8	6.06			
Newborn calf management						
Along adults	67	13	19.40	41.45	5,295-324.5	<0.0001
Separated adults	169	1	0.59			
Veterinary care						
Yes	140	9	6.42	1,250	0.4056-3.854	0.7849
No	96	5	5.20			
Raw milk consumption						
Yes	205	11	5.36	0,486	0.1272-1.859	0.3899
No	31	3	9.67			
Contact of calves with adult feces						
Yes	133	12	9.02	5,008	1.095-22.91	0.0258
No	103	2	1.94			

*Odds Ratio; P<0.05 indicates a significant difference.

The herd-level prevalence was 85% (38/40, 95% CI: 82.16–87.84%) and the animal-level prevalence was 50.42% (119/236, 95% CI: 49.51–51.32%). In addition, all the animals examined on three farms were reactive to BLV. With regard to

the risk factors analyzed for BLV, the variables ‘blood transfusion’ ($p=0.0027$), ‘veterinary care’ ($p=0.0005$) and ‘reproductive management’ ($p=0.0319$) showed a significant association with the presence of anti-BLV antibodies (Table 2).

Table 2. Distribution of cows from dairy herds with antibodies against BLV, according to the evaluated risk factors, in the municipality of Lagoa Formosa, MG, Brazil, 2017.

Variable	Cows			OR*	CI 95%	P
	Total	Reagents	Frequency (%)			
Age						
20 - 48 months	62	27	43.54	0.6876	0.38 - 1.23	0.2377
> 4 years	174	92	52.87			
Disposable needles						
Yes	10	4	40.00	0.64	0.17 - 2.34	0.5373
No	226	115	50.88			
Reuse gloves						
Yes	161	87	54.03	1.58	0.90 - 2.74	0.1243
No	75	32	42.66			
Use oxytocin						
Yes	5	5	100	11.29	0.61 - 206.6	0.0599
No	231	114	49.35			
Blood transfusion						
Yes	83	53	63.85	2.32	1.34 - 4.038	0.0027
No	153	66	43.13			
Animal trade						
Yes	104	57	54.80	1.36	0.81 - 2.294	0.2410
No	132	62	46.96			
Veterinary care						
Yes	140	84	60.00	2.61	1.53 - 4.468	0.0005
No	96	35	36.84			
Reproductive management						
Natural mating	51	30	58.82	2.27	1.117 - 4.639**	0.0319
AI	102	57	55.88			
Natural mating + AI	83	32	38.55			

*Odds Ratio; **OR calculated for the greatest difference between proportions; AI: artificial insemination; $P < 0.05$ indicates a significant difference.

Asymptomatic cattle testing positive for MAP by ELISA are considered important sources of infection because they may discharge the bacillus intermittently into the environment, thereby contaminating water and food (Fecteau, 2017). The prevalence of antibodies against MAP detected in 20% of the herds in this study may be even higher given that the disease has a relatively long latency period of 2–10 years and levels of antibodies against MAP are detected only at the end of this period. Studies into the occurrence of MAP are rare in the state of Minas Gerais, and there is no available survey containing official data about the epidemiological situation in Brazil. Surveys conducted in other Brazilian regions have

shown higher MAP prevalence rates among herds than the one reported here (Yamasaki *et al.*, 2010; Vilar *et al.*, 2015).

In the Odds Ratio analysis, it was found that cows older than 4 years had a greater chance of becoming infected with MAP than cows between 20 and 48 months of age. The larger number of positive adult animals found in this study may also be attributed to the diagnostic method used here, since MAP antibody levels are identified only at the end of the incubation period of the disease, which may last from 2 to 10 years (Vilar *et al.*, 2015; Fecteau, 2017).

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The variable 'newborn calf management' was significant ($P < 0.0001$). In the herds where newborn calves were in frequent contact with adult cattle, an OR of 41.45 was identified. Preventing contact between young animals and adults may help to control the disease, since MAP transmission occurs mainly through the ingestion of pasture contaminated with adult feces or during the ingestion of colostrum or milk from feces-contaminated teats (Sá *et al.*, 2013; Vilar *et al.*, 2015). Serological surveys conducted in different regions of Brazil have shown mixed results with respect to the occurrence of leukosis, with an average rate of 27.60% of reactive animals (Starling *et al.*, 2013; Pinheiro-Júnior *et al.*, 2013; Ambrósio, 2015). The variable 'blood transfusion' was identified as a risk factor for BLV ($p = 0.0027$), and an OR of 2.32 was identified at the farms where this procedure was employed. BLV transmission is closely tied to management practices, especially the more technified ones.

Reproductive management was also identified as a risk factor for BLV. In the herds where the animals mated naturally, a 2.27-fold higher OR was found than in herds in which reproduction was achieved through a combination of artificial insemination and natural mating. Flores (2007) states that transmission may occur through natural mating, which is a form of transmission of the virus from an infected bull to females and is due to the possible presence of lymphocytes in the reproductive tract and not necessarily to the presence of BLV in semen.

As for the variable 'veterinary service', the animals on farms where such care was available presented a 2.32-fold higher risk of having the infection ($p = 0.0005$). It is known that the main mechanism of BLV transmission is via the horizontal route, by iatrogenic transmission through procedures that enable contaminated blood to be transferred between animals (Flores, 2007). Consistent with this study, Pinheiro-Júnior *et al.* (2013) also identified a significant association ($P < 0.000$) between the variable 'veterinary care' and the occurrence of leukosis in bovine herds in the state of Alagoas.

Given the detection of 20% of herd-level seroprevalence of antibodies against MAP identified for the first time in the region under study and the 85% herd-level seroprevalence of antibodies against BLV, the importance of implementing specific sanitary hygiene measures that include elements of surveillance based on the risks identified in this study cannot be overstated. Moreover, advice for farm administrators about the economic losses these diseases can cause should serve as an incentive to promote a change in attitude to improve the implementation of effective control measures in herds. This work received financial support from CAPES (Coordination of Improvement of Higher-Level Personnel), CNPq - no. 64/2008 (National Council for Scientific and Technological Development) and PIBIC (Institutional Program of Scientific Initiation Scholarships) of the University Center of Patos de Minas, Minas Gerais, Brazil.

Keywords: paratuberculosis, bovine leukemia, IDGA, ELISA

RESUMO

Mycobacterium avium subesp. paratuberculosis (MAP) e o vírus da leucemia bovina (BLV) são agentes que causam grandes perdas econômicas nos rebanhos. O objetivo deste estudo foi avaliar a situação epidemiológica da paratuberculose bovina (PTB) e leucose enzoótica bovina (EBL) em rebanhos leiteiros de Lagoa Formosa, Minas Gerais, Brasil. Foram coletadas 236 amostras de sangue de vacas, as quais foram submetidas aos testes ELISA e imunodifusão em gel de ágar para detecção de anticorpos contra MAP e BLV. A soroprevalência de anticorpos contra MAP e BVL foi de 20% para os rebanhos e 6% para os animais e de 85% para os rebanhos e 50,42% para os animais, respectivamente. A presença dessas enfermidades deve servir como um alerta para os produtores e veterinários, para que concentrem maior atenção na implementação de medidas higiênico-sanitárias, incorporando elementos de vigilância com base nos riscos identificados no estudo.

Palavras-chave: paratuberculose, leucemia bovina, IDGA, ELISA

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